Directions for a practical course in chemical physiology / W. Cramer.

DIRECTIONS FOR A PRACTICAL COURSE IN CHEMICAL PHYSIOLOGY

W. CRAMER

DIRECTIONS
FOR A PRACTICAL COURSE IN
CHEMICAL PHYSIOLOGY

BY

W. CRAMER, PH.D., D.SC.

Lecturer on Chemical Physiology, University of Edinburgh

LONGMANS, GREEN & CO.
39 PATERNOSTER ROW, LONDON
NEW YORK, BOMBAY, AND CALCUTTA
1912

PREFACE

In writing this book for the use of medical students the author has departed from the method usually followed. The subject matter is practically limited to experiments and deductions from the experiments, and is not meant to supply the full and ordered information obtainable from a text-book of physiological chemistry. The arrangement of the work differs from that generally followed, in that the student is at the outset provided with substances familiar to him, such as a potato, an egg, lard, butter, &c. From these he prepares, by simple chemical manipulations, the proximate constituents and their decomposition-products, and studies their chemical reactions and physical properties. In this way he is introduced to the subject without interposing complex chemical conceptions, which the usual arrangement of dividing the subject into the study of carbohydrates, fats, and proteins necessarily involves.

In teaching practical physiological chemistry to students, who have had only an elementary training in organic chemistry, it seems on the whole more satisfactory and more scientific to impose no tax on the faith of the pupil, even if the work covered is less comprehensive than it might otherwise be. The knowledge acquired in such a course as outlined in this book is at least real, and questions beyond the scope of personal demonstrations by the student may well be left to treatment in lectures.

In order to induce the student to record his own observations, to draw his conclusions from them, and to correlate the facts observed in

the laboratory with the theoretical matter taught in the systematic lectures or in the text-books, the text is interspersed with questions which the student is supposed to answer.

In describing the experiments special care has been taken to refer to the numerous technical details that must be observed and the fallacies that must be excluded in order to obtain a trustworthy result.

CONTENTS

POTATO.

Experiment 1. Preparation of Starch from Potato.—Scrape a potato as finely as possible with a knife, collect the scrapings in a beaker, stir with about 100 c.c. water, and strain the water which contains a large part of the starch through a piece of unbleached muslin. Mix the residue with more water and strain again. After repeating this with several portions, collect the water in one vessel and allow the starch grains to settle to the bottom. Examine some starch grains microscopically, and sketch. Pour off the water, add more, and allow again to settle, repeating till the starch appears white.

Experiment 2. Preparation of Starch Solution.—Rub 2 grms. of starch to a thin paste with a little water, and pour the mixture slowly, with constant stirring, into 150 c.c. boiling water. Use the solution so obtained for experiments 3-18.

Tests for Starch.

Experiment 3.—(*a*) To some of the starch solution in a test tube add a drop of iodine solution. Note the result. (*b*) Heat the tube gradually. What change takes place? (*c*) Allow the solution to cool, and observe the result. (*d*) Make alkaline with a few drops of sodium hydroxide. Note the result. (*e*) Neutralise with a few drops of dilute hydrochloric acid. Explain the result.

Experiment 4.—Repeat the preceding test, first adding sodium hydroxide solution before testing with iodine. Record and explain the result.

Experiment 5.—To 5 c.c. (roughly a quarter of a test tube) of the starch solution, add three times its volume of saturated ammonium sulphate solution. Shake, and allow to stand for fifteen to thirty minutes. Filter and test separately the precipitate and the clear filtrate with iodine for starch.

Experiment 6.—To 5 c.c. of the starch solution add alcohol until a precipitate appears. Apply the iodine test for starch to the precipitate, and to the filtrate separately, and note the result.

COMPLETE HYDROLYSIS OF STARCH BY ACIDS. FERMENTATION OF GLUCOSE.

PREPARATION OF GLUCOSE AND ALCOHOL FROM STARCH.

Preparation of Glucose by Acid Hydrolysis of Starch.

Experiment 7.—To 100 c.c. of starch solution, in a flask, add 1 c.c. of concentrated hydrochloric acid, and boil for twenty minutes over a small flame. Add occasionally some water previously heated in a test tube to replace the water which has evaporated. (*a*) What change do you observe in the appearance of the starch solution? Cool the solution. Test 5 c.c. of the solution with iodine. (*b*) What has taken place? This solution is used for Experiments 8-16.

Preparation of Alcohol by Fermentation of Glucose.

Experiment 8.—(*a*) To about 15 c.c. of the glucose solution obtained in Experiment 7 add dilute sodium hydroxide until the reaction is almost neutral to litmus, but still slightly acid. Add a small quantity of fresh yeast, and gently shake so as to break up the yeast. With the emulsion of yeast in the glucose solution fill the closed limb of a fermentation tube, so that no air bubble is left, and place the tube for about an hour in a warm water-bath at 40° C. What takes place?

(*b*) Put the fermentation tube into the cupboard. After twenty-four hours remove the solution and filter. Examine the filtrate for alcohol by means of the iodoform test and the bichromate test. [*Tests for alcohol*—(*a*) *Iodoform test.* To the solution add a solution of iodine in potassium iodide until it is brown, then add just sufficient sodium hydroxide to decolorise the mixture. Warm gently. A yellow crystalline precipitate with the characteristic smell of iodoform is formed. (*b*) *Bichromate test.* Dissolve a crystal of potassium bichromate in the solution and add a little dilute sulphuric acid. Heat. The solution turns green, and the characteristic odour of aldehyde will be observed. What reaction has taken place?]

Tests for Alcohol.

Carefully neutralise, with sodium hydroxide, the rest of the solution obtained in Experiment 7, and perform the following tests for glucose.

Tests for Glucose.

Experiment 9.—(*a*) To 5 c.c. of sodium hydrate in a test tube add a solution of copper sulphate. A blue flaky precipitate forms which does not dissolve on shaking. What is it? Boil. Describe the result.

Explain and compare with the following :—

(*b*) **Trommer's Test.**—Add to 5 c.c. of the glucose or sugar solution an equal volume of strong sodium hydrate or potassium hydrate solution, then a dilute solution of copper sulphate, drop by drop, shaking after each addition, as long as the bluish precipitate formed continues to dissolve readily. If an excess of $CuSO_4$ is added, the precipitate remains, and must be dissolved by adding a few crystals of sodium potassium tartrate (Rochelle salt). The result is a deep blue solution. Why is no precipitate formed in this case? Heat the solution, and note the result. Explain the chemical changes which have taken place.

Experiment 10. Fehling's Test.—Prepare Fehling's solution. Dissolve in 80 c.c. of distilled water 7 grms. of copper sulphate, and label solution "A." Dissolve 34.5 grms. sodium potassium tartrate in 120 c.c. sodium hydroxide 10 per cent., and label solution "B." Mix "A" and "B," and fill the mixture into bottle labelled "Fehling's Solution."

Boil 5 c.c. of Fehling's solution in a test tube (it should remain clear), and then add a few drops of the glucose solution. Let it stand a while and note the result. Repeat the test without heating.

Experiment 11. Barfoed's Test.—To 5 c.c. of a solution of cupric acetate in acetic acid (Barfoed's reagent) add a few drops of the glucose solution, boil, and set aside. Examine against a dark background and note the result. How does Barfoed's test differ from Fehling's or Trommer's test?

Experiment 12. Böttger's Test.—To a quarter of a test tube of the glucose solution add a little solid bismuth subnitrate, and about double the quantity of sodium carbonate. Heat and keep boiling for two minutes. Describe and explain the result.

Experiment 13. Nylander's Test. — Prepare Nylander's reagent. Dissolve 4 grms. sodium potassium tartrate and 2 grms. bismuth subnitrate in 100 c.c. sodium hydroxide 10 per cent. Pour the reagent into bottle labelled "Nylander's Reagent." Add one part of Nylander's reagent to ten parts of sugar solution, and keep boiling over a small flame for two minutes. Compare with Böttger's test.

Repeat tests 8 to 12 with a very dilute glucose solution in order to test their delicacy. Tests 8 to 12 are reduction tests. Why are they called so, and what is the rationale of these tests? What happens if these tests are applied to starch solution?

Experiment 14. Osazone Test.—To about 10 c.c. of the glucose solution add 10 drops of phenylhydrazine and an equal amount of glacial acetic acid. Shake the test tube and keep in the boiling water-bath for ½ to 1 hour. Allow to cool slowly and examine crystals under the microscope, and sketch. They consist of glucosazone.

Experiment 15. Molisch's Test.—To a few c.c. of the solution add a few drops of an alcoholic solution of *a*-naphthol. Incline the test tube and allow about 5 c.c. of concentrated sulphuric acid to flow down the side of the tube so that the acid settles at the bottom of the tube. At the junction of the two liquids a green ring may at first be formed (if traces of nitrates are present in the sulphuric acid), and above this, in a short time, a reddish-violet ring. If the two liquids be mixed and cooled by gently shaking in running water, so that excessive heating is prevented, the mixture assumes a bluish-red or dark blue colour. [All carbohydrates and some proteins give this reaction. The test is used to demonstrate the presence of a carbohydrate group in proteins.]

Experiment 16.—Examine by means of a polarimeter the effect of the glucose solution on polarised light. (Demonstration.) Glucose rotates polarised light to the right.

PARTIAL HYDROLYSIS OF STARCH BY ACIDS.

PREPARATION OF DEXTRIN.

Preparation of Dextrin by Acid Hydrolysis of Starch.

Experiment 17.—Prepare dextrin from starch by heating in a porcelain dish, on a water-bath, half a spoonful of powdered starch, previously moistened with a few drops of dilute acid (made by adding a few drops of strong nitric acid to a test tube full of water). The starch must be stirred with a glass rod until it has turned yellow, or brown. Extract with cold water; filter. To a few c.c. of the solution thus obtained add some iodine solution, drop by drop, noting the colour after each drop until five drops have been added. If the colour obtained is very dark, dilute until the colour can be plainly distinguished. To another portion of the solution apply Trommer's, Fehling's, and Barfoed's tests. Record and explain.

Tests for Dextrin.

PARTIAL HYDROLYSIS OF STARCH BY FERMENTS.

PREPARATION OF DEXTRIN AND MALTOSE.

Preparation of Dextrin and Maltose by action of Saliva on Starch.

Experiment 18.—Prepare some dilute saliva by rinsing out the mouth for 1 minute with 5 c.c. warm water. Collect the washings in a beaker. Repeat. Filter the dilute saliva. Place a series of drops of iodine on a porcelain plate. Put some starch solution (about 5 c.c.) in a test tube and add about half a test tube full of saliva. Shake the mixture and place in the water-bath at 40° C. Every minute take out a drop with a glass rod and apply it to one of the iodine drops on the porcelain slab. What changes take place in the mixture? Keep the mixture in the water-bath for $\frac{1}{2}$ to 1 hour, until all the starch has been transformed into maltose. This solution is used for Experiment 19. By what constituent of the saliva is starch transformed into maltose?

Tests for Maltose.

Experiment 19.—With the solution of maltose obtained in Experiment 18 carry out the tests 9 (*b*) to 14. Note the results. How does maltose differ from glucose, and how can it be distinguished from it?

LARD, OLIVE OIL.

Solubility of Fats.

Melt a little lard in a porcelain basin on a boiling water-bath; with the melted fat carry out the following experiments:—

Experiment 20.—Add a few drops of the lard to each of four test tubes containing (1) alcohol, (2) ether, (3) chloroform, (4) water, and note the solubility of the fat in these solvents.

Experiment 21.—Let a drop of the alcoholic or ethereal solution fall on a piece of white paper and note the grease spot which remains after the solvent has evaporated.

Experiment 22.—To a test tube containing water add a few drops of the alcoholic solution of the fat. A precipitate appears. Explain.

Experiment 23.—Repeat Experiments 20 to 22 with olive oil instead of lard.

HYDROLYSIS OF LARD BY ALKALI (SAPONIFICATION).

PREPARATION OF FATTY ACIDS.

Preparation of Fatty Acids by Hydrolysis of Fats.

Experiment 24.—Slowly add about 5 c.c. of the melted lard to 50 c.c. of an alcoholic solution of caustic potash, contained in a flask, and kept on a boiling water-bath. Mix thoroughly and heat for ten to twenty minutes. Add a few drops of the mixture to some water in a test tube as in Experiment 22. No oil globules separate out. Why? (If oil globules are still seen, the reaction is not complete, and heating must be continued with more alcoholic soda.) When the reaction is complete, slowly pour the solution in the flask into a beaker containing 100 c.c. of warm water,

and mix thoroughly. To the watery solution (which contains soap), add some 25 per cent. sulphuric acid (1 part concentrated sulphuric acid slowly added to 3 parts of water) and heat on the water-bath until the melted fatty acids separate out as an oily layer floating on the top of the liquid. Cool. The fatty acids solidify and can then be removed and freed from adhering sulphuric acid by rinsing with cold water.

Properties of Fatty Acids and of Soaps.

Experiment 25.—Suspend some of the fatty acids in water and add dilute sodium hydroxide. The fatty acids dissolve. Soap is formed again. What is soap? Use the solution of soap thus obtained to demonstrate the following properties:—

(*a*) Shake up some of the soap solution with warm water. A soap lather is produced.

(*b*) To some soap solution add solid sodium chloride until the solution is saturated. The soap is precipitated: "salted out."

(*c*) Add a few drops of calcium chloride solution to some of the soap solution. A precipitate is formed. Of what?

The remainder of the soap solution is used to demonstrate the part played by soap in—

EMULSIFICATION OF FATS.

Emulsification of Fats.

Experiment 26.—Label two test tubes "(*a*)" and "(*b*)." Place in (*a*) some water and in (*b*) some soap solution. Add to each three drops of olive oil and shake. A permanent emulsion is formed in (*b*) but not in (*a*). Explain.

Experiment 27.—The same result is, of course, obtained if neutral fat containing some fatty acid is shaken up with a little dilute alkali solution. For instance: To half a test tube full of water add one drop of a 10 per cent. solution of sodium hydroxide and 2 c.c. of ordinary olive oil, which always contains some free fatty acid. An emulsion is formed. Explain.

A neutral fat, which does not contain any free fatty acids, will not give a permanent emulsion with dilute sodium hydroxide. This reaction may, therefore, be used to test for the presence of free fatty acids in a fat. These are present if a fat is rancid.

Another method of detecting whether a fat is rancid is as follows :—

Experiment 28.—To some alcohol in a test tube add a drop of phenolphthalein and one or two drops of dilute caustic soda, just enough to produce a red colour. Add this fluid to a solution of olive oil in alcohol. If fatty acids are present the red colour disappears. Why?

BUTTER.

Experiment 29.—Heat a little butter with a small quantity of alcoholic soda until a clear yellow solution is obtained. Pour the solution into a beaker containing hot water (no oil drops should be seen) and heat to expel the alcohol. Then acidify with dilute sulphuric acid and warm again, when the smell of butyric acid (and caproic acid) will be noticed. Compare briefly butyric acid and the higher fatty acids obtained from lard with regard to their solubility in water, their volatile nature, and their molecular weight.

A TISSUE FLUID: BLOOD SERUM.

The cellular elements of the blood are suspended in a fluid—the blood plasma. When blood is drawn from a blood vessel, this coagulates into a clot, which entangles the blood cells. On standing, the clot contracts and expresses a clear fluid—the *blood serum.*

For the following experiments ox serum is given out :—

Experiment 30.—Test the reaction of serum against litmus.

Determine the specific weight by means of a hydrometer. Wipe the instrument clean, and float it in the centre of the cylinder containing the serum, taking care that it does not touch the sides of the vessel. Place the eye level with the true surface of the serum (not the top of the meniscus around the shaft of the hydrometer), and read the division of the scale Record your result.

Proteins of Blood Serum

Experiment 31. Preparation of Globulin and Albumin.—To 10 c.c. of the serum add an equal volume of a saturated solution of ammonium sulphate, thus obtaining a half-saturated solution. Filter off the precipitate of *serum-globulin* which appears, and remove the filtrate, which contains *serum-albumin.* Wash the precipitate two or three times with a half-saturated ammonium sulphate solution, and dissolve it together with the ammonium sulphate which adheres to it in about 20 c.c. of water. This yields a clear solution of a globulin in a dilute salt (ammonium sulphate) solution. Label it "*Serum-globulin.*"

To the filtrate from the serum-globulin add solid ammonium sulphate until the solution is saturated, keeping the solution at a temperature of 20° to 30° C. in order to facilitate the dissolving of the ammonium sulphate. Higher temperatures must be avoided. Why? When the solution is saturated a precipitate of albumin is formed. Note the difference between the dead-white, heavy crystals of ammonium sulphate lying at the bottom of the vessel and the yellowish-white, light, flocculent precipitate of albumin which is suspended in the liquid. Filter. Wash the precipitate on the filter with a saturated solution of ammonium sulphate, and dissolve it in about 20 c.c. water. A clear solution of an albumin is obtained. Label it "*Serum-albumin.*"

Solubilities of Albumin and Globulin.

Experiment 32. Solubility of Albumin and Globulin in Water and in Concentrated Salt Solutions.—Add a few drops of the globulin solution to a large volume of distilled water in a beaker. A slight, cloudy precipitate is formed.

Do the same with the albumin solution. No precipitate is formed.

Saturate some of the globulin solution with solid sodium chloride. The globulin is precipitated.

Saturate some of the albumin solution with solid sodium chloride. The albumin is not precipitated.

Record in tabular form the solubilities of albumin and globulin according to the following table:—

Solvent.	Albumin.	Globulin.
Distilled Water - - - -		
Dilute NaCl Solution - - -		
Saturated NaCl „ - - -		
Half-saturated $(NH_4)_2 SO_4$ solution		
Full-saturated $(NH_4)_2 SO_4$ „		

GENERAL PROTEIN REACTIONS.

General Protein Tests.

Dilute 10 c.c. of serum with 90 c.c. of water, and with the diluted serum carry out the following tests by which proteins can be recognised:—

Experiment 33. Heat Coagulation:—

(*a*) Heat 5 c.c. in a test tube to boiling point. The solution becomes opalescent, but there is no definite coagulum.

(*b*) Heat 5 c.c. with 1 to 2 drops of dilute acetic acid. A coagulum is formed.

(*c*) Heat 5 c.c. with 2 drops of glacial acetic acid. No coagulum occurs. Acid albumin is formed, which does not coagulate on boiling.

(*d*) Heat 5 c.c. with 2 to 3 drops of dilute sodium carbonate solution. There is no coagulation. Alkali albumin is formed, which does not coagulate on boiling.

The globulins and albumins of blood serum are thus only coagulated by boiling when the solution is neutral or faintly acid. If the solution be alkaline, as serum generally is, the proteins are acted on by the alkali, as the temperature rises, and converted into *alkali-albumin*. If strongly acid, as in (*c*), the proteins are converted into *acid-albumin*.

These compounds, which are grouped together as *metaproteins*, are not coagulated on heating their solutions. They are precipitated on neutralising their solutions, and dissolve again in an excess of either acid or alkali.

Experiment 34. Colour Tests:—

(*a*) *Biuret Test.*—To a portion of the protein solution add sodium hydroxide so that the solution is strongly alkaline, then one or two drops of very dilute copper-sulphate solution. (An excess of copper-sulphate must be avoided.) The solution becomes violet.

On what group in the protein molecule does this test depend? Prepare some biuret by heating a few urea crystals in a dry test tube until the melted mass begins to solidify again. Allow to cool, dissolve in a little water, and apply the biuret test. What is the structural formula of biuret?

Study the effect of the presence of an ammonium salt on the reaction in the following way:—Apply the test to (*a*) water; (*b*) water to which some ammonium sulphate has been added; (*c*) serum; (*d*) serum to which some ammonium sulphate has been added. The following colours will be obtained:—

(*a*) Water - - - - -	faint blue
(*b*) Water + $(NH_4)_2SO_4$ - - -	deep blue
(*c*) Serum - - - - -	violet
(*d*) Serum + $(NH_4)_2SO_4$ - -	deep blue.

Only (*c*) gives the characteristic violet colour which indicates the presence of proteins. Ammonium salts therefore interfere with the test. This must

be borne in mind when the test is applied after having used ammonium salts for the separation of proteins. The difficulty can be overcome by using in such a case a very large amount of caustic soda before adding the copper sulphate. Try this with some serum to which ammonium sulphate has been added.

(*b*) *Millon's Test.*—To a portion of the protein solution add a few drops of Millon's reagent (solution of mercurous and mercuric nitrates). A precipitate forms, which, on heating, becomes brick-red. The red colour constitutes the essential part of the test.

On what group in the protein molecule does this test depend? Repeat with a dilute solution of phenol.

Apply Millon's test to the protein solution after having added some sodium chloride. What takes place? Explain.

Millon's test can be applied to insoluble proteins.

(*c*) *Xanthoproteic Test.*—To a few c.c. of the protein solution add one-third of its volume of concentrated nitric acid; a white precipate may or may not be produced (according to the concentration and nature of the protein). Boil. The precipitate or liquid turns yellow. Cool the test tube, and carefully add excess of ammonia, so as to form a layer above the nitric acid. An orange colour is produced at the junction. This constitutes the essential part of the test. Mix the ammonia with the acid by shaking and note that the yellow colour of the solution deepens.

On what group in the protein molecule does this test depend? Repeat the test with benzine.

This test can be applied to insoluble proteins.

Experiment 35. Precipitation by Strong Mineral Acids (Nitric Acid or Heller's Test).—Place 5 c.c. of the protein solution in a test tube, and by means of a pipette add 1 or 2 c.c. of strong pure nitric acid very carefully to the bottom of the solution, so that it forms an under layer. A white ring of coagulated protein appears at the junction of the two fluids. (Allow ten minutes if the reaction is slow in appearing.)

Experiment 36. Precipitation by Alkaloidal Reagents. — Proteins in acid solution are precipitated by reagents which precipitate alkaloids; *e.g.* :—

(*a*) *Hydroferrocyanic Acid.*—Make a portion of the protein solution distinctly acid with acetic acid; then add a few drops of potassium ferrocyanide solution. A precipitate is formed. Why is it necessary to carry out this test in this way, instead of adding free hydroferrocyanic acid to the protein solution?

(*b*) *Picric Acid.*—To some protein solution add picric acid drop by drop. Note that a precipitate forms around each drop as it falls in. On shaking, this precipitate dissolves at first. If sufficient picric acid has been added the precipitate remains.

(*c*) *Salicylsulphuric Acid.*—To a portion of the protein solution add a few drops of salicylsulphuric acid solution. A precipitate is formed.

A number of other alkaloidal reagents (tannic acid, phosphotungstic acid, &c.), give similar precipitates with protein solutions.

What is an alkaloid? Why do albumins and globulins ln acid solution behave like alkaloids towards these reagents?

Experiment 37.—Test the delicacy of the protein tests 33 to 36 by applying them to (*a*) 2 c.c. serum diluted with 98 cc. water (1 : 50 dilution), (*b*) 1 c.c. serum diluted with 99 c.c. water (1 : 100 dilution). Record your results.

Experiment 38. Precipitation by Alcohol.—To the protein solution add an excess of alcohol. A precipitate is formed. Allow the precipitate to stand in contact with alcohol for half an hour. Decant the alcohol and add water to the precipitate. It has become insoluble in water, having been coagulated by the alcohol. What is the difference between "precipitation" and "coagulation"?

SERUM CONSTITUENTS OTHER THAN PROTEIN.

Experiment 39.—Remove the serum proteins by heat coagulation in the following manner:—Dilute 20 c.c. of serum with 100 c.c. of water; heat the neutral solution to boiling point, stirring constantly. Make faintly acid

with a few drops of dilute acetic acid, and filter. If the heat coagulation has been carried out correctly (see Exp. 33), the filtrate will not give the biuret test, *i.e.*, it will be free from proteins.

Concentrate the filtrate to about 20 c.c., and apply to it the following tests :—

(*a*) Test for chlorides with silver nitrate and nitric acid.

(*b*) Test for phosphates with ammonium molybdate and nitric acid.

(*c*) Test for sulphates with bariumchloride and hydrochloric acid.

(*d*) Test for a reducing sugar with Fehling's or Trommer's test.

Record your results.

EXAMINATION OF TISSUE PROTEINS: MUSCLE AND GLAND.

Experiment 40. Proteins of Muscle.—In a mortar rub thoroughly 10 grms. of muscle (best from white fish) with 5 grms. of sodium chloride, then to the pulp add 50 c.c. of water, so as to make a 10 per cent. solution of NaCl, Stir the mixture well and strain through muslin. Label the solution, "*Salt Extract* (A)."

Rub the residue with 0.2 per cent. NaOH solution in the mortar and again strain. Label this solution, "*Soda Extract* (B)." A residue containing collagen is left on the muslin.

Salt Extract (A).—Determine that this extract contains protein by applying the colour tests for protein (Test No. 34). Record the results.

Determine that it is a protein coagulable by heat by heating the *neutral extract* with the addition of a few drops of acetic acid (Test 33). (Note the conditions necessary for this test as explained in Test 33). Record the results.

Determine whether the protein is a globulin or an albumin by testing its solubility in water (test 32), and in ammonium sulphate solution (Test 31). Record the result.

Nucleoprotein. *Soda Extract* (B).—On careful addition of acetic acid to this extract a precipitate forms which is redissolved in a large excess of the acid. It is the nucleoprotein of muscle.

Determine that the nucleoprotein solution gives the colour tests for proteins (No. 34).

Experiment 40a. Proteins of Glands.—Repeat this experiment with glands (pancreas) instead of muscle. Note and record how these two tissues differ with regard to the relative amounts of (1) albumin and globulin (salt extract), (2) nucleoprotein (soda extract).

FLOUR AND BREAD.

Experiment 41. Flour.—Knead some flour with a little warm water to form a stiff dough and allow to stand for about fifteen minutes. Place the dough in a muslin bag and continue kneading in a basin of water; starch grains pass through. If the water is poured from the basin into a beaker the deposit of starch grains which settle can be easily seen. Prove their chemical nature by examining microscopically, and by boiling some grains with water and applying the iodine test to the watery solution thus obtained. Remove the grains from the water by filtration, and test the filtrate with iodine for the presence of dextrin (see Exp. 17) and with Fehling's and Nylander's tests for glucose (Tests Nos. 10 and 13). Continue kneading the dough under the tap until all the starch has been removed. A yellowish sticky mass remains. This is gluten, the chief protein of wheat. Prove its protein nature by applying the ordinary colour tests for solid proteins (No. 34 *b*, *c*).

Gluten.

Experiment 42. Bread.—Grate some bread finely, and extract, first with cold water, then with boiling water. Strain the watery extract through muslin.

Examine :—

1. Cold water solution for glucose, starch grains, dextrin.

2. Hot water solution for glucose, starch, dextrin. In order to demonstrate the presence of dextrin when starch is also present in solution, remove the latter by half saturating the solution with ammonium sulphate. The dextrin passes into the filtrate.

3. The residue is gluten and can be identified as a protein by the colour tests (No. 34) as in Experiment 40.

Record the results. What difference is there between the carbohydrates of flour and those of bread?

What changes do the carbohydrates of flour undergo in the process of baking, and how do you account for these changes?

Experiment 43.—Extract some crust of bread with water and test for starch, dextrin, and glucose.

Note that in the crust glucose is present in traces only. Why?

EGG.

Demonstration of Osmosis.—Two eggs, from which the shells have been removed by immersion in hydrochloric acid, are weighed. The one is immersed in distilled water, the other in a 10 per cent. salt solution. Note the change in volume and weight.

Demonstration of a semi-permeable membrane.

Separation of Constituents of Egg.—Break a raw egg and collect separately in two beakers the white and the yolk of the egg. The white of the egg consists mainly of proteins, while the yolk, besides other protein substances and some fat, contains lipoids (*e.g.*, lecithin, cholesterin).

Lipoids of Egg-Yolk.

Experiment 44. Yolk. Preparation of Lecithin and Cholesterin.—Mix the yolk thoroughly with about 20 c.c. of ether, pour into a flask, and close flask. Shake vigorously, and allow it to stand for some time In the meantime the solution of egg albumin may be prepared as directed below (in Exp. 45).

(N.B.—*In working with ether all gas flames in the neighbourhood must be extinguished.*)

Pour off from the residue the ethereal, deeply coloured solution into a porcelain capsule, and extract the residue again with 20 c.c. of ether. The ethereal extracts are concentrated by placing the capsule in the fume chamber in a previously heated water-bath (use no flame!). Acetone is then added until a distinct precipitate is formed. The precipitate is a crude mixture of phosphorised fats. This mixture is frequently described by the name of its main constituent—"lecithin." The filtrate contains cholesterin. Remove the precipitate by filtration, concentrate the filtrate on the water-bath. Cholesterin separates out.

(*a*) LECITHIN.

Lecithin.

Dissolve in alcohol the precipitate on the filter. The bulk of it dissolves. Drop the alcoholic solution into water and stir. A white pre-

cipitate of "lecithin" is formed. Compare the solubility of lecithin in water, alcohol, and ether with that of ordinary fats in the same solvents and record. Boil the watery emulsion with caustic soda. It becomes clear. Notice the smell of Trimethylamine. On acidifying, fatty acids separate out.

Explain the change which has taken place.

(*b*) CHOLESTERIN.

Cholesterin. Dissolve some of the cholesterin which has separated out from acetone in a very little boiling alcohol, and allow to cool slowly. Examine microscopically the crystals which separate out, and sketch. Add a drop of strong sulphuric acid; the edges of the crystals turn red.

Remove all the water from a test tube by washing it out with alcohol and ether. Place some chloroform in this dry test tube and dissolve in it some cholesterin. Add an equal bulk of concentrated sulphuric acid and shake gently. The chloroform solution which rises to the top turns red, the acid at the bottom of the test tube shows a green fluorescence.

The necessity for using a dry test tube is shown by the fact that the red colour of the chloroform solution disappears if it is poured into a wet test tube.

Proteins of Egg-White. **Experiment 45. White of Egg.**—Cut up the egg-white with a pair of scissors. The viscid fluid thus obtained is faintly yellow in colour, alkaline in reaction, and has a specific gravity of 1.045. It contains about 10 per cent. of proteins, the greater proportion is egg albumin.

Egg Globulin. A small quantity of the filtered egg white, on dilution with nineteen times its volume of water, yields a well-marked white precipitate—egg globulin—which readily dissolves on the addition of a few drops of a saturated solution of sodium chloride. If the greater part of the egg white be similarly diluted with water, and filtered from the precipitate of globulin the filtrate approximately represents a $\frac{1}{2}$ per cent. solution of ovalbumin.

Egg Albumin. With the solution of ovalbumin carry out the protein tests (Nos. 33 to 38).

Prove that the chief protein is an albumin by determining its solubility in distilled water, half saturated ammonium sulphate solution, fully saturated ammonium sulphate solution (see Test 32).

That egg white does not contain any carbohydrates can be shown by applying the tests for carbohydrates to the watery extract (the ovalbumin solution may be used) from which all the protein has been removed by heat coagulation. Record.

Apply Molisch's test (Exp. 15). A positive result is obtained, showing that while no free carbohydrate is present, the protein of the white of the egg contains a carbohydrate group chemically bound in its molecule.

MILK.

Milk.

Experiment 46.—Test the fresh milk with litmus paper. The reaction is either faintly alkaline or amphoteric (*i.e.*, turns blue litmus red and red litmus blue).

Determine the specific gravity of milk. Record.

Why has skimmed or separated milk a higher specific gravity than fresh milk?

Fat.

Experiment 47. Separation of Constituents.—Extract the fat by shaking 5 c.c. of milk in a test tube with twice its volume of ether. Pour off the ether and allow to evaporate until only a few drops are left. Pour these on to a filter paper. A greasy spot indicates the presence of fat.

Caseinogen.

Dilute 20 c.c. of milk with 80 c.c. of water. Add carefully dilute acetic acid drop by drop, stirring the fluid after each drop has been added, until a flocculent precipitate is formed and the solution in which it floats appears clear. An excess of acid must be carefully avoided as it would dissolve the precipitate. The precipitate consists of caseinogen—the main protein of milk—with adherent fat. Filter. With the precipitate, carry out the experiments described below under caseinogen (Exp. 48).

Lactalbumin.
Lactoglobulin.

The filtrate from the caseinogen, which still gives the tests for proteins, contains (besides small amounts of lactalbumin and lactoglobulin) lactose —the carbohydrate material of milk. Remove the albumin and globulin by heat coagulation in the following way:—

Carefully neutralise the acid filtrate with dilute sodium carbonate solution. Heat the neutral solution to boiling point and add a few drops of dilute acetic acid. If the heat coagulation is carried out correctly, the lactalbumin and lactoglobulin separate out as a flocculent precipitate which is removed by filtration, and the filtrate does not give any tests for protein.

Lactose.

When all the protein has been removed, the filtrate may be used for the experiments described below under lactose. It contains, besides lactose, inorganic phosphates of calcium, which can be detected by the ordinary test for phosphates. Label the filtrate "*Lactose.*"

Experiment 48. Caseinogen.—Dissolve the precipitate of caseinogen obtained in Experiment 47 in dilute sodium carbonate solution. The adherent fat remains suspended. Filter through a wet filter paper, and apply to the caseinogen solution the colour tests for proteins. Record.

Determine the solubility of caseinogen in half-saturated ammonium sulphate solution. Record.

Determine whether caseinogen is a protein coagulable by heat. (See Exp. 33.) Record.

Experiment 49. Lactose.—Evaporate some of the filtrate obtained in Experiment 47, and labelled "Lactose," to a syrup on the water-bath, and allow to stand in the cold until the sugar has crystallised out.

To another portion of the filtrate labelled "Lactose" apply the following tests for sugar:—

Fermentation test (No. 8).

Reduction tests: Trommer or Fehling (Nos. 9 and 10); Böttger or Nylander (Nos. 12 and 13); Barfoed (No. 11).

Osazone test (No. 14).

Experiment 50.—Apply the same tests to a solution of cane sugar which is given out.

Demonstration of the action of the different sugars on polarised light before and after hydrolysis (boiling with dilute HCl). Why is hydrolysed cane sugar called invert-sugar?

From Experiments 8, 9, 10, 11, 12, 13, 14, 19, 49 and 50, construct the following table :—

Tests for Sugars.

REACTIONS OF SUGARS.

	Nature of Sugar.	Trommer or Fehling.	Nylander.	Barfoed.	Fermentation.	Form of Osazone.
Monosaccharide -	Glucose -					
Disaccharide	Maltose -					
	Lactose -					
	Sucrose (Cane Sugar)					

Verify the table by applying the various tests to the solutions of the different sugars which are given out.

Action of Rennet on Milk.

Experiment 51. Clotting of Milk.—To 5 c.c. of milk in a test tube add 1 c.c. of a neutral solution of rennet ferment (prepared by extracting a calf's stomach with glycerine). Place the mixture in a water-bath, kept at 37° to 40°. After a few minutes a firm clot forms, from which, on standing, a clear fluid exudes. The clot contains casein and fat. What is casein? The clear fluid—the whey—contains all the other constituents of the milk.

Experiment 52. Prove that the formation of the clot depends upon the presence of soluble Calcium Salts.—Precipitate all the calcium present in 5 c.c. of milk by adding 2 c.c. potassium oxalate solution (1 per cent.). Then add 1 c.c. of rennet and place in water-bath. No clot forms if all the calcium has been precipitated. Then add to the unclotted milk 2 c.c. $CaCl_2$ solution (2 per cent.). A flocculent precipitate is produced. Explain the results.

CHEMICAL EXAMINATION OF TISSUES.

From the foregoing experiments it follows that the three main groups of organic substances which may be present in tissues and tissue-fluids namely, *proteins*, *carbohydrates*, and *fats*, can be separated from each other by extraction with different solvents. The various substances of which each group is composed can then be recognised by their specific reactions.

The following general rules for the chemical examination of tissues and tissue-fluids can be deduced from the work hitherto performed :—

The organs must be minced.

1. *Proteins.*

(*a*) Extraction with dilute salt solution dissolves—

1. *Native Proteins* (*Albumins*, *Globulins*).—They give the tests for proteins. They are coagulated by heat. In order to ascertain whether an albumin or a globulin is present, determine the solubility in distilled water, in half-saturated and in fully saturated ammonium sulphate solution. If both are present they can be separated by their different solubility in ammonium sulphate solution.

2. *Mucin.*—It gives all the tests for proteins. It is precipitated by acetic acid, and is insoluble in excess of this acid (see Exp. 53). On boiling with dilute HCl it yields a reducing substance, glucosamin. This last test can be carried out only if sufficient material is obtained.

The saline extract will also contain carbohydrates and extractives (see below).

(*b*) Extraction with 1 per cent. sodium carbonate or sodium hydrate solution dissolves *Nucleoprotein*. It gives all the protein tests. It is coagulable by heat. It is precipitated by dilute acetic acid, but soluble in excess of this acid. This alkaline extract will also contain *Mucin* (see above).

II. *Fats and Lipoids.*—The tissue is dried and extracted with ether, which dissolves—

Fats.
Phosphorised fats.
Cholesterin.

Some lipoids, for instance, *cerebrosides*, *phorphorised cerebrosides* (*protagon*), &c., can be dissolved only by extraction with hot alcohol.

III. *Carbohydrates.*—Extraction with hot water will dissolve all the carbohydrates :—*Starch*, *glycogen* (see below under liver), *dextrin*, *sugars*. These may be recognised by their tests. The extract may contain a small amount of protein substances (test by means of biuret test) these must be removed by precipitation or heat coagulation before the tests for carbohydrates can be applied. The watery extracts also contain *inorganic salts* and *organic extractives*, such as *urea*, *creatin*, *purin-bases*, &c. The commercial meat extracts consist mainly of such organic extractives.

The method is essentially the same if tissue-fluids, exudates, &c., are to be examined, except that the tests for proteins are applied directly to the fluid. If any proteins are present, they must be removed before examining for carbohydrates. Fat is extracted by shaking the fluid with ether, which, after allowing the two fluids to separate, is removed by means of a pipette, or by decantation.

If contents of the alimentary canal are to be examined, products of protein digestion may be present, and must be tested for by the method described under digestion (see below).

DIGESTION.

I. SALIVA.

Experiment 53.—Collect about 10 c.c. of saliva in a beaker. Test its reaction to litmus. Record.

Mucin.

Add dilute acetic acid to some saliva in a test tube. A stringy precipitate of mucin is formed. It is insoluble in excess of acetic acid.

Prepare some dilute saliva as described in Experiment 18. With this dilute saliva carry out the following experiment:—

Digestion by Saliva.

Experiment 54. Prove that Saliva contains an Amylolytic (Diastatic) Ferment: Ptyalin.—To 5 c.c. of starch solution (1 per cent.) in a test tube add 5 c.c. of dilute saliva. Mix by shaking and place in a water-bath kept at 37° to 40°. Place a series of drops of dilute iodine solution on a porcelain slab. Every half minute remove a drop of the salivary digest by means of a glass rod, and apply it to one of the iodine drops. At first a blue colour will be produced, then a reddish violet colour, then a light brown colour, finally no colour will appear. (State what chemical changes are indicated by the changes in colour.) The point when first a colour fails to appear is called the "achromic point"; the time necessary to reach the achromic point is called the "chromic period." The determination of the chromic period is a means of estimating quantitatively the activity of the ferment, provided that a starch solution of known and constant concentration is used. State the chromic period of the saliva used for this experiment. After the achromic point has been reached, apply Trommer's or Fehling's test to the salivary digest. Reduction occurs. Which sugar is present?

Properties of Ptyalin.

Experiment 55. Prove that Ptyalin does not act in an Acid Medium.—To 5 c.c. of dilute saliva add five drops of very dilute HCl (0.4 per cent.), and mix this with 5 c.c. of starch solution. Then determine the "chromic period" as detailed above. Record your result. What bearing has the effect of dilute HCl on the digestion of carbohydrates in the stomach?

Experiment 56. Prove that the Ferment Ptyalin is destroyed by Heat.—Heat 5 c.c. of dilute saliva to boiling point, cool, and mix with 5 c.c. of starch solution. Determine the "chromic period" as detailed above. Record your results.

Experiment 57. Prove that the activity of the Ferment Ptyalin is arrested or retarded by Cold.—Mix 5 c.c. of dilute saliva with 5 c.c. of starch solution and place the mixture into a beaker filled with cold water. Determine the "chromic period."

II. GASTRIC DIGESTION.

GASTRIC JUICE.

Gastric Digestion.

An extract of the gastric mucous membrane is supplied.

Experiment 58.—Test the reaction to litmus. It is acid. The acid which is present in gastric juice is hydrochloric acid.

The following Experiments (59 to 66) should be carried out at the same time.

Properties of Pepsin.

Experiment 59. Prove that the gastric juice contains a proteolytic ferment: Pepsin.—Place small shreds of fibrin (what is fibrin?) in three or four different test tubes, labelled "A." Add about 5 c.c. of the gastric juice to each, and place the test tubes in a water-bath which is kept between 35° and 40°. The fibrin swells up, becomes transparent and dissolves after half an hour or an hour. The time will be longer if more than a small shred of fibrin is placed in each tube. Reserve the contents of test tubes for Experiment 74.

Experiment 60. Prove that the action of this ferment is dependent upon the presence of a trace of free HCl (about 0.2 per cent.).—Place a small shred of fibrin in a test tube, labelled B, and add about 5 c.c. of gastric juice, which has previously been neutralised by adding carefully, drop by drop, dilute NaOH until the solution is neutral to litmus. Place in water-bath. The fibrin remains unaltered.

Experiment 61. Prove that HCl alone cannot digest fibrin.—Place a small shred of fibrin in a test tube, labelled C, and add about 5 c.c. of dilute HCl of the same concentration (0.2 per cent.) as the acid present in the gastric juice. Place in water-bath. The fibrin swells up, becomes transparent, but does not dissolve. This can be verified by testing the filtered fluid for the presence of protein by means of the biuret or the xanthoproteic test.

Experiment 62. Prove that the ferment is destroyed by heat.—Place a small shred of fibrin in a test tube, labelled D, and add about 5 c.c. of gastric juice which has been boiled and then cooled. Place in water-bath. The fibrin swells up, becomes transparent, but does not dissolve.

Experiment 63. Prove that at a low temperature the activity of the ferment pepsin (in the presence of HCl) is arrested.—Place a small shred of fibrin in a test tube, labelled E, add about 5 c.c. of the gastric juice, and place the test tube in a beaker filled with cold water. The fibrin does not dissolve.

Experiment 64. Prove that this result is not due to destruction of the ferment as in Experiment 62, but to the inactivity of the pepsin at a low temperature. Describe the experiment and record the result.

Experiment 65. Prove that in the process of peptic digestion hydrochloric acid combines with the protein.—Place about 5 c.c. of gastric juice in a test tube, labelled F, and add a large shred of fibrin. Place test tube in water-bath, and allow to digest for about half an hour. Then add one drop of Töpfer's reagent, which will give a brownish-pink colour. Compare this colour with the deep pink colour obtained on adding one drop of Töpfer's reagent to some gastric juice in which no protein has been digested.

The difference in colour is due to the fact that in the latter case the gastric juice contains all the HCl as "free HCl," while the gastric juice in which fibrin has been digested contains part of its HCl as "combined HCl" (*i.e.*, in combination with protein).

Töpfer's reagent like Günzburg's reagent is sensitive only to free HCl. If no free acid is present a yellow colour is obtained. (It does not, however, distinguish between lactic acid and hydrochloric acid, as Günzburg's reagent does; see Experiment 69.)

Experiment 66. Prove that the gastric juice contains Rennin.—Repeat Experiments 51 and 52 with gastric juice. Determine how the activity of rennin is affected by acting (*a*) in a neutral medium; (*b*) in a faintly acid medium; (*c*) in a faintly alkaline medium; (*d*) by boiling; (*e*) by cold.

GASTRIC CONTENTS.

Acids in Gastric Contents.

1. **Acids.**—Gastric contents have, as a rule, an acid reaction. This may be due either to hydrochloric acid, which is secreted by the gastric mucous membrane, or to organic acids, such as lactic acid, butyric acid, &c., which have been formed by the decomposition of the food in the stomach. The presence of butyric acid can be detected by the characteristic smell. Special tests are necessary in order to distinguish between hydrochloric acid and lactic acid.

The following tests should be carried out with the solutions of 0.3 per cent. HCl, and of 0.3 per cent. lactic acid, which are supplied. These acids show a similar behaviour towards the usual indicators such as litmus, phenolphthalein, congo-red, Töpfer's reagent (dimethyl-amino-azo-benzene). (What is an indicator?) With certain indicators, however, such as Günzburg's reagent, they show differences (see Exp. 69). They also differ in their solubility in ether in which lactic acid dissolves (see Exp. 72). It is thus possible to distinguish between hydrochloric acid and lactic acid. These acids may be present in the gastric juice either *free* or in *loose combination with proteins*. Some indicators (*e.g.*, phenolphthalein), on titration, indicate the presence of both "free" and "combined" acid. Others, such as Töpfer's reagent, Günzburg's reagent, congo-red, are sensitive only to "free acid," and do not indicate acid combined with protein. Why?

Experiment 67.—To 10 c.c. of water in a test tube add two drops of caustic soda and one drop of phenolphthalein. A dark pink colour is produced, indicating that the solution is alkaline. Divide the solution into two equal parts and add to the one 5 c.c. of dilute HCl, to the other 5 c.c. of dilute lactic acid. The solution becomes colourless in each case.

Experiment 68. Congo-red.—Apply a drop of each acid to paper stained with congo-red. A blue colour is produced. Note the difference in colour produced by the two acids. Allow the congo-red paper, which has thus been acted upon, to dry. Place the paper in a dry test tube and extract with ether. The blue colour produced by lactic acid disappears from the paper, which turns red again. Why? The blue colour produced by HCl remains.

Experiment 69. Günzburg's Test for Free Hydrochloric Acid.—Evaporate four or five drops of Günzburg's reagent (which consists of an alcoholic solution of phloroglucin and vanillin) on a lid of a porcelain crucible over a small flame until the mixture just begins to become dry. Charring must be avoided. A brown residue remains.

To four or five drops of Günzburg's reagent add about 10 drops of hydrochloric acid and evaporate to dryness in the same way. A carmine red colour appears.

To four or five drops of Günzburg's reagent add about ten drops of lactic acid and evaporate to dryness in the same way. A brown residue remains.

Günzburg's test distinguishes not only between hydrochloric acid and and lactic acid, but also between "free hydrochloric acid" and "combined hydrochloric acid." This important test indicates minute traces of free HCl.

Experiment 70. Uffelmann's Test for Lactic Acid.—To 10 c.c. of 2 per cent. carbolic acid add one drop of ferric chloride. A blue colour appears. Add to this a few drops of lactic acid: the blue colour is replaced by a distinct yellow. Carry out the same test with dilute HCl. The blue solution becomes colourless without any yellow colouration.

Experiment 71.—Mix equal quantities of HCl and lactic acid and repeat Experiments 68, 69 and 70. Record the results.

Experiment 72. Separation of Hydrochloric and Lactic Acid.—If both acids are present, free HCl can still be recognised by Günzburg's test, but it is difficult to obtain satisfactory results with the tests for lactic acid. In order to test for lactic acid, it is preferable to separate the two acids. This can be done as follows:—To 5 c.c. of the fluid add an equal amount of ether. Close the test tube with the finger and shake vigorously. The lactic acid passes into the ether. Allow the two fluids to separate, remove the supernatant ether by means of a pipette and allow the ether to flow into a beaker containing a small amount of hot water. (All flames in the neighbourhood must be extinguished.) The ether evaporates, leaving a watery solution of lactic acid, to which the various tests are applied.

Experiment 73.—Apply Tests 68, 69, 70, 72 to gastric contents or to a gastric digest.

Products of Peptic Digestion.

2. Products of Peptic Digestion.—After the fibrin shreds in the test tubes A (from Experiment 59) have dissolved completely, the contents are collected in a beaker. The reaction is acid : test with litmus. Examine this peptic digest as follows :

Experiment 74.—Neutralise carefully, with dilute NaOH. A precipitate of *acid-albumin* may appear, when the solution is just neutral. An excess of NaOH must be avoided, as it would dissolve the acid-albumin. Remove the precipitate of acid-albumin by filtration. Boil the filtrate to remove any undigested coagulable protein that may be present. Filter, and use the filtrate for the examination of the products of peptic digestion : *albumoses* and *peptones.*

Albumoses.

Apply *tests for proteins*—biuret, xanthoproteic, Millon's, hydroferrocyanic acid. Note that in the xanthoprotein test the yellow precipitate which is formed in the cold, dissolves on heating and reappears on cooling.

Precipitation by Alcohol.—Add to some of the digest an excess of alcohol. Allow the precipitate which forms to stand in contact with alcohol for some time. Decant alcohol, the precipitate dissolves in water. Compare with native protein and state the difference.

Solubility in Ammonium Sulphate Solution.—Determine solubility in saturated ammonium sulphate solution, by saturating the digest with *solid* ammonium sulphate. A precipitate of albumoses appears.

Peptones.

If digestion has proceeded sufficiently far, the filtrate from this precipitate will still give the biuret test (an excess of strong alkali must be used in applying this test here ; why ?) and the xanthoproteic test. Note that in the latter test the nitric acid does not give a yellow precipitate at all, but gives only a yellow solution.

The following scheme summarises the method of examining a peptic digest :—

Neutralise carefully. If a precipitate appears, *acid albumin* is present.

Filter, if necssary, and boil. If a precipitate appears, *unaltered coagulable protein* is present.

Filter, if necessary, and saturate with solid ammonium sulphate. If a precipitate appears, *albumoses* are present.

Filter, if necessary, and apply to filtrate biuret and xanthoproteic tests. If tests are positive, *peptones* are present.

Experiment 75.—Examine by means of this scheme the different peptic digests which are given out, and state how far digestion has proceeded in these digests.

Experiment 76. — Demonstration. Dialyse globulins, albumins, albumoses, and peptones in parchment tubes against water.

Properties of Proteins.

From these experiments, and from Experiments 31 to 38 summarise the properties of different proteins according to the following table :—

PROPERTIES OF PROTEINS.

Variety of Protein.	Biuret Test.	HNO_3.		Solubility in $(NH_4)_2SO_4$	Dialysis Indicating Colloidal Nature.	Alcohol.	Coagulation by Heat.
		Precip.	Heat.				
Globulins -							
Albumins -							
Albumoses							
Peptones -							

III. PANCREATIC DIGESTION.

PANCREATIC JUICE.

Pancreatic Digestion.

An alkaline extract of pancreas is supplied.

Determine experimentally the following points, and record your results. (For the arrangement of these experiments see experiments on saliva and gastric juice).

Experiment 77. Does the extract contain a proteolytic ferment (trypsin)? (Digestion of fibrin, see Experiment 59.)

Experiment 78.—If so, how is the activity of the ferment affected—(*a*) by boiling; (*b*) by cold; (*c*) by acting in a faintly acid medium.

Experiment 79.—Does the extract contain an amylolytic ferment (amylopsin)? (Digestion of starch, see Experiment 54.)

Experiment 80.—If so, how is the activity of the ferment affected—(*a*) by boiling; (*b*) by cold; (*c*) by acting in a faintly acid medium.

Experiment 81. Does the extract contain a lipolytic ferment (steapsin)? (For this experiment a fresh extract is necessary, as lipase is rapidly destroyed.) Proceed as follows:—

Boil 10 c.c. of fresh milk in order to destroy any lactic acid bacilli which may be present. Why is this necessary? Cool and add 2 c.c. of *neutral* extract of pancreas and a few drops of litmus solution. Owing to the alkalinity of the milk, the mixture should now be faintly alkaline as indicated by the blue colour of the litmus.

Place the mixture in a water-bath at 37° to 40°. If a lipolytic ferment is present the blue colour changes to red owing to the formation of fatty acids from the fat of the milk.

PRODUCTS OF TRYPTIC DIGESTION OF PROTEIN.

Experiment 82.—Prepare a pancreatic digest by allowing pancreatic extract to act on fibrin for two or three hours.

Examine the tryptic digest according to the scheme given in Experiment 74. Note, however, that digestion has proceeded in an alkaline medium, so that any precipitate which appears on neutralising with dilute acid must be alkali-albumin, not acid-albumin, as in the case of a peptic digest. Record your results.

LIVER.

Glycogen.—The student is supplied with—

Presence of Glycogen in Liver.

A. Small pieces of the liver of an animal which has been fed for some time on a diet rich in carbohydrates (carrots). The liver was placed in alcohol immediately after death.

AA. Small pieces of the liver of an animal fed on the same diet, but the liver was placed in alcohol twenty-four hours after death.

AAA. Small pieces of the liver of a fasting animal. The liver was placed in alcohol immediately after death.

B. Pieces of kidney, placed in alcohol immediately after death.

With A carry out the following tests :—

Experiment 83.—Cut up a small piece into shreds, place the shreds into a test tube. Add 10 c.c. of water and heat slowly to boiling point. Cool thoroughly. Decant from the shreds and divide the solution into three parts.

1. To one part add two drops of iodine solution. A brown colour results, indicating the presence of glycogen.

Care must be taken not to add too much of the iodine solution, since the iodine solution itself has a similar colour. It is useful, therefore, to carry out a control experiment in a test tube which contains the same amount of water as the glycogen solution to be tested. If two drops of iodine solution are added to the water a yellow colour is obtained.

The brown colour obtained by adding iodine to the glycogen solution disappears on heating, and reappears on cooling. It disappears on making the solution alkaline. It reappears on neutralising the alkaline solution. Compare with starch. (Experiment 3.)

2. To another part apply Fehling's test. No reduction results.

3. A third portion is boiled with dilute HCl for five minutes. Neutralise and apply Fehling's test. Reduction occurs. Explain. To which group of carbohydrates does glycogen belong?

The presence of large amounts of glycogen in a tissue can be demonstrated rapidly by applying iodine directly to the tissue as follows :—

Experiment 84.—To a piece of liver A apply a drop of iodine solution by means of a glass rod. After a minute remove the iodine solution by rinsing the tissue under the tap. A deep brown colour appears where the iodine has acted. Record your result. Carry out the test with AA, AAA and B. Record your results. The colour is very faint in AA and AAA, and may even be absent. Why? It is absent in B. Why?

Why must the tissues be preserved in alcohol and not in a watery solution (of formalin, for instance), if one wishes to test for glycogen?

Iron in Liver.

Experiment 85. Iron in Liver.—To a test tube containing 10 c.c. of water add a few drops of ferric chloride. To this ferric chloride solution add a few drops of potassium ferrocyanide. A blue colour results. What has been formed?

Explain the reaction which has taken place in the form of a chemical equation.

Experiment 86.—Keep a piece of liver in a potassium ferrocyanide solution for a few minutes. Then add some dilute HCl (0.5 per cent.). A faint blue colour appears.

Experiment 87.—Apply the same test to a piece of liver from a case of pernicious anæmia, in which there is an excessive destruction of red blood-corpuscles. A distinct blue colour appears. Explain the results.

BILE.

Ox or sheep bile is supplied.

Bile.

Experiment 88.—Note the green colour. The colour is due to the pigments biliverdin and bilirubin. What is the relation between biliverdin and bilirubin?

Test the reaction with glazed litmus paper by allowing a drop of bile to lie on the paper for about a minute, and removing it with some water. Record. Note taste.

Acidify some bile with acetic acid. A precipitate of a mucinoid protein appears. It is insoluble in excess of the acid.

Experiment 89.—Fill a test tube with water. Sprinkle some flowers of sulphur on the surface. The particles float.

Fill a test tube with alcohol and sprinkle some flowers of sulphur on the surface. The particles sink. This is due to the fact that alcohol has a lower surface-tension than water. What is surface-tension?

Fill a test tube with water to which some bile has been added. Sprinkle some flowers of sulphur on the surface. The particles sink. What effect has bile on the surface-tension of water?

Experiment 90.—Into two funnels place filter-papers. Moisten the one with water, the other with diluted bile. Pour some oil into each. The oil passes through the filter-paper moistened with bile, but not through that moistened with water. Why?

Experiment 91. Effect of Bile on Emulsification.—Shake up some olive oil containing some fatty acids—(*a*) with water, (*b*) with 1 per cent. sodium carbonate, (*c*) with some bile. In (*a*) no emulsion is formed, in (*b*) and (*c*) an emulsion is formed. Emulsification is more complete in (*c*) than in (*b*). Explain.

What bearing has this fact on the absorption of fat from the intestine?

Bile Salts.

Experiment 92. Bile Salts.—Pettenkofer's test. Dilute some bile with water, and in 5 c.c. of this diluted bile dissolve a fragment (less than half) of a crystal of cane sugar. Shake. When the cane sugar has dissolved incline the test tube and allow 5 c.c. of concentrated sulphuric acid to flow down the side of the tube, so that the acid settles to the bottom. Gently shake the test tube from side to side. A purple colour develops where the acid mixes with the bile, both in the fluid and in the froth which was formed as the result of shaking.

Note 1.—Care must be taken that in mixing the bile with the sulphuric acid, the temperature does not rise above 70°.

Note 2.—A solution of cane sugar alone, if mixed with concentrated sulphuric acid in the same way, gives a black ring at the junction. Carry out this test with cane sugar alone. In testing for bile pigment it is therefore necessary to avoid an excess of cane sugar.

Why is cane sugar necessary for this test, and by what substance can it be replaced?

This test cannot well be applied to urine, since urine contains other substances which give a similar colour reaction. If applied to urine it is best carried out in the following modification, which should here be carried out with bile.

Experiment 93.—Dissolve a small fragment of a cane sugar crystal in dilute bile, then filter repeatedly through a filter-paper. Allow the filter-paper to dry by placing it over the radiator. To the dried paper apply a drop of concentrated sulphuric acid. After a few seconds a violet colour appears, which can best be seen in transmitted light. (Normal urine gives a reddish or brownish colour.)

Bile Pigments.

Experiment 94. Bile Pigments.—Gmelin's test. Place in a test tube 5 c.c. of strong nitric acid containing some nitrous acid (*i.e.*, fuming yellow nitric acid). With a pipette carefully add 5 c.c. of dilute bile, so that the bile floats on the top of the nitric acid. Gently shake the test tube from side to side. At the junction of the two liquids a series of colours appears, spreading up into the bile. (At the same time a white ring of the precipitated mucin of the bile is seen. This, however, has nothing to do with the bile pigment.) The test depends upon the oxidation of bilirubin, which gives rise to biliverdin and other pigments. The play of colours is best seen with bile which has a brown colour. Why? In that case the colours seen from the bile to the acid are green, blue, red, and yellow.

The test can also be carried out as follows:—

A drop of dilute bile is spread out on a white porcelain slab in a thin film. A drop of fuming nitric acid is placed on it. A play of the various colours is seen round the drop of nitric acid.

Another modification of the same test is to filter the dilute bile repeatedly through a filter-paper. Allow the filter-paper to dry superficially and then place a drop of fuming nitric acid on it. A similar play of colours is obtained.

Experiment 95. Huppert's Test for Bile Pigments.—Render some dilute bile alkaline with a little caustic soda or sodium carbonate; add an equal amount of barium chloride or calcium chloride solution. A precipitate forms which carries down the pigment. Filter; wash the precipitate in the filter-paper with water. Transfer the precipitate to a test tube and add to it about 5 c.c. of alcohol. Add five drops of concentrated HCl and shake. The precipitate dissolves. Add two drops of ferric chloride. Heat the alcoholic solution. The solution becomes green. This is the most reliable test for bile pigment.

Cholesterin in Bile.

Experiment 96. Cholesterin.—In a capsule evaporate 10 c.c. of bile to dryness on the water-bath. Cool and extract the residue with ether. Decant the ethereal solution into a beaker and again extract with ether. Decant again, collect the ethereal extracts and allow the ether to evaporate. (*All gas flames must be extinguished.*) When the ether has been evaporated a residue remains, which should be dissolved in a few drops of warm alcohol. Examine microscopically the crystals which separate out on cooling, and apply the tests for cholesterin.

Under certain pathological conditions cholesterin separates out from bile. What is the result?

BLOOD.

Blood.

Defibrinated blood is supplied for the following experiments. The composition of blood serum has already been studied (see Exps. 30 to 39).

The Coagulation of Blood.—Demonstration of the methods of obtaining fibrin, defibrinated blood, plasma, serum. Demonstration of the chemical factors concerned in blood-coagulation: fibrinogen, thrombin calcium salts.

Experiment 97. Reaction.—Apply a drop of defibrinated blood to glazed litmus paper. Allow it to remain for a minute and then wash off with water. Why is it necessary to use glazed litmus paper?

Determine in the same way the reaction of freshly drawn blood obtained by pricking the finger.

Experiment 98. Laking of Blood. Hæmolysis.—Label three test tubes A, B, and C. Place in each 1 c.c. (=twenty drops) of defibrinated blood. To B add 5 c.c. of water, to C two or three drops of ether. Then fill all three test tubes with 0.9 per cent. NaCl solution, and compare.

In A the solution is opaque, because the blood-corpuscles have remained intact; in B and C it is transparent, because the blood-corpuscles have been laked. Explain the results.

Experiment 99. Guaiac Test.—Dilute five drops of blood with about 10 c.c. of water, add some hydrogen peroxide and float on the surface a few drops of an alcoholic tincture of guaiac resin, so that the latter forms a resinous ring above the diluted blood. A blue colour gradually develops above the resinous ring. In order to make the test more sensitive a little ether may be added. The blue colour will be taken up by the ether.

Experiment 100.—Determine the greatest dilution of blood with which this test can still be obtained.

The test is given by boiled blood and by the isolated blood-pigment. It is not specific for blood, but is obtained also with milk, saliva, and other substances. Explain the reaction.

Oxygen Capacity of Blood.

Experiment 101. Determination of Oxygen-Capacity of Blood.—The oxygen-capacity is the maximum amount of oxygen that can be held by blood. It is dependent on the amount of hæmoglobin present.

The apparatus consists of a burette, which is inverted in a tall jar filled with cold water, and is connected by tubing to a bottle containing a small test tube. The bottle is placed in a vessel (water-bath) filled with cold water. The water surrounding the bottle should have the temperature of the room.

Place some blood in a porcelain dish and stir with a glass rod, until it becomes completely saturated with oxygen. By means of a pipette run exactly 20 c.c. of blood into the bottle. Add to it 30 c.c. of dilute ammonia (1 : 500). The water lakes the blood; the ammonia is added in order to absorb the carbonic acid formed by the action of potassium ferricyanide on oxyhæmoglobin. In the small test tube place 4 c.c. of saturated potassium ferricyanide solution. Put the test tube into the bottle, taking care that the ferricyanide is not spilt. Close the bottle with the stopper, and put it into cold water.

Test whether the apparatus is air-tight by raising the burette; if there is no leakage the column of water in the burette remains standing at a higher level than the water in the jar.

Allow the apparatus to stand for five minutes, so that all parts of it acquire the same temperature. Then open for a few seconds the clip on the tubing, which connects the inside of the bottle to the outer air. The pressure inside the apparatus is now the same as the pressure outside. Close the clip. The level of the water inside the burette is then at the same height as the level of the water in the jar. Read off the height in the burette with the water inside and outside at the same level.

Now tilt the bottle so that the ferricyanide solution is upset, and shake gently as long as gas is evolved. When no more gas is evolved

replace the bottle in the water and wait five minutes. What reaction has taken place?

Read the burette in the same way as before, *i.e.*, with the water inside and outside at the same level. (Why is this necessary?) The difference in readings gives the amount of oxygen held by 20 c.c. of defibrinated blood. Record the result.

N.B.—In measuring gases, as in this experiment, the temperature must be kept as constant as possible. (Why?) The apparatus should therefore be touched with the hands as little as possible, and no source of heat (gas flame, bunsen, &c.), must be near it.

ABSORPTION SPECTRA OF HÆMOGLOBIN AND ITS DERIVATIVES.

Spectroscopic Examination of Blood Pigments.

Solutions of hæmoglobin and its derivatives give characteristic absorption spectra, which can be examined with a spectroscope.

What is an absorption-spectrum?

Examine the spectroscopes provided. Note that the width of the slit at one end can be adjusted,* and that the spectrum can be focussed by adjusting the eyepiece. The sharpness of the spectrum is dependent upon the width of the slit, and is the sharper the narrower the slit.

Experiment 102. Solar Spectrum.—Direct the spectroscope to the sky. If the slit and the eyepiece are properly adjusted, fine vertical dark lines can be seen in the spectrum. These are Fraunhofer's lines. How are they produced?

The lines are designated by letters. Line B is in the red, line D in the yellow, line E in the green, line F in the blue. Their position in the spectrum is constant, and they can be used to locate certain parts of the spectrum.

Experiment 103. Spectrum of white Light.—Now direct the spectroscope to a luminous gas-flame (or to any other source of artificial light). The spectrum is still visible, but it does not show Fraunhofer's lines.

Explain why Fraunhofer's lines are absent.

* In some spectroscopes the width of the slit is fixed.

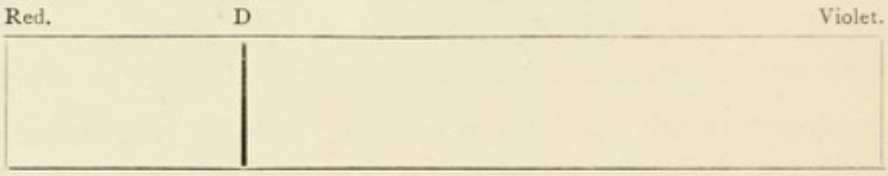
Experiment 104. Spectrum of Sodium in white Light.—Introduce into the gas-flame an asbestos stick soaked in sodium chloride, so that a sodium flame is produced. A bright yellow band is seen in the yellow part of the spectrum, and if the slit of the spectroscope is sufficiently narrow this yellow band can be seen to consist of two sharply defined narrow lines. These lines are in the same position as the black line D of the solar spectrum.

Most of the absorption spectra of hæmoglobin and its derivatives lie in the neighbourhood of the D line, and their position with reference to the D line can be easily recognised by using a sodium flame as described in Experiment 104.

NOTE.—*By convention the spectrum is usually arranged in such a way that the red end is on the observer's left-hand side.*

In observing the spectrum of any fluid the degree of dilution is of importance.

Record diagrammatically the absorption spectra of oxyhæmoglobin hæmoglobin, carboxyhæmoglobin, methæmoglobin, and hæmochromagen, on charts, using the following chart as a model :—



Experiment 105. Oxyhæmoglobin—Take some defibrinated blood in a test tube and run in water slowly from the tap, holding the tube obliquely under the end of the pipe ; allow the water to continue running after the tube is full. Thus one obtains a solution of oxyhæmoglobin diluted in such a way that the upper part of the tube contains almost pure water, the lower part a very concentrated solution of the pigment, and the middle part all gradations between the two. The corpuscles are of course laked.

Adjust the spectroscope as described above ; place the upper end of the test tube, which must be quite dry, against the slit, holding the tube by the lower end with the left hand.

On looking through the spectroscope probably no bands will be seen.

Gradually raise the tube so as to bring a stronger solution of oxyhæmoglobin in front of the slit. Two bands will appear, one narrower than the other and nearer the red end of the spectrum. In stronger and stronger solution these two bands fuse into one, and broaden out so as to obscure the whole spectrum.

Prepare a solution of oxyhæmoglobin so that the two bands can be clearly seen. Direct the spectroscope towards a sodium flame. It will be seen that both bands lie to the right of the D line, the left band lying close to the D line.

Experiment 106. Hæmoglobin. (Reduced hæmoglobin).—Reduce the solution of oxyhæmoglobin prepared in Experiment 105, which shows the two bands of oxyhæmoglobin distinctly. Reduction is performed by adding a reducing agent—five drops of ammonium sulphide, or five drops of Stokes' solution, made alkaline with a few drops of ammonia (see below). Note that the scarlet-red colour of oxyhæmoglobin (arterial blood) gives place to the bluish-red colour of hæmoglobin (venous blood). Examine spectroscopically. The spectrum now shows a single broad band, which overlaps the space enclosed by the two bands of oxyhæmoglobin, and is fainter than either.

Locate its position with reference to the D line. It is immediately to the right of the D line.

Preparation of Stokes' Solution.—Dissolve 2 grms. of tartaric acid in a little water. In another small quantity of water dissolve 2 grms. of ferrous sulphate. Mix the two solutions and make the mixture up to 100 c.c. with water. Fill into bottle labelled "Stokes' solution." Before use a few drops of ammonia are added, so that the solution is just alkaline.

Experiment 107.—Close with the finger the test tube containing hæmoglobin as prepared in Experiment 106, and shake vigorously for two to three minutes. Note that the colour of the solution changes from bluish-red to scarlet-red. Examine at once with the spectroscope; the two bands of oxyhæmoglobin have reappeared. (Why?) Allow the test tube to stand for two to three minutes; reduction takes place again owing to the presence of excess of the reducing agent, and the single band of hæmoglobin is again seen in the spectrum.

Experiment 108. Carboxyhæmoglobin.—Prepare some CO-hæmoglobin by filling a test tube with coal gas, and adding some diluted blood; close the tube with the thumb, and shake. Notice the pink colour. This pink colour persists even if the pigment is much diluted with water. (Contrast with oxyhæmoglobin, which becomes yellow if much diluted with water.)

Examine CO-hæmoglobin spectroscopically, diluting the solution until two absorption bands are distinctly visible. The two bands are apparently similar to those of oxyhæmoglobin. Verify by locating the position of the bands with reference to the D line, using a sodium flame. The two bands have almost the same position immediately to the right of the D line.

Carboxyhæmoglobin is not acted upon by reducing agents: Distinction from oxyhæmoglobin.—Add five drops of ammonium sulphide. No change takes place: the bands persist. Add five drops of Stokes' solution, made alkaline with ammonia. Same result.

Experiment 109. Methæmoglobin.—To 5 c.c. of water add four drops of blood. To the strong solution of oxyhæmoglobin thus prepared add a few drops of potassium ferricyanide. The solution becomes brown, and the spectrum shows a distinct band in the red. Locate by means of the D line, using a sodium flame: the band is to the left of the D line. This is the characteristic band.

With this concentrated solution there is marked absorption in the blue end of the spectrum. Dilute with an equal bulk of water; two faint bands appear in the green.

Add to the dilute solution of methæmoglobin a few drops of ammonium sulphide. The colour changes to red. Examine immediately with the spectroscope. At first the spectrum of oxyhæmoglobin appears, then that of reduced hæmoglobin.

What is the difference between methæmoglobin and oxyhæmoglobin?

Experiment 110. Alkaline hæmatin. Hæmochromogen (or reduced alkaline hæmatin).—Prepare alkaline hæmatin by warming some diluted blood with caustic soda. Warm gently at first, then heat to near boiling point, but do not allow to boil. The colour changes to brown. Cool.

The spectrum is indistinct. It shows a faint band in the red to the left of the D line.

Now add a few drops of a reducing agent (ammonium sulphide is preferable); the very distinct spectrum of hæmochromogen appears. It consists of two bands in the green. Locate by means of the D line, using a sodium flame. The two bands are to the right of the D line, as in the case of the spectra of oxyhæmoglobin or CO-hæmoglobin, from which the bands of hæmochromogen differ by lying further towards the blue end of the spectrum. There is a distinct space between the left band of hæmochromogen and the D line, while in the case of oxyhæmoglobin and CO-hæmoglobin the left band abuts against the D line.

If the solution of hæmochromogen is diluted, the left band persists longer, being the stronger band, so that in very weak solutions only this band is seen.

Experiment 111.—The formation of hæmochromogen is a very delicate test for blood. Dilute blood until the spectrum of oxyhæmoglobin cannot be readily seen. Convert this very dilute solution of oxyhæmoglobin into hæmochromogen as detailed in Experiment 110. The formation of hæmochromogen can be detected by the presence of the left band.

URINE.

CONSTITUENTS OF NORMAL URINE.

Normal Urine.

Each student is expected to provide for these experiments about 200 c.c. of his own urine.

Colour.—The colour is of a transparent yellow. The froth which forms on shaking soon disappears. Normal urine contains no sediment when passed. Deposits may form later (see below).

Experiment 112. Reaction.—The urine is acid to litmus. Test. It may react acid to blue litmus, and alkaline to red litmus: "amphoteric reaction." Two or three hours after a meal it may have an alkaline reaction. Why?

On standing urine becomes alkaline unless kept sterile. This is due to "ammoniacal fermentation," produced by the micrococcus ureæ which converts urea into ammonium carbonate.

Experiment 115. Specific Gravity.—Determine the specific gravity by means of a urinometer as described in Experiment 30. The urinometers are graduated for a temperature of 15° C.

The specific gravity of normal urines varies between 1.015 and 1.025, and gives a rough indication of the amount of solids present in the urine. The last two figures of the specific gravity multiplied by 2.33 give approximately the solids in grammes per litre.

Example: specific gravity 1.020.

20 : 2.33 = 46.6 grammes of solids per litre of urine.

Volume.—The volume varies. The average volume is 1,400 c.c. per day.

CHLORIDES.

Chlorides in Urine.

Experiment 114.—To 15 c.c. of urine add a drop or two of concentrated nitric acid (if the urine is alkaline add nitric acid till reaction is acid) and five drops of silver nitrate solution (2 per cent.). A coherent clump of silver chloride is precipitated if the chlorides are present in normal quantity. If the chlorides are much diminished, as in febrile conditions, the precipitate is more or less diffuse, according to the diminution. Why is nitric acid added? Add ammonia; the precipitate dissolves.

What is the average quantity of chlorides in normal urine? What is the source of the chlorides in urine.

SULPHATES.

Sulphates in Urine.

Experiment 115. (*a*) **Inorganic Sulphates.**—To 10 c.c. of urine add about 3 c.c. of barium chloride solution. A thick precipitate forms, which consists of the phosphate and sulphate of barium. Acidify with a few drops of concentrated HCl. The barium phosphate dissolves and an opaque milkiness remains indicating the presence of inorganic sulphates. If the precipitate which remains is thick the inorganic sulphates are in excess.

Experiment 116. (*b*) **Ethereal Sulphates.**—To 10 c.c. of urine add barium chloride as long as a precipitate continues to form. Make alkaline with a few drops of sodium carbonate solution and filter. Acidify the filtrate, which is now free from inorganic sulphates, with concentrated HCl and boil for three minutes. A faint cloud of barium sulphate is formed on standing, indicating the presence of ethereal sulphates. If ethereal sulphates are present in excess, a distinct precipitate is formed, which settles to the bottom.

What is the average daily quantity of sulphates in urine? What is the source of the sulphates in the urine? What does an excess of ethereal sulphates indicate?

Experiment 117.—Prepare an ethereal sulphate as follows:—

Warm ten drops of absolute alcohol with five drops of concentrated sulphuric acid. After cooling, make alkaline with 10 per cent. sodium

hydrate; add barium chloride as long as a precipitate continues to be formed; then heat to boiling point and filter. The filtrate contains barium ethyl-sulphate. To it add half its volume of concentrated hydrochloric acid, and boil. A precipitate of barium sulphate forms. Compare the solubility in water of barium sulphate and barium ethyl-sulphate.

PHOSPHATES.

Phosphates in Urine.

Experiment 118.—To a test tube full of urine add a little strong ammonia and heat. A white precipitate of the phosphates of calcium and magnesium—"earthy phosphates"—forms. (Such a precipitate is often found in alkaline urine as a crystalline deposit. See below, under Deposits.) Filter. The filtrate contains the phosphates of sodium and potassium.

A. *Precipitate.* "*Earthy Phosphates.*"—Place the precipitate in a test tube with some water and add a few drops of dilute acetic acid. It dissolves. The presence of phosphoric acid in this solution can be verified by the ordinary tests, for instance by the formation of ammonium phosphomolybdate. To the acid solution add some nitric acid and about 5 c.c. of ammonium molybdate. Heat gently over small flame to about 60°. The solution turns yellow and a yellow crystalline precipitate is formed. What is the yellow precipitate?

B. *Filtrate. Alkaline "Phosphates."* — To the filtrate add a little magnesia mixture and warm gently. A white precipitate is formed, indicating the presence of phosphates in the filtrate. What is this precipitate? The presence of phosphates may also be determined by testing with ammonium molybdate as detailed above.

Note the difference in the bulk of this precipitate and that of "earthy phosphates" obtained on adding ammonia to the urine. Are the "earthy" or the "alkaline phosphates" present in larger amount in urine?

Experiment 119. Formation of deposit of ammonium-magnesium phosphate. ("**Triple Phosphate**").—Set some urine aside in a beaker for two or three days. Ammoniacal fermentation occurs (the reaction of the urine becomes alkaline; test with litmus) and a

crystalline deposit of ammonium-magnesium phosphate settles out. Examine microscopically. Sketch. Crystals of calcium phosphate may also be present, and may be recognised by the stellar arrangement of the the crystals.

Experiment 120.—On heating neutral urine a precipitate of earthy phosphates is often formed. Try this. It dissolves on the addition of a few drops of acetic acid. This is important in testing for albumin by the heat test.

The explanation for the appearance of this precipitate is given by the following experiment :—

Experiment 121.—Treat a solution of calcium chloride with sodium phosphate, and then with excess of sodium carbonate. Calcium phosphate is precipitated. Add acetic acid, drop by drop, till the precipitate just dissolves: acid calcium phosphate is formed. Heat: calcium phosphate is precipitated again owing to the alteration of the reaction as the carbon dioxide is evolved. The precipitate dissolves on adding a drop or two of dilute acid.

Experiment 122. Precipitation of Phosphates by Uranium Nitrate.—To a little urine add a few drops of acetic acid and some sodium acetate, and then uranium nitrate. A precipitate forms. In order to complete precipitation the urine must be heated to boiling point.

This reaction is used for the quantitative estimation of phosphates.

What is the average daily quantity of phosphates in the urine?

What is the source of phosphoric acid in the urine?

By which other channel are calcium and magnesium excreted?

DEPOSITS OF PHOSPHATES IN URINE.

Urinary Deposits of Phosphates.

From alkaline urine deposits of phosphates may separate out. All these deposits are easily soluble in acetic acid.

Earthy Phosphates.—Amorphous granules of $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$.

Ammonium-Magnesium Phosphate.—$MgNH_4PO_4$. (Triple phosphate.) Large colourless prisms in the shape of "knife-rests" or "coffin lids," or in the shape of feathery crystals.

Calcium-Hydrogen Phosphate.—$CaHPO_4$. Prismatic crystals arranged in rosettes. These may also occur in acid urines.

DEPOSIT OF OTHER CALCIUM SALTS IN URINE.

From *acid* urines :—

Urinary Deposits of Calcium Salts.

Calcium oxalate either in the form of highly refractive octahedra, "envelope" shape, or in the form of ovoid bodies, "dumb-bell" shape. They are insoluble in acetic acid ; soluble in hydrochloric acid. A considerable sediment of calcium oxalate is pathological.

From *alkaline* urine :—

Calcium carbonate.—Spherical or ovoid crystals with concentric striation. Readily soluble in acetic acid with effervescence. Common deposit in the urine of herbivorous animals.

AMMONIA.

Ammonia.

Experiment 123.—Make urine alkaline with sodium carbonate solution, and warm. Note the smell of ammonia. A moist piece of red litmus paper held over the mouth of the tube turns blue.

Within what limits does the average amount of ammonia excreted by a normal person in twenty-four hours vary? How can it be increased? How can it be diminished?

PREPARATION OF UREA FROM URINE.

Preparation of Urea.

Experiment 124.—Place about 100 c.c. of urine in a porcelain capsule and evaporate in a water bath to about 20 c.c., so that the urine is now concentrated to a syrup. Cool by floating the capsule on cold water. Filter into a beaker and place beaker in cold water. Slowly add while stirring an equal volume of cold 50 per cent. nitric acid (pure). (Why can fuming yellow nitric acid not be used?) The mixture must be kept cold. Crystals of urea nitrate separate out. Filter off the crystals ; dry them by pressing between successive sheets of filter paper. When the crystals are dry, mix with excess of barium carbonate, and add a little alcohol to form a paste. The urea nitrate is decomposed, giving barium nitrate, CO_2 and urea. Dry the mass on the water bath, extract with alcohol and filter. Concentrate on

the water bath the alcoholic filtrate to a small volume. Cool. Urea crystallises out in long needles. Examine microscopically. Sketch the crystals. If no immediate crystallisation occurs, allow the alcoholic solution to stand over night.

Is urea or urea-nitrate more soluble in water?

Within what limits does the amount of urea excreted by a normal person in twenty-four hours vary? How can it be increased? How can it be diminished?

PREPARATION OF URIC ACID FROM URINE.

Preparation of Uric Acid and of Urates.

Experiment 125.—To 25 c.c. of urine in a beaker add 5 c.c. of strong HCl and allow to stand for twenty-four hours. Examine microscopically the crystals of uric acid which separate out. Sketch the crystals. Note that the crystals are deeply pigmented with urinary pigment.

To some of the crystals add caustic soda. The crystals dissolve. To the alkaline solution add excess of strong HCl. The crystals are again formed after some time.

The crystals give the murexide test (see below, Experiment 132).

PREPARATION OF AMMONIUM URATE FROM URINE.

Experiment 126.—To 25 c.c. of urine add 2 drops of ammonia and solid ammonium chloride, stirring all the time until the solution is saturated. Avoid an excess of the salt. A gelatinous, amphorous precipitate of ammonium urate is formed. Examine microscopically. All the uric acid present in the urine is completely precipitated as ammonium urate. This reaction is therefore used for the quantitative estimation of uric acid.

The deposit gives the murexide test (see below).

What is the source of the uric acid excreted in urine?

Within what limits does the quantity of uric acid excreted in twenty-four hours by a normal person vary? How can it be increased? How can it be diminished?

UREA.

Urea.

What is the structural formula for urea?

Prepare biuret from urea (see Exp. 34*a*).

Experiment 127. Solubility.—Determine the solubility of urea in (*a*) water; (*b*) cold alcohol; (*c*) cold ether. Record the results.

Prepare a dilute watery solution of urea for the following experiments:

Experiment 128—To 5 c.c. of the urea solution add some sodium hypobromite. Bubbles of nitrogen are evolved.

State in the form of an equation the process which takes place. This reaction is used for the quantitative estimation of urea. It is, however, given, not only by urea, but by all substances having amido groups and by ammonium salts.

Repeat the test, using a dilute solution of ammonium sulphate instead of the urea solution. Record the result.

Experiment 129.—To some urea solution add some yellow nitric acid, which contains nitrous acid. Bubbles of nitrogen and of CO_2 are evolved.

This reaction also is given by all substances having an amido group.

State in the form of an equation the process which takes place.

Experiment 130.—Boil some urea solution with caustic soda. Notice the smell of ammonia which is evolved.

State the reaction which takes place.

URIC ACID.

Uric Acid.

What is the structural formula for uric acid?

Experiment 131. Solubility.—Determine the solubility of uric acid in (*a*) water, cold and hot; (*b*) alcohol, cold and hot; (*c*) caustic soda or sodium carbonate, cold and hot; (*d*) dilute HCl, cold and hot.

Dissolve some uric acid in warm caustic soda, acidify with dilute HCl, and allow to cool slowly. Uric acid crystallises out. Examine microscopically. Sketch the crystals.

What is formed when uric acid is dissolved in caustic soda?

Experiment 132. Murexide Test.—To a small crystal of uric acid on a chip of thin porcelain or a crucible lid, add two drops of strong nitric acid. Evaporate over a *small* flame, in a fume chamber, to complete dryness. A red deposit remains. Add with a glass rod a drop of *very dilute* ammonia. The residue turns to a violet colour.

Experiment 133. Uric Acid reduces Fehling's Solution.—Dilute some Fehling's solution and heat to boiling point. To the boiling solution add repeatedly a few drops of a solution of uric acid in caustic soda, heating after each addition. Red cuprous oxide separates out. If the urate is present in excess a white precipitate of cuprous urate will be formed at the same time.

The presence of uric acid may therefore be a fallacy in examining urines for sugar by means of Fehling's test, especially if the boiling is prolonged. This can be shown to be the case as follows :—

Experiment 134.—Heat simultaneously in two test tubes equal volumes of normal urine and of half-diluted Fehling's solution to boiling point. When boiling mix the contents. Pour one-half of this mixture of urine and Fehling's solution into a test tube and allow to cool. This will remain blue if the urine is normal. Boil the other half for about three minutes : if sufficient urates are present the solution may become decolourised and acquire a brown colour owing to the reduction of the cupric salt by urates.

Experiment 135.—Boil 5 c.c. solution of uric acid in caustic soda with 1 c.c. of Nylander's reagent. No reduction takes place.

Experiment 136.—The presence of uric acid is therefore no fallacy if Nylander's reagent is used. Demonstrate this by adding to some urine (5 c.c.) one-tenth of its volume ($\frac{1}{2}$ c.c = 10 drops) of Nylanders reagent, boil over a small flame for three minutes. No reduction to metallic bismuth occurs.

DEPOSITS OF URIC ACID AND URATES.

Urinary Deposits of Uric Acid and Urates.

From *acid* urines a crystalline deposit of uric acid may separate out. It has a sandy red colour, and is therefore called "cayenne pepper deposit." This deposit corresponds to the one obtained experimentally in Experiment 125. It is recognised by its crystalline form and by the murexide test.

From *acid* urines a deposit of urates (mainly sodium urates) may separate out. It may be amorphous or crystalline. It has a pinkish red colour, and is therefore called "brick dust deposit." This deposit is found

frequently in concentrated urines on cooling. It dissolves on heating the urine, and thus differs from deposits of phosphates.

The deposit is soluble in hot water or hot acids. On adding hydrochloric acid to the watery solution, and allowing to cool, crystals of uric acid separate out. The deposit gives the murexide test. It is not readily soluble in cold acetic acid.

From *alkaline* urines a deposit of ammonium urate may separate out in the form of yellow or brownish spheres, with or without projecting spicules —"hedgehog crystals." They dissolve in hydrochloric acid; on standing uric acid crystallises out of the acid solution. The deposit gives the murexide test.

A considerable sediment of uric acid or urates does not necessarily indicate a high uric acid content.

CREATININE.

What is its structural formula?

Creatinine.

Experiment 137. Weyl's Test.—To 5 c.c. of urine add a few drops of a freshly prepared solution of sodium nitroprusside. Then render alkaline with caustic soda. A red colour results. Strongly acidify with acetic acid. The solution is decolourised,

Experiment 138. Jaffe's Test.—To 5 c.c. of urine add picric acid. Then render alkaline with caustic soda. The solution becomes red in colour.

What is the average amount of creatinine excreted by a normal person in twenty-four hours?

Creatinine like uric acid is capable of slightly reducing Fehling's solution. It has at the same time the power to keep in solution a small amount of cuprous oxide. If, therefore, a dilute glucose solution containing creatinine is tested with Fehling's solution, a brown clear solution instead of a red or yellow deposit may be obtained. This may also occur if a urine containing only a small amount of sugar is examined by Fehling's test.

INDICAN.

Indican.

Experiment 139.—To about 10 c.c. of urine add an equal volume of strong *fuming* HCl, one or two drops (not more!) of a 5 per cent. solution of calcium hypochlorite and 3 c.c. chloroform. Close the mouth of the tube with the thumb, cautiously invert a few times, and allow the mixture to stand for a few minutes. The chloroform becomes blue. Note the change in the colour of the urine.

The amount of indican present is proportional to the depth of colour of the chloroform extract. A rough estimate of the amount of indican present can be obtained by comparing the depth of the blue colour with a blue standard solution, *e.g.*, Fehling's solution.

From what substance is the indican formed?

What relation does the amount of indican present in the urine bear to intestinal putrefaction?

Repeat the test, using about five to ten drops of ferric chloride solution, instead of the calcium hypochlorite. The same result is obtained.

Repeat the test, with one or two drops of calcium hypochlorite, as detailed above. After the chloroform has become blue add a few more drops of calcium hypochlorite and invert the tube repeatedly. What takes place?

Why is the addition of calcium hypochlorite necessary for this reaction, and by what substances can it be replaced?

Why must an excess of calcium hypochlorite be avoided?

Is the addition of chloroform necessary for the formation of indican?

METABOLISM.

I. QUANTITATIVE ESTIMATION OF CERTAIN NITROGENOUS CONSTITUENTS OF THE URINE.

Quantitative Urine Analysis.

Each student is expected to collect accurately a twenty-four hour specimen of his own urine, and to measure and record its volume.

The urine should be protected against ammoniacal fermentation by the addition of 2 c.c. of a 5 per cent. solution of thymol in chloroform, which is supplied. A sample (about 150 c.c.) of the total twenty-four hours' urine should be kept on hand for the experiments.

Decinormal solutions of acid and alkali are supplied.

NORMAL SOLUTIONS.

A normal solution is one which contains the equivalent weight in grammes of a given substance dissolved in 1,000 c.c. of water.

What is meant by the term "equivalent weight"?

As an example take hydrochloric acid. The equivalent weight of Cl = 35.5, H = 1, therefore HCl = 36.5. Therefore a normal HCl solution contains 36.5 grms. HCl in 1,000 c.c. of water, a decinormal solution contains 3.65 grms. HCl in 1,000 c.c. of water, and 1 c.c. of a decinormal HCl solution contains 3.65 mgs. HCl.

Normal solution is written $\frac{n}{1}$, decinormal $\frac{n}{10}$, and so on.

The same holds good for, say, NaOH, or for H_2SO_4. Note that for a dibasic acid, such as H_2SO_4 the equivalent weight is half the molecular weight. What is the equivalent weight for NaOH? for H_2SO_4? for NH_3?

Since normal solutions contain equivalent weights dissolved in equal volumes, it follows that each c.c. of any $\frac{n}{10}$ acid will require for neutralisation exactly 1 c.c. of any $\frac{n}{10}$ base. Try this as follows:—

Experiment 140.—Run from a burette into a beaker exactly 10 c.c. of $\frac{n}{10}$ acid. Dilute with about 20 c.c. of distilled water, add two drops of an indicator (phenolphthalein). Then add slowly from a burette $\frac{n}{10}$ alkali, until the addition of one drop will just produce a purple colour.

Record your result.

Experiment 141. — Repeat the titration, using rosolic acid as an indicator instead of phenolphthalein.

Total Nitrogen Estimation.

Experiment 142. Estimation of the Total Nitrogen by Kjeldahl's Method.

Principle.—First Stage. Acid Incineration.—By boiling with concentrated sulphuric acid all organic nitrogenous compounds are converted into ammonium sulphate. This reaction is accelerated by the addition of a small amount of copper sulphate which acts as a catalyser. (What is a catalyser?)

Second Stage. Distillation.—The ammonium sulphate formed in the first stage of the process is decomposed by the addition of an excess of caustic soda. The ammonia, which is set free, is distilled into a measured amount of standard $\frac{n}{10}$ sulphuric or hydrochloric acid. The amount of the acid that has been neutralised by the ammonia is found by subsequent titration with standard $\frac{n}{10}$ sodium hydrate.

Process—First Stage.—From the twenty-four hours' urine take exactly 5 c.c. by means of a pipette and place in a Kjeldahl flask in such a way that the urine does not touch the sides of the neck of the flask. Add about 10 c.c. concentrated sulphuric acid (*measured with a measuring cylinder, not with a pipette*), and a small crystal of copper sulphate. Place the Kjeldahl flask in the fume-chamber and heat. After all the water is driven off a more or less violent reaction will take place. Continue the boiling until a clear, almost colourless solution is obtained (30 to 45 minutes in the case of urine).

In the meantime arrange the apparatus for distillation by connecting the upper end of a condenser with a spurting bulb, while the lower end is connected with an "adapter," a glass tube which passes down into a receiving flask.

Second Stage. Distillation.—Allow the incinerated urine to cool. In the meantime place in receiving flask from a burette an accurately measured quantity (between 25 c.c. and 50 c.c.) of $\frac{n}{10}$ acid, *e.g.*, 30 c.c. Note the amount of acid placed in the receiving flask. When the Kjeldahl flask has cooled add distilled water to the contents, so that about a third of the flask is filled. (The first addition of water must be done carefully, because the Kjeldahl flask contains concentrated sulphuric acid.) Add two drops of an indicator, *e.g.*, rosolic acid (which gives a red colour when alkaline) to the contents both of the Kjeldahl flask and of the receiving flask. Now add a pinch of talc to the contents of the Kjeldahl flask (in order to prevent bumping), then make the contents strongly alkaline by the addition of strong caustic soda, and at once close the Kjeldahl flask by inserting the spurting bulb. (*Note.*—The addition of soda is best carried out through a funnel so that the mouth of the flask remains dry, otherwise it will be difficult to keep the spurting bulb in position.) See that the tube connected with the lower end of the condenser dips into the acid.

Now heat, at first carefully, then with a full flame. Continue boiling until all the ammonia is distilled over. This will last from 30 to 45 minutes. The distillation is complete when a piece of red litmus held against the mouth of the adapter no longer turns blue.

Note 1.—In distilling great care must be taken to prevent a "sucking back" of the contents of the adapter into the distilling flask. This will occur as soon as the pressure in the distilling flask is allowed to fall by removing the flame or by careless heating. If there is any danger of "sucking back," the receiving flask should be lowered, so that the adapter does not dip into the acid.

Note 2.—If during distillation the contents of the receiving flask turn pink, indicating that the reaction has become alkaline, a measured amount of standard acid must be added at once. Otherwise ammonia will be lost.

When distillation is complete, remove first the receiving flask, then turn out the gas. Titrate the acidity of the contents of the receiving flask, and from that calculate the total amount of nitrogen excreted in twenty-four hours.

Calculation. Example :—

Total volume of twenty-four hours urine = 1,550 c.c.
Amount taken for estimation = 5 c.c.

Amount of $\frac{n}{10}$ acid placed in receiving flask = 30 c.c.

Amount of $\frac{n}{10}$ alkali used in titration = 17.8 c.c.

Therefore amount of $\frac{n}{10}$ acid neutralised by ammonia = 12.2 c.c.

Now 1 c.c. $\frac{n}{10}$ acid = 1 c.c. $\frac{n}{10}$ ammonia.

Since 1,000 c.c. $\frac{n}{10}$ ammonia contain 1.7 grms. NH_3, 1 c.c. $\frac{n}{10}$ ammonia contains 1.7 mg. NH_3.

Therefore 1 c.c. $\frac{n}{10}$ acid = 1.7 mg. NH_3.

Further, 1.7 mg. NH_3 contains 1.4 mg. N, because the atomic weights are N = 14, H3 = 3.

Therefore 1 c.c. $\frac{n}{10}$ acid indicates 1.4 mg. N.

Therefore the amount of N in milligrammes is obtained if the number of c.c. of $\frac{n}{10}$ acid, neutralised by ammonia, is multiplied by 1.4.

In this case 5 c.c. of urine contain 12.2 × 1.4 mg. N = 17.08 mg. N.

From this the total amount of nitrogen excreted in twenty-four hours can be calculated (in this case = 5.29 grms.)

Ammonia and Acidity Estimation.

Experiment 143. Estimation of Acidity and of Ammonia.—This method is based on the fact that ammonium salts (and also other substances containing an amido group) react with formaldehyde in such a

way, that the ammonia and formaldehyde form a complex organic compound (hexamethylentetramin or urotropin), while the acid which was combined with the ammonia is liberated. The amount of acid set free is determined by titration, and is a measure of the ammonia present.

In carrying out this estimation the urine is at first neutralised by adding from a burette *n*/10 alkali. The amount of *n*/10 alkali added is a measure of the acidity of the urine. Then neutral formaldehyde is added. Owing to the liberation of acid which takes place when the formaldehyde has combined with the ammonia the urine acquires again an acid reaction. This second acidity is titrated again with *n*/10 alkali, and this second titration is a measure of the amount of ammonia present. At least two estimations should be carried out.

Process.—Dilute 25 c.c. of urine with an equal volume of water, add 15 grms. of finely powdered neutral potassium oxalate in order to precipitate all the calcium salts, and four or five drops of phenolphthalein. Shake thoroughly for one or two minutes, and, whilst the solution is still cold from the effect of the oxalate, titrate with *n*/10 NaOH until a permanent pink tint remains. Record the number of cubic centimetres added. This is a measure of the acidity.

Now dilute 10 c.c. of formalin with two volumes of water. This mixture will be slightly acid owing to the presence of some formic acid in the formaldehyde. It must be made neutral to phenolphthalein by adding *n*/10 NaOH until a faint permanent pink colour appears. Add this neutral formaldehyde to the urine. The urine becomes acid again and the colour disappears. Run the *n*/10 NaOH into the mixture until a permanent pink tint is again obtained. Record the number of cubic centimetres added in this second titration. This is a measure of the ammonia.

Calculate from your results :—

1. The *acidity* of the urine expressed in terms of *n*/10 acid (*a*) as percentage ; (*b*) for the total twenty-four hours' quantity.

2. The *ammonia* in grammes excreted in twenty-four hours.

3. The fraction of total nitrogen which is excreted as ammonia. Express the fractions in terms of percentage of total nitrogen.

Calculation. Example :—

Total volume of urine = 1,550 c.c.
Total N. excreted in twenty-four hours = 5.29 grms.
Amount taken for estimation = 25 c.c.
First titration, 2.2 c.c. *n*/10 alkali.
Second titration, 7.7 c.c. *n*/10 alkali.

1. *Acidity.*—Since 2.2 *n*/10 alkali neutralises 25 c.c. of urine, its acidity is 2.2 c.c. *n*/10 acid.

The acidity of 100 *c.c. of urine is therefore* 8.8 *c.c.* n/10 *acid.*

The acidity of the total urine is $\frac{8.8 \times 1{,}550}{100}$ *c.c.* = 136.4 *c.c. n*/10 *acid.*

2. *Total* NH_3.—Since 1 c.c. *n*/10 alkali = 1 c.c. *n*/10 NH_3, 25 c.c. urine contains 7.7 c.c. *n*/10 NH_3.

1 c.c. *n*/10 NH_3 = 1.7 mg. NH_3.

Therefore 7.7 c.c. *n*/10 NH_3 = 7.7 × 1.7 mg. NH_3 = 13.39 mgs. NH_3.
This is the amount present in 25 c.c. urine.
The amount excreted in twenty-four hours is therefore—

$$\frac{1{,}550 \times 13.39}{25} \text{ mg. } NH_3 = 0.830 \text{ grms. } NH_3.$$

3. *Ammonia N.*—Now 17 grms. NH_3 contain 14 grms. N.

Therefore 0.830 grms. NH_3 contain $\frac{14 \times 0.830}{17}$ grms. N = 0.683 grms. N.

Of 5.29 grms. of total N, 0.683 grm. N are excreted in form of NH_3.

Of 100 grms. N of total N, $\frac{0.683 \times 100}{5.29}$ grms. are excreted in form of NH_3 = 12.9 grms. N.

12.9 *per cent. of total N is excreted in the form of Ammonia.*

Urea Estimation.

Experiment 144. Estimation of Urea.—In the following method urea is estimated by measuring the amount of nitrogen liberated from the urine by sodium hypobromite. From 1 grm. urea 354 c.c. of nitrogen are evolved. As stated in Experiment 128 some other substances also liberate nitrogen if acted upon by hypobromite, so that the method gives only approximate results.

Apparatus.—Connect a bottle containing a short test tube and closed with a rubber stopper, with an inverted burette standing in a tall glass cylinder filled with water. The apparatus is the same as the one used for the estimation of the oxygen capacity of the blood (Exp. 101).

The bottle is standing in a bath filled with water. The water in the jar and in the bath should have room temperature.

Method.—Place 20 c.c. of hypobromite solution in the bottle, without letting it touch the mouth of the flask. Run, with a pipette, 5 c.c. of urine accurately measured into the small test tube, and place the test tube into the bottle, taking care not to upset any of the urine into the hypobromite Put in stopper tightly, place the bottle in the water-bath, connect one tube with the burette, leaving the second tube open. After five minutes close the second tube with a clip. Read the burette with water-level outside and inside equal.

Take the bottle out of the water-bath, and tilt, so that the urine mixes with the hypobromite. Gently shake bottle from side to side, holding it upright so that the froth does not enter the tube. Tilt again and shake again. Place the bottle back in water-bath. After five minutes read the burette again with water-level outside and inside equal. The difference in the readings gives the amount of N evolved. Record your results and calculate from them: (1) Amount of urea excreted in twenty-four hours; (2) that fraction of the total N which is excreted as urea.

Calculation. Example:—

Amount of urine used, 5 c.c.; N liberated 8.7 c.c.
Volume of urine excreted in twenty-four hours, 1,550 c.c.
Amount of N excreted in twenty-four hours, 5.29 grms.

1. *Total Urea.*—Since 354 c.c. N are liberated by 1 grm. urea, 8.7 c.c. N are liberated by $\frac{8.7}{354}$ grms. urea = 0.0245 grm. urea.

Since 5 c.c. of urine contain 0.0245 grm. urea, 1,550 c.c. urine contain $\frac{0.0245 \times 1{,}550}{5}$ grms. urea = 7.61 grms. urea.

2. *Urea N in Percentage of Total N.*—Urea (molecular weight = 60) contains N_2 (molecular weight = 28).

Since therefore 60 grms. urea contain 28 grms. nitrogen, 7.61 grms. urea contain $\frac{7.61 \times 28}{60}$ grms. N = 3.55 grms. nitrogen.

This amount of N excreted in twenty-four hours in the form of urea represents a fraction of the total N excreted in twenty-four hours. This fraction expressed in terms of percentage of total N is $\frac{3.55 \times 100}{5.29} = 67.10$ per cent.

Result.—*The amount of urea excreted in twenty-four hours is* 7.61 *gs. The urea nitrogen represents* 67.10 *per cent. of the total nitrogen.*

Collect the results from Experiments 142 to 144 in tabular form as under :—

NITROGEN DISTRIBUTION IN TWENTY-FOUR HOURS' URINE.

Date.	Volume.	Total N.	Urea.		Ammonia.	
			Absolute Amount.	N. Per Cent.	Absolute Amount.	N. Per Cent.

II. QUANTITATIVE ESTIMATION OF CHLORIDES IN URINE.

Chlorides ; Quantitative Estimation.

Experiment 145. Estimation of Chlorides (Volhard's Method).—The principle of this method consists in precipitating all the chlorides with an excess of a standard silver nitrate solution. The excess of silver

nitrate used is determined by adding a standard solution of ammonium sulphocyanate in the presence of a ferric salt. The sulphocyanate solution precipitates the soluble silver nitrate as silver sulphocyanate. As soon as all the silver nitrate is precipitated the addition of another drop of sulphocyanate solution will produce a red colour, since the ammonium sulphocyanate will now react with the ferric salt and form red ferric sulphocyanate.

The standard sulphocyanate solution is made up in such a way that 1 c.c. of it will just completely precipitate 1 c.c. of the standard silver nitrate solution (13 grms. in 1,000 c.c.).

The standard silver solution is made up in such a way that 1 c.c. will completely precipitate 10 mg NaCl (29.04 grms. $AgNO_3$ in 1,000 c.c.).

Method.—With a pipette place 10 c.c. of urine in a beaker, dilute with about 90 c.c. of distilled water, add 5 c.c. of a five per cent. solution of ammonia iron alum and 5 c.c. of dilute pure nitric acid. Then add with a pipette a measured excess of standard silver nitrate solution (20 c.c. of standard $AgNO_3$ will, as a rule, be sufficient). Now run in at once from a burette the standard sulphocyanate solution stirring all the time, until the reddish tint of the ferric sulphocyanide first extends through the whole liquid. Note the number of c.c. of sulphocyanate solution used, and calculate in terms of sodium chloride (1) the amount of chlorides in 100 c.c. urine; (2) the amount of chlorides excreted in twenty-four hours.

Calculation.—The amount of ammonium sulphocyanate solution used gives at once the excess of silver nitrate used beyond the quantity required to precipitate all the chlorides in 10 c.c. urine. If this excess is deducted from the total amount of silver nitrate added, one obtains the number of c.c. of standard silver nitrate solution necessary to precipitate all the chlorides.

Each c.c. of the standard silver solution equals 10 mgs. NaCl.

Example.—

Urine used, 10 c.c.; total volume, 1,550.;
Standard $AgNO_3$ added 20 c.c.;
Sulphocyanate solution used 7.4 c.c.;

therefore excess of $AgNO_3$ solution = 7.4 c.c.

$AgNO_3$ solution necessary to precipitate all chlorides = 20 c.c. − 7.4 c.c. = 12.6 c.c.

Since 1 c.c. $AgNO_3$ = 10 mgs. NaCl.

12.6 c.c. $AgNO_3$ = 126 mgs NaCl.

Since 10 c.c. of urine contain 0.126 grms. NaCl,

100 c.c. of urine contain 1.26 grms. NaCl.

Result.—*The total amount of chlorides excreted in twenty-four hours is in terms of NaCl:*—

$$\frac{1.26 \times 1{,}550}{100} = 19.53 \text{ grms.}$$

PATHOLOGICAL METABOLISM.

I. QUANTITATIVE ESTIMATION OF HYDROCHLORIC ACID IN GASTRIC CONTENTS.

In certain pathological conditions the gastric cells secrete more hydrochloric acid than they do normally, while in certain other conditions the amount of hydrochloric acid secreted by these cells is below the normal. The existence of such abnormal conditions can be recognised by a quantitative chemical examination of the gastric contents collected, by means of the stomach tube, an hour after a test meal of a fixed composition (for instance, dry toast and a large cup of tea without milk or sugar) has been given.

The chemical examination consists in estimating quantitatively :—

1. The total amount of hydrochloric acid secreted.

   Hydrochloric acid may exist in the gastric contents in three forms—(*a*) free HCl ; (*b*) HCl combined with proteins and organic bases ; (*c*) HCl combined with inorganic bases, as NaCl.

2. The "total acidity" which is a measure of the "physiologically active HCl.," provided that no other acids are present.

   The physiologically active HCl comprises free HCl and HCl combined wlth proteins, &c.

3. The "free acidity," which is a measure of the "free HCl."

Why are butter, milk, and sugar excluded from the test meal ?

Estimation of Physiologically Active HCl.

Experiment 146a. Estimation of Total Acidity.—Place into a beaker by means of a pipette exactly 10 c.c. of the filtered gastric contents. Add two drops of phenolphthalein. Titrate with $\frac{n}{10}$ sodium hydrate until a purple colour is just produced. Take the burette reading.

Record your result.

Calculation.—From your result calculate the number of c.c. of $\frac{n}{10}$ alkali necessary to neutralise 100 c.c. of gastric contents. From this calculate the weight in grammes of HCl which the total acidity represents.

Example.—6.5 c.c. $\frac{n}{10}$ alkali neutralise 10 c.c. gastric contents.

Therefore 100 c.c. gastric contents are neutralised by $6.5 \times 10 = 65$ c.c. $\frac{n}{10}$ alkali.

Now 1 c.c. $\frac{n}{10}$ alkali = 1 c.c. $\frac{n}{10}$ HCl; 1 c.c. $\frac{n}{10}$ HCl contains 3.65 mgs. HCl.

Therefore 100 c.c. gastric contents contain 65×3.65 mgs. HCl = 237.25 mgs. HCl = 0.237 grms. HCl.

Result.—The total acidity of the gastric contents is represented by 0.237 per cent. HCl.

Estimation of Free HCl.

Experiment 146b. Estimation of Free Acidity.—10 c.c. of filtered gastric contents are placed in a beaker by means of pipette. Add two or three drops of Töpfer's indicator. The solution turns red. Titrate with $\frac{n}{10}$ alkali until the red colour is replaced by a lemon-yellow colour. (If the lemon-yellow colour appears at once when the indicator is added, free acid is absent).

Take burette reading. Record the result.

Since Töpfer's indicator reacts only to free acid which is not combined with protein, the result indicates the amount necessary to neutralise the free acidity of 10 c.c. of the gastric contents.

Calculation is carried out as above.

Explain the results obtained. Refer to Experiment 65.

Estimation of Total HCl Secreted.

Experiment 146c. Estimation of Total Amount of Hydrochloric Acid Secreted.—To 20 c.c. of the filtered gastric contents apply Volhard's method for the estimation of chlorides as given in Experiment 145, and express the result in terms of HCl.

By deducting the "free acidity" from the "total acidity" the amount of "HCl combined with proteins and organic bases" is arrived at.

By deducting the "total acidity" from the total HCl secreted the amount of "HCl combined with inorganic bases" is arrived at.

Collect the results obtained from Experiments 146a, 146b, and 146c in tabular form as under :—

<table>
<tr><td rowspan="3">Total HCl secreted by gastric cells :
=</td><td>Free HCl :
=</td><td rowspan="2">Physiologically active HCl :
=</td></tr>
<tr><td>HCl combined with proteins and organic bases :
=</td></tr>
<tr><td colspan="2">HCl combined with inorganic bases :
=</td></tr>
</table>

II. ABNORMAL CONSTITUENTS OF URINE.

Of the substances which may appear in the urine in pathological conditions, *proteins*, *blood pigments*, *bile pigments and bile salts*, *glucose*, *acetone*, *diacetic acid*, occur most frequently. The fact that these substances are dissolved in urine, a fluid having such a complex composition, introduces certain fallacies in the application of the reactions by which the substances mentioned can be recognised if present alone in a simple watery solution.

In the case of proteins, for instance, the fact that the urine has a colour of its own excludes the use of the colour reactions (biuret, xanthoproteic, &c.) as tests for the presence of proteins in urine. The guaiac test for blood is given also by pus, and a variety of other substances which may occur in urine. In the case of sugar it has been pointed out already that Fehling's solution may be reduced by other substances than glucose or lactose. In order to avoid these fallacies, as many different tests as possible should be carried out before deciding whether a certain substance is or is not present in the urine.

Some drugs are excreted in the urine, and may introduce further fallacies. The previous treatment of the patient must therefore also be taken into consideration.

The following tests for abnormal constituents should be carried out at first on normal urines, then on normal urines to which a very small amount of the abnormal constituent has been added; lastly, on pathological urines obtained from the infirmary.

PROTEINS.

The proteins of the blood-plasma (globulin, albumin, fibrinogen) are the proteins most frequently met with in the urine. Clinically no distinction is drawn between these different proteins, and the presence of any of them is spoken of clinically as "albuminuria."

Tests for Protein in Urine, and their Fallacies.

Experiment 147. Tests for Protein in Urine.—For all the following tests the urine must be quite clear. If not clear, filter.

(*a*) *Heat Test.*—The urine must be neutral or very faintly acid. Heat to boiling point. A precipitate may appear which may consist of either

earthy phosphates or protein. Add three drops of dilute acetic acid to the hot solution *and heat again to boil.* If the precipitate be one of earthy phosphates it will dissolve. If a flocculent precipitate remains after the addition of the acid, the presence of protein is indicated.

Fallacies.—If the urine is alkaline when heated, or if too much acid is added, alkali-albumin or acid-albumin is formed, which is not coagulated by boiling and remains in solution. A small amount of protein will therefore not be indicated by this test, unless it is carried out carefully.

(*b*) *Precipitation by Strong Mineral Acids. Heller's Test.*—Place two or three c.c. of pure concentrated nitric acid in a test tube. Incline the test tube and from a pipette allow the urine to flow slowly down the side, so that it forms a layer above the nitric acid. If albumin is present a white opaque ring appears in the urine at the junction of the two fluids. The test is very delicate, and is given by a dilution of 1 : 50,000. If only traces of albumin are present, the ring may appear only after a minute.

Fallacies.—If concentrated urines are examined by this test, a white ring, usually less defined, may be formed, which may be due to the precipitation of uric acid or urea nitrate. If that is suspected, dilute the urine with three times its volume of water, and repeat the test with the diluted urine. If the ring was due to uric acid or urea nitrate the ring will not appear with the diluted urine.

After the administration of drugs containing resins a white ring may appear owing to the precipitation of the resins by the acids. This ring will disappear on the addition of alcohol to the urine, while a ring due to protein will persist.

Note.—A coloured ring may also appear at the junction of the acid and urine. This is due to the formation of indigo-red in urine rich in indican. It has no relation to the presence of albumin.

(*c*) *Precipitation by Alkaloidal Reagents :—*

Precipitation by Hydroferrocyanic Acid.—Render 5 c.c. of urine distinctly acid with one to two c.c. of acetic acid. Add drop by drop potassium ferrocyanide. If the urine remains clear no albumin is present. If a precipitate forms protein is present.

Precipitation by Salicylsulfonic Acid.—To 5 c.c. of urine rendered slightly acid add drop by drop salicylsulfonic acid. If protein is present a precipitate forms.

Precipitation by Picric Acid.—Place in a test tube 5 c.c. of picric acid solution. Allow a drop of urine to fall from a pipette into the picric acid. If protein is present, a cloud will form round the drop.

Fallacies.—If alkaloids are administered in large doses, for instance quinine, they may be excreted in the urine and form a precipitate with these reagents.

In some pathological conditions it will be found that the heat test is negative, while the other tests give a positive result. Then albumoses are present in the urine. It will be found then that the white ring formed in Heller's test dissolves on heating, and reappears on cooling. This condition of *albumosuria* is rare.

REDUCING SUGARS.

The sugar which is found most frequently is *glucose*. This occurs in *diabetes mellitus*. If proteins are present they must be removed by heat coagulation before testing for reducing sugars.

Tests for Glucose in Urine and their Fallacies.

Experiment 148. Tests for Glucose.—(*a*) *Trommer's Test.*—To 5 c.c. of urine add 1 c.c. of caustic soda. Then add drop by drop cupric sulphate, shaking after the addition of each drop. On the addition of each drop a flaky precipitate of cupric hydrate forms at first, which, if sugar is present, dissolves on shaking, giving a deep blue solution. Continue to add cupric sulphate, drop by drop, until a little cupric hydrate remains undissolved. Heat the upper part of the solution. A yellow precipitate forms if a reducing sugar is present.

(*b*) *Fehling's Test.*—Heat in two test tubes equal volumes of urine and of Fehling's solution to boiling point. The Fehling solution must remain clear on boiling. Otherwise it has become decomposed, and a fresh solution must be prepared. When both solutions are boiling, remove the test tubes from the flame and pour the one into the other without further heating. Allow the mixture to stand. If much sugar is present yellow

cuprous oxide separates out at once. With less sugar present the colour gradually changes. This is followed by the appearance of a yellow precipitate, which may not begin to appear until the tube has cooled.

If little sugar is present repeat the test with Fehling's solution half diluted with water.

Note.—Although the principle of Trommer's and Fehling's test is the same, it is best to use both in testing for reducing sugars, as there are certain advantages and disadvantages connected with either of them.

Glycuronic Acid.

Fallacies.—The fallacies due to the presence of uric acid and creatinine have already been discussed (see Exps. 134, 138). Further: glycuronic acid reduces cupric sulphate. This acid may occur in the urine as the result of administration of certain drugs, such as chloral, morphia, camphor, chloroform, antipyrin, antifebrin, or it may occur as the result of excessive intestinal putrefaction. (Explain its appearance in both these conditions.) If the test for indican shows that a large amount of indican is present, it points to the latter possibility. Glycuronic acid (what is its structural formula?) can be distinguished from glucose by its failure to ferment with yeast.

(*c*) *Nylander's Test.*—To some urine in a test tube (wide tubes are most convenient) add one-tenth of its volume of Nylander's reagent. Boil over a small flame for three minutes and allow to cool. If reducing sugar is present the urine darkens to a deep brown, and eventually a fine black precipitate settles out. Normal urine shows only a slight darkening.

Fallacies.—The same as in Trommer's or Fehling's test, except that uric acid and creatinine do not reduce Nylander's reagent. On the whole this test is more reliable than Fehling's or Trommer's test.

(*d*) *Fermentation.*—For this test the urine must be clear and as fresh as possible. It must not have entered into ammoniacal fermentation. Test reaction. If alkaline, make faintly acid with a few drops of very dilute acid (tartaric acid). Boil the urine for two minutes and cool under the tap. Place a small piece (size of a pea) of yeast in the cooled urine in a test tube and shake up gently, so that an emulsion of yeast in the urine is formed. Fill the fermentation tube with this emulsion, so

that the closed limb contains no air bubbles. Allow the tube to stand in a warm place (best at 37°), and examine after one hour, and again after twenty-four hours. The appearance of a bubble (of CO_2) in the closed limb indicates the presence of glucose.

Fallacies.—This is the most reliable and important test for glucose in the urine, since glucose is the only fermentable substance which may occur in the urine. The only possible fallacies arise from possible irregularities in the behaviour of the yeast. Firstly, the yeast may be inactive, so that no fermentation takes place although glucose is present. Secondly, the yeast may undergo self-fermentation, that is, it may give off gas, even in the absence of glucose. These two fallacies can be excluded by the following two control experiments, carried out at the same time with the same yeast.

One tube contains an emulsion of yeast in a dilute glucose solution. Absence of fermentation in this tube would indicate that the yeast is inactive, and therefore useless.

The other tube contains an emulsion of yeast in normal urine. If more than a very small air bubble is found in the closed limb of this tube the yeast undergoes self-fermentation, and the results obtained are not reliable.

(*e*) *Phenylhydrazine Test.*—To 10 c.c. of urine add 10 drops of phenylhydrazine and an equal amount of glacial acetic acid. Shake the test tube to ensure a thorough mixing, and keep in the *boiling* water bath for one hour. Allow to cool slowly. If crystals separate out, examine microscopically. If glucose is present the characteristic crystals of the glucosazone should be seen. If only small amounts of glucose are present this test may be negative.

Note.—The value of this test is that it enables one to distinguish glucose from other reducing sugars, especially lactose, which may be present. The other sugars also form osazones which, however, have a different crystalline form.

(*f*) *Polarimetric Examination.*—If a polarimeter is available the urine should be examined with regard to its action on polarised light. If glucose is present, the urine will produce dextrorotation. If the urine is too deeply coloured it should be decolourised by shaking with solid lead acetate and filtering until the filtrate is clear.

Lactose, its Distinction from Glucose.

Lactose is sometimes present in the urine. It gives the same tests as glucose, but can be distinguished from it by the form of the osazone crystals and by its inability to ferment.

Method of Testing for Reducing Sugars.

Method of Testing Urine for Reducing Sugars.—Proceed as follows:—Test first with Trommer's and with Fehling's test. If both are undoubtedly negative no further tests are necessary. If they are positive Nylander's test, Phenylhydrazin test, and fermentation must be carried out before any reliable conclusion can be arrived at. A polarimetric examination is desirable.

Quantitative Estimation of Glucose.

Experiment 149. Estimation of Glucose by Fehling's Method.—This titration is based on the principle that a definite amount of copper salt, in this case the amount contained in 10 c.c. of Fehling's solution, is reduced to the red cuprous oxide by a constant amount of glucose (0.05 grm. glucose). When all the blue copper sulphate has been reduced to red cuprous oxide, which falls out, the end-point of the reaction is reached, and the blue colour of the supernatant fluid, which is due to the presence of copper sulphate, totally disappears. The volume of urine used is then known to contain 0.05 grm. glucose.

Method.—Run exactly 10 c.c. of Fehling's solution from a burette into a porcelain basin, dilute with about 40 c.c. of water, and heat to boiliug. While the Fehling's solution is kept boiling gently, run in the urine from a burette. Add $\frac{1}{2}$ c.c. of the urine at a time in short intervals. A red precipitate appears suspended in a fluid which is, at first, deep blue. As more and more of the urine is added the blue colour of the fluid in which the precipitate is suspended becomes fainter and fainter. Finally the red precipitate will be seen suspended in a fluid which is colourless (or slightly yellow). The point when the fluid, in which the precipitate is suspended, first becomes colourless indicates the end-point of the titration.

The first titration gives only a rough estimate of the amount of urine necessary to reduce 10 c.c. of Fehling's solution. If the urine contains so much glucose that less than 5 c.c. of urine have been used in this first titration, the urine must be diluted until about 6 to 12 c.c. of urine are required to arrive at the end-point. (If, for instance, 2.8 c.c. of urine have been used

in the first titration, the urine should be diluted three times, *i.e.*, 20 c.c. of urine with 40 c.c. of water). Empty the burette containing the original urine, wash out with the diluted urine, and then fill with the diluted urine.

In order to determine the end-point accurately, subsequent titrations are carried out in such a way that instead of adding the urine in small portions, almost all the urine necessary to produce complete reduction is added in a bulk to the Fehling's solution. Continue boiling after the addition, and complete the reaction until the supernatant fluid is colourless by adding the urine in small quantities (three drops at a time). The end point is best seen, if, after allowing the red precipitate to settle, the basin is slightly tilted so that the supernatant fluid appears against the white background of the porcelain.

Carry out one preliminary and two subsequent titrations. If the two subsequent titrations agree to within 0.5 c.c., take their average, and calculate from it the amount of sugar present in 100 c.c. of urine.

Note.—The fallacy to guard against in this titration is the oxidation by air. If, for instance, a titration has been completed, and the red precipitate with the supernatant colourless fluid is allowed to stand in contact with the air, a blue colour will again appear in the supernatant fluid owing to oxidation and solution of the cuprous oxide. If, therefore, the end point of the reaction has once been reached, subsequent appearance of a blue colour must be neglected.

For the same reason the whole titration should be carried out as quickly as possible.

Calculation. Example:—

1. Preliminary titration. 10 c.c. Fehling are reduced by 3.3 c.c. of urine. Dilute 20 c.c. of urine with 40 c.c. of water. Fill burette with urine thus diluted 1 : 3.
2. Titration. 9.1 c.c. urine.
3. Titration. 8.8. c.c. urine.

   Average, 8.95 c.c. urine.

8.95 c.c. diluted urine contain 0.05 grm. glucose.

100 c.c. diluted urine contain $\frac{0.05 \times 100}{8.95}$ grm. glucose = 0.56 grms. glucose.

Result.—*The original urine was three times stronger, and contained therefore in* 100 *c.c. urine* 1.68 *grms. glucose.*

From this the amount of glucose excreted in twenty-four hours can, if necessary, be calculated.

ACETONE AND DIACETIC ACID.

These two substances occur usually together, since acetone is formed by decomposition from diacetic acid, which is primarily formed. They frequently occur in diabetes.

What are the structural formulæ for these two substances

ACETONE.

Note the odour.

Tests for Acetone in Urine.

Experiment 150.—(*a*) *Lieben's Iodoform Test.*—To 5 c.c. of urine add a few drops of caustic soda until the reaction is alkaline. Add two drops of iodine solution. If acetone is present the characteristic smell of iodoform appears; the urine becomes turbid after standing for some time (the length of time depending on the amount of acetone present), and a yellowish crystalline sediment of iodoform forms. Examine microscopically. (Hexagonal plates, sometimes arranged in stars).

Fallacies.—The test is given also by alcohol and aldehyde.

(*b*) *Gunning's Iodoform Test.*—Same as Lieben's test, but instead of caustic soda, ammonia is added, which leads to the formation of a black precipitate of nitrogen iodide, which disappears after some time. If acetone is present, iodoform is formed as in Lieben's test. This test has the advantage of being given only by acetone, not by alcohol or aldehyde.

(*c*) *Nitroprusside Test.* (*Legal's Test*).—To 5 c.c. of urine add a few drops of a freshly prepared solution of nitroprusside. Then make alkaline with caustic soda. A red colour appears whether acetone is present or not. (To what constituent of the urine is this colour due?) Acidify with acetic acid. If acetone is present the colour will remain or become violet. If the urine is normal it becomes yellow (see Exp. 137).

This test may be modified as follows:—

Add nitroprusside as above, then carefully add some strong ammonia

so that it forms a layer above the urine A magenta colour at the point of contact indicates the presence of acetone.

Normal urine gives an orange colour.

DIACETIC ACID.

Tests for Diacetic Acid in Urine.

Note.—In testing for diacetic acid the urine must be fresh, as diacetic acid is easily decomposed on standing (into what?).

Experiment 151. *Gerhard's Test.*—To 5 c.c. of urine add, drop by drop, ferric chloride as long as a precipitate of ferric phosphate continues to form. A claret-red colour is produced if diacetic acid is present.

If, owing to the presence of the ferric phosphate precipitate, the colour is difficult to recognise, filter.

Fallacies.—Carbolic acid, salicylates, antipyrin, &c., give a similar colour. They can be distinguished from diacetic acid, because the colour produced by the latter disappears on boiling, while the colour produced by the drugs named persists.

Why is the colour produced by diacetic acid destroyed by boiling?

Diacetic acid may be extracted from urine, acidified with hydrochloric or sulphuric acid, by shaking with ether. Remove the ether with a pipette, and add to the ether ferric chloride. A claret-red colour appears if diacetic acid is present.

Formation of Acetone from Diacetic Acid.—To some normal urine free from acetone add a little diacetic acid. Boil with dilute caustic soda. Acetone is formed. Note the odour and prove the absence of diacetic acid and the presence of acetone by appropriate reactions.

State the reaction which has taken place in the form of a chemical equation.

BLOOD PIGMENT.

Tests for Blood in Urine.

Experiment 152.—(*a*) *Colour.*—If a relatively large amount of blood is present the urine has a red colour. Smaller quantities give to the urine an opaque reddish-brown appearance, "smoky urine."

Examine microscopically the deposit, if any, for the presence of red blood corpuscles.

(*b*) *Colour of Earthy Phosphate Precipitate.*—Render 5 c.c. of urine strongly alkaline with caustic soda, and boil. A precipitate of earthy phosphates will separate out on standing. This precipitate is normally greyish-white. If blood is present it is brownish-red in colour from the hæmatin which is carried down with it.

Fallacies.—Cascara sagrada, rhubarb, senna, give to the precipitate a similar colouration.

(*c*) *Guaiac Test.*—To 5 c.c. of urine add 2 drops of tincture of guaiac, 1 c.c. of hydrogen peroxide, and shake. A blue colour appears in the fluid if blood is present. This test is a very sensitive one.

Fallacies.—Iodides and pus also give a blue colour.

(*d*) *Spectroscopic Examination.*—Examine urine spectroscopically as described in Experiments 105-111. If blood is present the spectrum of methæmoglobin is usually seen. This may have been formed from hæmoglobin on standing after the urine has been passed, or it may have been passed as such.

If the absorption spectrum is not distinct, prepare hæmochromogen by boiling with caustic soda and reducing with ammonium sulphide. If hæmoglobin or methæmoglobin are present the characteristic spectrum of hæmochromogen will be seen.

Spectroscopic examination is free from fallacies, but it does not indicate traces of blood as the guaiac test does. It is therefore possible that the guaiac test is positive, while spectroscopic examination is negative.

BILE.

Tests for Bile Constituents in Urine.

Experiment 153. Bile Pigments.—(*a*) *Colour.*—If bile pigments are present the urine has a brownish or greenish colour. The latter colour is present especially if the urine has been standing for some time, so that the red bilirubin has become oxidised to the green biliverdin.

(*b*) *Gmelin's Test.*—The following modification is best adapted for the examination of urine. Filter urine repeatedly through the same paper

Unfold the paper and put a drop of yellow nitric acid on it. A ring of colours appears, if bile pigments are present.

This test is not delicate.

(*c*) *Huppert's Test.*—Render 10 c.c. of urine alkaline with caustic soda or sodium carbonate solution, and add barium chloride or calcium chloride solution drop by drop under shaking. A precipitate forms, which carries down with it the bile pigment. Continue adding calcium chloride until the fluid in which the precipitate is suspended has the colour of normal urine. Collect the precipitate on a filter, wash with water. Place the filter paper with the precipitate in a porcelain basin, add 10 c.c. of alcohol, and while stirring add five to ten drops of strong hydrochloric acid, until the precipitate is dissolved. Pour the yellowish solution into a test tube ; add two drops of ferric chloride and heat. A green colour changing to blue appears, if bile pigments are present.

This is the most reliable test for bile pigments.

Experiment 154. Bile Salts.—*Pettenkofer's Test* for bile salts is best carried out as follows :—Dissolve in the urine a small fragment of a crystal of cane sugar. Filter the urine thus prepared through filter paper. Dry the paper. Allow a drop of concentrated sulphuric acid to fall on the paper. After half a minute the paper shows in transmitted light a violet colour if bile salts are present. Normal urine gives a reddish or brownish colour.

SOME PATHOLOGICAL DEPOSITS.

Morphological elements such as pus cells, epithelial cells, casts, &c., are not dealt with here.

Cystin.—Hexagonal plates, insoluble in water and acetic acid, soluble in hydrochloric acid and in ammonia.

Leucin and Tyrosin.—Usually occur together, especially in diseases of the liver.

Leucin is deposited in spheres having a radial and concentric striation. It is slightly soluble in water. Soluble in acids and alkalis.

Tyrosin is deposited in sheaves of fine white needles. The appearance of the crystals is very much like that of glucosazone, except that the latter is yellow. Tyrosin is very slightly soluble in water, soluble in acids and alkalis. It gives a red colour with Millon's test.

What are the structural formulæ for cystin, leucin, and tyrosin? What are they formed from?

Calcium Oxalate.—See under normal urines (p. 51.) Is pathological only if present in excess.

Experiment 155.—Examine urines of cases from the infirmary for pathological constituents, and report on the form given out.

Experiment 156.—Examine urines of cases from the infirmary for total N, urea, ammonia, acidity, chlorides, sulphates, indican, and report on the form given out.

FORM FOR REPORT

Name of Patient	
Diet and Treatment (Drugs) during previous twenty-four hours	
Total Volume of Urine excreted in twenty-four hours	
Colour	
Deposit, if any	
Specific Gravity	
Reaction	
Total N excreted in twenty-four hours	
Urea. Amount excreted in twenty-four hours	
,, Percentage of urea N to total N	
Ammonia. Amount excreted in twenty-four hours	
,, Percentage of ammonia N to total N	
Acidity of total Urine	
Chlorides. Amount excreted in twenty-four hours	
Sulphates*	
Ethereal sulphates*	
Indican*	

1 c.c. $\frac{n}{10}$ acid = 1.7 mg. NH_3 = 1.4 mg. N ; 1 c.c. standard $AgNO_3$ = 10 mg. NaCl ; 10 c.c.

* State by + or − whether test is positive or negative. A doubtful result may be indicated

† State here your general conclusion ; for instance : "Glucose absent, although Fehling's

Abnormal Constituents.			
Test.		Result.*	Conclusion.†
Albumin.	Heat		
	Nitric Acid		
	Picric Acid		
	Hydroferrocyanic Acid		
	Salicylsulfonic Acid		
Reducin Sugar.	Trommer		
	Fehling		
	Fermentation		
	Nylander		
	Osazone		
	Quantitative Estimation		
Blood.	Guaiac		
	Earthy Phosphates		
	Spectroscopic Examination		
Diacetic Acid.	Gerhard		
Acetone.	Iodoform		
	Nitroprusside		
Bile.	Gmelin		
	Huppert		
	Pettenkofer		

standard Fehling's solution = 50 mg. glucose ; 1 g. urea liberates 354 c.c. N.
by + ?, a strong positive result by + +, and so on.
and Trommer's slightly positive."