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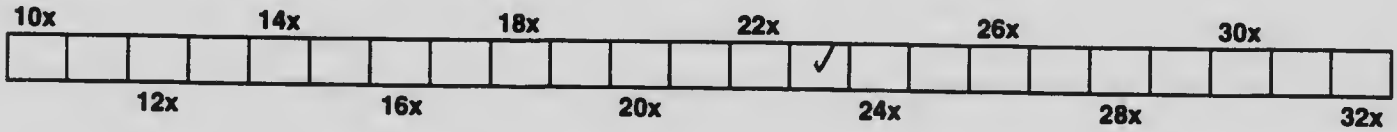

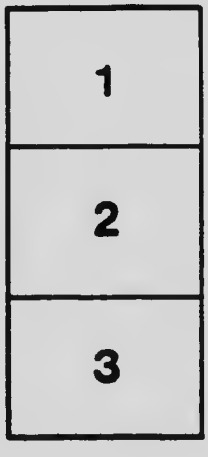

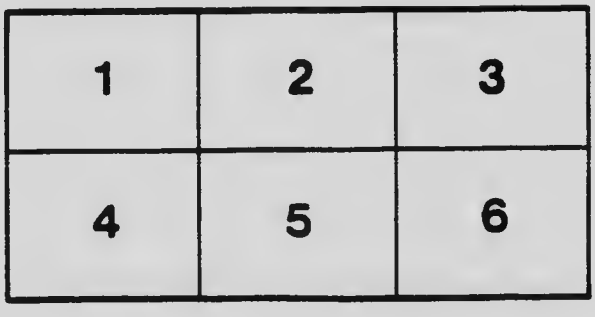

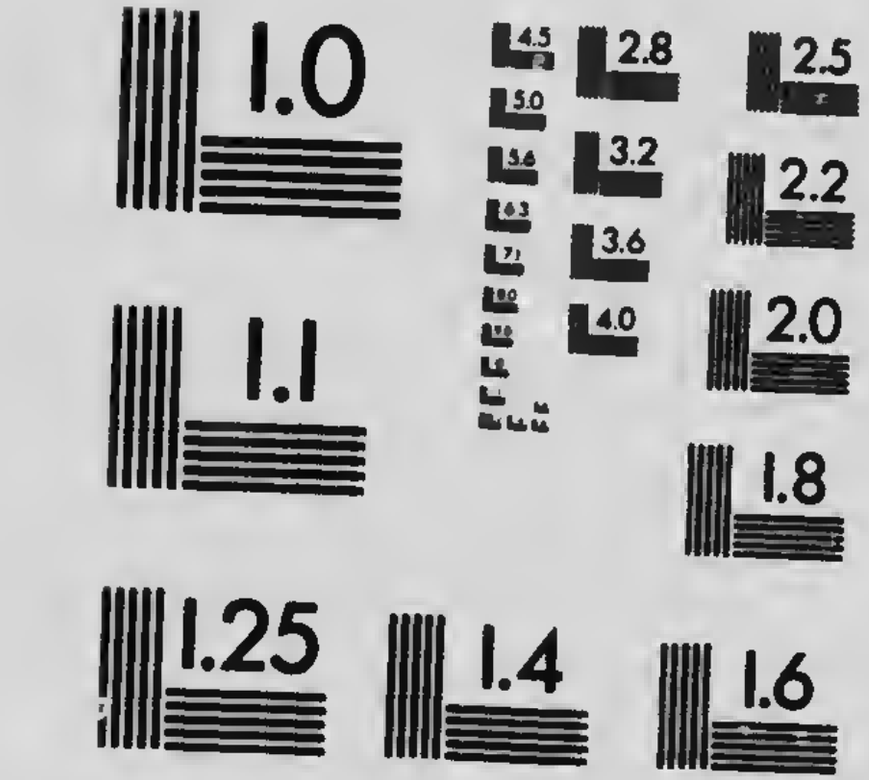

ORGANIC AND PHYSIOLOGICAL CHEMISTRY

LOTHROP

A LABORATORY COURSE

IN

ORGANIC AND PHYSIOLOGICAL CHEMISTRY

BY

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PREFACE

These experiments are intended to cover the laboratory work given to the students in the Medical Faculty of Queen's University in the second year. The material has been selected with two aims in view: First, the tests have been limited to those which experience has shown to be essential to a thorough appreciation of the technique of the organic and physiological chemistry which comes within the scope of the medical practitioner's work. Second, the author has endeavored to investigate and select only the more recent and approved methods. In view of the increasing importance of laboratory technique in the modern practice of medicine, the importance of these two factors cannot be over-emphasized and it is the hope of the author that by frequent revision the manual may be maintained to this standard.

The tests have been presented in such a way as to permit inductive observation. By such a method the student acquires the ability to observe closely and interpret correctly the results observed. The continuity and the theory of the subject must be presented in the lectures and conferences.

Grateful acknowledgment is made to Prof. William J. Gies for the experiments developed by him in the Laboratory of Biological Chemistry at the College of Physicians and Surgeons of Columbia University. Many suggestions were also obtained from the laboratory manual by Drs. Ruttan and Harding of McGill University, to whom the author expresses his indebtedness.

Thanks are due to Prof. Paul E. Howe of the Department of Biological Chemistry of Columbia University and to Dr. Walter H. Eddy, Head of the Biology Department of the New York City High School of Commerce, for criticism of the manuscript. The author also wishes to express to his wife his appreciation of her very material assistance in connection with proof reading and other details of production.

Queen's University,
Faculty of Medicine,
September 25, 1915.

CONTENTS.

DETECTION OF THE ELEMENTS IN ORGANIC COMPOUNDS.

Carbon.—Dry thoroughly about a horn spoonful of pulverized copper oxide by heating in an evaporating dish and, while still warm, mix with it a small amount of cane sugar. Transfer to a dry hard-glass test tube and insert a rubber stopper with an L-tube connection. Heat the mixture cautiously and conduct the gas into about 10 cc. of baryta water in a test tube. What is the ppt. which appears in the baryta water? Note the deposit on the sides of the hard glass test tube. Of what does it consist? Indicate the reactions.

Hydrogen.—Heat gently a small amount of cane sugar in a dry test tube. Note the condensation of liquid in the cooler parts of the tube; remove some of it on a glass rod and bring it in contact with a little *anhydrous* copper sulfate in an evaporating dish. Explain the result. Charring of the substance on heating indicates the presence of carbon.

Nitrogen.—*As ammonia.* Grind thoroughly in a mortar a small amount of pulverized egg albumin with 10-15 times its amount of soda lime. Transfer to a dry test tube and heat carefully. Note the odor of the gas evolved and its effect on wet red litmus paper.

As cyanide. Dry a piece of sodium the size of a pea by pressing between filter papers. Transfer to a *dry* test tube and add a very small amount of pulverized egg albumin. Heat gently at first, gradually increasing the temperature until the end of the tube is glowing and fumes cease to come off. Dip the hot end of the tube into 10-15 cc. of water in an evaporating dish. Break up the charred mass with a stirring rod and warm to favor solution. Filter through a small wet filter paper. If decomposition has been complete, the filtrate at this point should be colorless; if brown, the experiment should be repeated. Divide the filtrate into two portions, reserving one portion for the detection of sulfur as sulfide. To the other portion add a few drops

each of NaOH and ferrous sulfate and a single drop of ferric chloride. Boil for a minute and, after cooling, acidify with HCl. The solution becomes bluish green and a ppt. of Prussian blue may be obtained. The nitrogen is converted successively into sodium cyanide, sodium ferrocyanide and ferric ferrocyanide. Write the equations.

Sulfur.—*As sulfide.* To a portion of the solution retained in the preceding experiment add a few drops of a freshly prepared solution of sodium nitroprusside. A violet color indicates the presence of alkaline sulfide.

Place a few drops of the solution on a silver coin. Explain the production of a black stain.

To the remainder of the solution add a drop or two of lead acetate solution and acidify with acetic acid. Black lead sulfide is formed.

As sulfate. Mix in a mortar a small amount of caseinogen with 3-4 times its bulk of fusion mixture. Transfer to a crucible and heat cautiously. Gradually raise the temperature and continue the fusion until the mass is practically colorless. Allow the crucible to cool, place it in a small beaker and add enough water to cover it. Acidify with nitric acid and warm until solution is complete. To what forms have the sulfur and phosphorus in the caseinogen been converted? Filter the solution and to a portion in a test tube add a few drops of barium chloride solution. What is pptd.?

Phosphorus.—Pour about 10 cc. of the solution obtained in the preceding experiment into a test tube, add an equal volume of molybdic solution and a few drops of nitric acid and warm to body temperature. What compound is pptd.?

Iron.—Add a drop or two of conc. nitric acid to a small amount of dried blood in a crucible. Heat *cautiously* until the mass is charred and then to a red heat for several minutes. Cool, add about 5 cc. of dilute HCl and boil for a minute. Divide the solution into two portions; to one add potassium ferrocyanide solution and to the other, ammonium sulfocyanate solution. Note the results and write the equations.

TYPICAL SUBSTANCES IN THE ALIPHATIC SERIES OF ORGANIC COMPOUNDS (STRAIGHT CHAIN SERIES).

HYDROCARBONS.

Methane.—Heat a horn spoonful of crystallized sodium acetate in an evaporating dish over a small flame until the mass is dehydrated. Powder the fused mass in a mortar and then grind it thoroughly with 3-4 times its bulk of soda lime. Transfer the mixture to a hard glass test tube fitted with a rubber stopper and delivery tube. Rap the tube carefully in order to form a space for the passage of the gas. Heat the tube gently and, after the air has been expelled, collect the gas in test tubes over water. Write the equation.

Bring the mouth of one of the tubes in contact with a flame. Is the gas inflammable? Write the equation. Pour a little baryta water into the tube and shake. Explain the result.

Determine whether the gas is lighter or heavier than air.

When the hard glass test tube has cooled, add HCl to the residue. Of what does it consist?

Kerosene.—Determine the solubility of kerosene (a mixture of hydrocarbons containing from 10 to 16 carbon atoms) in water, alcohol and ether.

Note the specific gravity of kerosene.

Place a lighted match in about 5 cc. of kerosene in an evaporating dish. Does it continue to burn? Warm the liquid on a water bath to about 40° and again apply a lighted match. Compare and explain the results.

Paraffin wax.—Determine the solubility of paraffin wax in water, alcohol and ether.

Drop small pieces of the wax into test tubes containing, successively, conc. sulfuric acid, conc. nitric acid and 10% KOH. Does a reaction occur in any case?

HALOGEN DERIVATIVES.

Chloroform.—Shake about 10 cc. of chloroform with an equal volume of water. Pour off the wash water. Determine its reaction to litmus and test for chloride. Explain the results.

To about 1 cc. of chloroform add 5 cc. of alcohol and a few drops of KOH. Warm. Apply the test for chloride. Compare with the preceding test and explain the difference in results.

Iodoform.—Dissolve a horn spoonful of potassium carbonate in 50 cc. of water, add 10 cc. of alcohol and warm on a water bath to 70°. Add several small amounts of iodine, stirring after each addition until the iodine has dissolved. If too much iodine has been added and a brown color persists, add enough potassium carbonate to discharge the color. Cool, filter and wash the iodoform with water.

Dissolve a small quantity of the crystals in warm alcohol, transfer a drop of the alcoholic solution to a microscope slide and, after the evaporation of the alcohol, examine the crystals under the microscope. What is the shape of the crystals?

Dry the remaining iodoform by heating in a porcelain dish on a water bath. Transfer to a dry test tube and heat gradually to a high temperature. Note the color of the fumes and the appearance of the residue.

ALCOHOLS.

Ethyl alcohol.—(1) Set fire to about 5 cc. of alcohol in a small beaker. As soon as the burning stops, cover the beaker with a watch glass. Note the condensation of moisture on the watch glass and the cooler parts of the beaker (source?). Add a few cc. of baryta water and shake. Explain the result.

(2) *Iodoform test.* To a few drops of ethyl alcohol in half a test tube of water add a few drops of iodine solution and enough KOH to decolorize the mixture. Shake and warm gently to about 70°. Note the odor and the character of any ppt. that may form.

(3) *Oxidation.* Acidify a solution of potassium dichromate with dilute sulfuric acid and add a few drops of ethyl alcohol. Gently heat the mixture. Note the change of color and the odor of acetaldehyde. Indicate the equation.

(4) *Ethyl acetate test.* To about 1 cc. of ethyl alcohol add an equal volume of conc. sulfuric acid and a small amount of sodium acetate. Warm gently and note the odor. Write the equation.

Methyl alcohol.—Repeat tests (2-4) using *methyl* instead of ethyl alcohol. Note any variations.

Sodium ethylate.—Into 10 cc. of *absolute* alcohol in an evaporating dish drop a small piece of sodium which has been freed from oil by pressing between filter papers. Compare the action of the sodium on the alcohol with its action on water. Identify the gas evolved by collecting a little in an inverted tube and bringing the mouth of the tube in contact with a flame. Continue adding small pieces of sodium until no further action occurs. Write the equation.

Evaporate the solution to dryness on a water bath. To the dry residue add about 50 cc. of water. Test the reaction of the solution to litmus and apply to it the iodoform test. Write the equation.

ALDEHYDES.

Preparation. (1) Introduce into a dry hard glass test tube a horn spoonful of dry calcium formate and insert a rubber stopper with an L-tube connection. Heat to dull redness, having the end of the delivery tube under the surface of about 5 cc of water in a test tube.

(2) Repeat the experiment using a mixture of dry calcium acetate and calcium formate.

Retain the solution in each tube and label for use later. Write equations showing the aldehyde formed in each case.

Formaldehyde.—(1) *Silver mirror test.* Thoroughly clean a test tube by boiling in it a small amount of KOH and

washing well with water. To about 5 cc. of silver nitrate in the cleaned tube add ammonium hydroxide, drop by drop, until the pptd. silver oxide just redissolves. Add a few drops of formaldehyde solution and heat the tube in a boiling water bath for some time. What is the effect of the aldehyde on the alkaline solution of silver oxide? Indicate the equation.

(2) *Action on Fehling's solution.* Dilute about 1 cc. of Fehling's solution with about 4 cc. of water and boil. There should be no discoloration of the solution. Add a few drops of formaldehyde and heat in a boiling water bath for several minutes. Explain the result.

(3) *Action on Benedict's solution.* To about 5 cc. of Benedict's solution add a few drops of formaldehyde and boil for about a minute. Does reduction occur?

(4) *Schiff's test.* To about 5 cc. of fuchsin solution, which has been decolorized with sulfurous acid, add a little formaldehyde and warm slightly. A violet-red color indicates the presence of an aldehyde.

(5) *Aldehyde resin test.* Add a few cc. of formaldehyde to 10 cc. of KOH solution and boil vigorously in a porcelain dish for about 5 minutes. Does any change occur?

(6) *Jorissen test.* To a test tube full of water add 5 drops of 1% formaldehyde solution and mix. To about 10 cc. of the very dilute formaldehyde add a few drops of KOH and 2 cc. of a 0.1% phloroglucin solution. Note the color produced.

(7) *Resorcin test.* To about 3 cc. of the very dilute formaldehyde solution [prepared in test (6)] add a drop of resorcin solution and shake thoroughly. Carefully pour about 2 cc. of conc. sulfuric acid down the side of the inclined tube. Note the color of the ring formed at the junction of the two liquids.

(8) *Leach's test for formaldehyde in milk.* To about 10 cc. of milk preserved with formaldehyde in a casserole add 10 cc. of conc. HCl and a drop of 5% ferric chloride solution. Gradually heat the mixture nearly to boiling. Note the color produced.

Repeat the test with fresh milk and compare the results.

Acetaldehyde.—Repeat tests (1) and (4-7) using acetaldehyde and note any variations from the results obtained with formaldehyde.

Identify the compound present by applying tests (1), (4), (6) and (7) to the solutions obtained by distilling calcium formate and the mixture of calcium formate and acetate.

Chloral and chloral hydrate.—Repeat tests (1), (2) and (4) using a solution of chloral hydrate.

Place a level horn spoonful of chloral hydrate in a test tube, cover with conc. sulfuric acid and warm carefully. Remove the chloral which floats on the surface with a pipette and transfer it to a dry test tube. Add a drop of water. The hydrate is reformed with the evolution of heat.

To a solution of chloral hydrate add a little KOH and warm by holding the tube in the hand. Note the odor. Write the equation.

Apply the chloride test to about 2 cc. of chloral hydrate solution. Explain the result. Add a small amount of zinc dust to 2 cc. of the chloral hydrate solution and boil the mixture for about two minutes. Filter, apply the chloride test to the filtrate and explain the result.

KETONES.

Acetone.—Introduce into a dry hard glass test tube a horn spoonful of calcium acetate. Insert a rubber stopper and delivery tube. Heat gradually to a high temperature, collecting the distillate in 5 cc. of water in another test tube. After the tube has cooled add HCl to the residue. What does the effervescence indicate? Write the equation. Apply to the distillate the iodoform and sodium nitroprusside tests.

(1) *Iodoform test.* To half a test tube full of water add a few drops of acetone, then iodine solution and KOH until the solution is decolorized. Note the odor and the immediate

formation of iodoform. Why is the solution not heated to 70° as in the similar test on alcohol? Write the equation.

(2) *Sodium nitroprusside test.* To a very dilute solution of acetone add a few drops of freshly prepared sodium nitroprusside solution and make alkaline with KOH. Note the color produced. Acidify with acetic acid; is there any change in color?

(3) *Bisulfite compound.* To about 5 cc. of a saturated solution of sodium bisulfite add a little acetone and shake thoroughly. The reaction takes place with the evolution of heat. Allow to cool and describe the compound which separates. Write the equation.

(4) *Oxidation to acetic acid.* To about 5 cc. of dilute sulfuric acid add a few drops of acetone. Warm slightly and add potassium permanganate solution a little at a time until a permanent pink color is produced. Explain the reaction.

ORGANIC ACIDS.

Formic acid.—Carefully neutralize a formic acid solution with ammonium hydroxide (do not allow the solution to become alkaline) and use the solution of ammonium formate in tests (1) and (2).

(1) To a silver nitrate solution add some of the ammonium formate solution and warm. Explain the reaction.

(2) Add a few drops of ferric chloride solution to the solution of ammonium formate. Note the color produced. Boil. A ppt. of basic ferric formate is formed.

(3) To a small amount of calcium formate in a dry test tube add a little conc. sulfuric acid. Heat and hold a lighted match at the mouth of the tube. Write the equation.

Acetic Acid.—(1) To about 2 cc. of acetic acid add a few drops of alcohol and an equal volume of conc. sulfuric acid. Mix by pouring from one tube to another. Note the fruity odor. Write the equation. Compare test (4), page 5.

(2) Add ammonium hydroxide to 10 cc. of acetic acid in a small beaker until the reaction is slightly alkaline to litmus. Remove the excess of ammonia by boiling. Cool and add to the ammonium acetate solution a few drops of ferric chloride. A blood red color is produced (no ppt. should appear at this point). Boil half the solution and to the other half add HCl. Note the results and write all the equations.

Butyric acid.—To about 2 cc. of dilute butyric acid add a few drops of alcohol and an equal volume of conc. sulfuric acid. Note the odor and compare with that of ethyl acetate.

Stearic acid.—Determine the solubility of stearic acid in water, warm alcohol and ether. Determine the reaction of the alcoholic solution to litmus.

Dissolve a small amount of stearic acid in about 2 cc. of hot alcohol, pour the hot solution on a watch glass and allow it to cool. Make a microscopic examination of the crystals.

Oxalic acid.—(1) Heat a crystal of oxalic acid on a crucible lid. Is the presence of carbon shown by charring?

(2) Dissolve a small amount of oxalic acid in conc. sulfuric acid and warm. Hold a lighted match at the mouth of the tube. Carbon dioxide and carbon monoxide are formed. Write the equation. Compare with formic acid, test (3), page 8.

(3) Acidify a solution of oxalic acid with sulfuric acid, warm and add potassium permanganate solution. Explain what occurs. Carbon dioxide is liberated. Write the equation.

(4) To a solution of oxalic acid or an oxalate add a few drops of calcium chloride solution. What is pptd.? Determine the solubility of the ppt. in acetic and hydrochloric acids.

Lactic acid.—(1) To a test tube of water add 5-10 drops of ferric chloride. To half of the solution add a few drops of lactic acid and compare the color with that of the control solution. The color is due to the formation of ferric lactate.

(2) *Uffelmann's reaction.* To a small amount of Uffelmann's reagent in a test tube add a few drops of lactic acid. Note the color change.

(3) Acidify a solution of lactic acid with sulfuric acid and add an equal volume of ether. Shake and, after the liquids have separated, pour off the ether into an evaporating dish. Allow the ether to evaporate, dissolve the residue in a little water and apply the Uffelmann test. What was the action of the ether?

Tartaric acid.—(1) To a conc. solution of sodium potassium tartrate (Rochelle salt) add a few drops of acetic acid and then potassium chloride solution. Does pptn. occur? Acid potassium tartrate (cream of tartar) is formed.

(2) To a solution of sodium potassium tartrate add a little silver nitrate. Dissolve the ppt. by adding ammonium hydroxide drop by drop; warm the solution. Compare test (1), page 5.

(3) Add a few drops of KOH to a solution of copper sulfate. What compound is pptd.? Add an equal volume of sodium potassium tartrate solution. A dark blue solution results. Fehling's solution (page 6) is prepared in a similar manner. Soluble sodio-potassium cupro-tartrate is formed. Write the formula.

AMIDES.

Urea (carbamide).—(A) *Synthesis.* Dissolve 10 grams of potassium cyanate in 25 cc. of water. Prepare also a solution of about 10 grams of ammonium sulfate in 25 cc. of water. When completely dissolved, mix the two solutions in an Erlenmeyer flask and boil for about five minutes. Pour into an evaporating dish and evaporate to dryness on a water bath. To the dry residue add 25 cc. of alcohol and extract thoroughly. Filter into a small beaker, evaporate almost to dryness and set the beaker aside, covered with a watch glass, for the crystallization of the urea. After an interval of at least 24 hours, pour off the supernatent liquid and dry the crystals

between folds of filter paper. Identify the urea by applying tests (3), (4) and (6). Write all the equations and the structural formula for urea.

(B) *Reactions.* (1) Test the solubility of urea in water, alcohol and ether.

(2) Boil a few urea crystals with about 5 cc. of KOH. What gas is evolved? Write the equation.

(3) Heat carefully a little urea in a dry test tube. Note the odor and the effect on wet red litmus paper of the gas which is given off after the urea has melted. Write the equation. Continue the heating until the melted mass begins to solidify. After cooling the tube, add about 5 cc. of water and divide the solution into two portions. To one portion add an equal volume of KOH and a drop of copper sulfate solution; to the other portion add an equal volume of "Biuret reagent." Compare the colors produced.

To 5 cc. of an aqueous solution of urea add an equal volume of biuret reagent. Explain the result.

(4) Dissolve a crystal of urea in a drop of water on a microscope slide. Allow a drop of conc. nitric acid to come in contact with it. Examine the crystals of urea nitrate under the low power of the microscope. Draw the crystals.

(5) Repeat test (4) but use saturated oxalic acid solution instead of nitric acid. Draw the crystals of urea oxalate.

(6) To a little urea solution add a few drops of sodium nitrite solution and a drop or two of dilute HCl. What gases are given off? Write the equation.

(7) To some urea solution add a little sodium hypobromite solution. Explain what occurs and write the reaction.

CARBOHYDRATES.

MONOSACCHARIDES.

Glucose.—(1) *Molisch's reaction.* To about 5 cc. of glucose solution add 2 drops of alpha-naphthol solution and shake thoroughly. Incline the tube and pour carefully down the side of the tube about 2 cc. of conc. sulfuric acid. What is the color of the ring that appears at the line of contact? This reaction is given by all substances containing a carbohydrate group.

Repeat the test using a very dilute solution of furol instead of the glucose solution. Explain why carbohydrates give the reaction.

(2) *Moore's test.* Boil some glucose solution with an equal volume of KOH solution. Describe the result. Compare test (5), page 6.

(3) *Silver mirror test.* Repeat test (1), page 5, using a glucose solution instead of formaldehyde.

(4) *Trommer's test.* To 5 cc. of glucose solution add half its volume of KOH, mix and add, drop by drop with thorough shaking, a very dilute solution of copper sulfate. Continue adding the copper sulphate until the solution becomes a deep blue. Heat to boiling. Explain the reaction and indicate the equation.

(5) *Fehling's test.* Dilute 1 cc. of Fehling's solution with 4 cc. of water and boil. There should be no discoloration of the solution. To the warm solution add an equal volume of glucose solution and heat to boiling. Allow the tube to cool and examine the deposit.

Quantitatively dilute the glucose solution and determine the sensitiveness of Fehling's solution.

(6) *Benedict's test.* To 5 cc. of Benedict's solution add *eight drops* of glucose solution. Boil for a minute or two and allow the tube to cool spontaneously. The entire solution will be filled with a precipitate which may be red, yellow or green, depending upon the amount of sugar present.

Determine the sensitiveness of Benedict's solution and compare with Fehling's solution.

(7) *Barfoed's test.* Heat 5 cc. of freshly prepared Barfoed's solution in a boiling water bath and add about 1 cc. of glucose solution to the hot solution. Heat the tube in a boiling water bath for *five* minutes. Examine by reflected light. Under these conditions Barfoed's solution is reduced by monosaccharides only.

(8) *Nylander's test.* To about 5 cc. of glucose solution add 1 cc. of Nylander's solution and heat in a boiling water bath for five minutes. Describe the color changes. Explain.

(9) *Safranine test.* To 2 cc. of 0.1% safranine solution add an equal volume of glucose solution and 2 cc. of KOH. Mix thoroughly. Boil carefully, avoiding shaking. The safranine is reduced to a leuco-base and the red color changes to a light yellow. After the mixture is cold, shake vigorously. Does the red color return? Explain the reaction.

(10). *Picramic acid test.* Mix equal volumes of saturated picric acid and 10% KOH solutions (2 cc. each) and add about 4 cc. of glucose solution. Boil for a minute. What color is produced?

(11) *Phenylhydrazine reaction.* Fill a test tube to the depth of a quarter of an inch with phenylhydrazine hydrochloride and add twice as much solid sodium acetate. Add 10 cc. of glucose solution, dissolve by warming and filter into a clean test tube if necessary. Heat in a boiling water bath for at least half an hour. Allow the tube to cool slowly in a beaker of warm water. Examine the osazone crystals under the microscope; sketch the crystals. Why is the sodium acetate added?

(12) *Fermentation test.* Rub up a small fragment of compressed yeast in a mortar with a little water. Mix this suspension with 10 cc. of glucose solution in a hard glass test tube. Insert a rubber stopper and delivery tube and set aside until the next laboratory exercise with the delivery tube dipping into a solution of calcium hydroxide in another test tube.

Examine the solution for alcohol by the iodoform test (test (2), page 4) and note the ppt. in the lime water. Explain.

Arabinose.—Repeat tests (1), (5) and (6), using a solution of arabinose (a pentose).

Bial's test. To 5 cc. of Bial's reagent in a test tube add about 2 cc. of arabinose solution and heat until the mixture just begins to boil. Allow to cool and note the color of the solution and of any ppt. that may form.

Tollen's test. Mix equal volumes (2 cc.) of conc. HCl and 1% phloroglucin solution and add a little arabinose solution. Heat the mixture in a boiling water bath and note the color produced.

DISACCHARIDES.

Sucrose.—Repeat tests (1), (2) and (5-7) using a solution of sucrose and compare with the results obtained with glucose. Explain the differences observed.

Inversion. To 5 cc. of sucrose solution add 2 *drops* of conc. sulfuric acid and boil for one minute. Cool and neutralize with KOH. Apply Benedict's test. Explain the result.

Seliwanoff's test. Add a few drops of the neutral solution obtained in the preceding experiment to about 5 cc. of Seliwanoff's reagent and heat the solution to boiling. A red coloration indicates the presence of laevulose. Account for its presence in the solution.

Maltose.—Repeat tests (1), (2), (5-7) and (11), using a solution of maltose, and compare the results with those obtained with glucose and sucrose.

Lactose.—Repeat tests (1), (2), (5-7) and (11), using a solution of lactose, and compare the results with those on glucose, sucrose, and maltose.

POLYSACCHARIDES.

Starch.—Suspend some starch granules in a drop of water on a microscope slide, cover with a cover slip and examine

under the microscope. Allow a drop of very dilute iodine solution to run under the cover slip; note what happens to the starch grains.

Heat to boiling in a casserole 200 cc. of water. Stir up in a small beaker a level horn spoonful of starch with 10 cc. of water. Pour the milky suspension slowly into the boiling water while stirring constantly. Boil for a minute. Starch paste is formed which is to be used in the tests given below.

(1) *Iodine test.* To a test tube full of water add 5-10 drops of starch paste and a few drops of iodine solution. Divide the blue solution equally between four test tubes and treat as follows:

(a) Keep the first portion as a "control" for color comparison.

(b) Heat the second portion to boiling. Cool. Again heat to boiling and cool a second time.

(c) To the third portion add a few drops of KOH. Acidify with HCl.

(d) Add silver nitrate solution to the fourth portion.

Note what occurs in each case and draw conclusions.

(2) *Action of alcohol.* To 5 cc. of starch paste add two volumes of alcohol. Filter, evaporate the filtrate to one-half its volume to remove the alcohol and test the solution with iodine solution after cooling. Is starch present?

(3) *Action of ammonium sulfate.* To 5 cc. of starch paste add an equal volume of *saturated* ammonium sulfate solution. Shake and allow to stand for five minutes. Filter through a dry paper and test the filtrate with iodine.

(4) *Hydrolysis of starch.* To 50 cc. of starch paste in an Erlenmeyer flask add 2 cc. of dilute HCl and heat to boiling. Remove about 2 cc. of the mixture with a pipette, dilute with a little water and apply the iodine and Benedict tests to the solution. (The solution must be neutralized with sodium carbonate before applying the Benedict test; why?). Boil the solution *very* gently and at five minute intervals remove some

of the solution and repeat the iodine and Benedict tests. Keep the tubes in the order in which the tests are made and compare the results. After the solution has boiled for 30 minutes, neutralize and apply the phenylhydrazine test. What sugar has been formed? What is the action of the acid on the starch?

Dextrins.—(1) *Iodine test.* To a solution of dextrin add iodine solution, drop by drop, noting the color produced by each addition.

(2) *Action of alcohol.* To 5 cc. of dextrin solution add *four* volumes of alcohol. Filter, evaporate the filtrate on a water bath to 5 cc. and test with iodine solution. Compare with the similar experiment with starch.

(3) *Action of ammonium sulfate.* To 5 cc. of dextrin solution add an equal volume of saturated ammonium sulfate solution. Shake and allow to stand for five minutes. Does pptn. occur? Saturate the solution by boiling with an excess of solid ammonium sulfate. Cool, filter and test the filtrate with iodine solution. Compare with the similar test on starch.

(4) Test dextrin with Benedict's solution. Why does reduction occur? Repeat the test with 5 cc. of dextrin solution that has been boiled with a few drops of dilute HCl for some minutes and then neutralized. Is the reduction more pronounced?

Cellulose.—(1) *Solubility.* Test the solubility of small pieces of filter paper in boiling water, dilute HCl and 10% KOH.

To 5 cc. of Schweitzer's reagent in a test tube add a small piece of cotton and stir until the cellulose is dissolved. Acidify the solution with acetic acid and note the pptn. of the cellulose in an amorphous form.

(2) *Molisch's reaction.* Suspend a bit of filter paper in 5 cc. of water and apply the Molisch test. Let the tube stand for some time if necessary.

(3) *Iodine test.* Add a drop of iodine solution to a shred of absorbent cotton. Does it differ from starch and dextrin?

(4) *Hydrolysis of cellulose.* Place a small sheet of filter paper in an Erlenmeyer flask and add 5 cc. of conc. sulfuric acid. Allow to stand for 15 minutes, shaking occasionally. Dilute with about 200 cc. of water and boil for 30 minutes, keeping the volume of water constant. Cool, neutralize a portion (using at first a small piece of *solid* KOH to prevent dilution) and test for glucose.

(5) *Nitration of cellulose.* Pour carefully 10 cc. of conc. sulfuric acid into 5 cc. of conc. nitric acid in a small beaker. Allow to stand until *cold*. Immerse in the acid mixture a small amount of absorbent cotton and stir slowly with a glass rod for *one* minute. (*Caution!* The cotton must not remain in contact with the acid longer than the specified time as a very explosive compound is formed by longer contact). Remove from the acid on the end of a glass rod and wash out all traces of the acid by stirring in several changes of water. After all the acid has been removed stir up once in alcohol, squeeze thoroughly and allow to dry. Dissolve half in the smallest amount of a mixture of 3 parts of ether and 1 of alcohol. Pour a little of the solution on the hand and allow it to evaporate. What remains?

Cautiously ignite the remaining portion and compare its inflammability with some of the original cotton.

Detection of carbohydrates in unknown mixtures.—From the preceding tests work out a scheme for the detection of carbohydrates in a mixture and apply it in the analysis of at least two "unknown" solutions. To facilitate the preparation of a scheme record the results of the tests in tabular form.

LIPINS.

(1) *Solubility.* Test the solubility of olive oil (use only a few drops for each test) in water, cold alcohol, hot alcohol, chloroform and ether.

Pour a few drops of the ethereal solution on a piece of paper and allow the ether to evaporate. Note the character of the stain which remains.

Dissolve a small amount of lard in ether in a test tube and add an equal volume of alcohol. Pour the solution into a dry watch glass and allow the solvents to evaporate spontaneously. Make a microscopic examination of the residue. Does it show a crystalline structure?

(2) *Reaction.* Test the reaction of *fresh* olive oil with litmus strips and with phenolthalein (alcoholic solution of the fat).

Repeat the tests using *rancid* olive oil. After adding the phenolthalein to the alcoholic solution of rancid oil, add, drop by drop, a *very dilute* KOH solution until a permanent pink color is obtained. Explain the result.

(3) *Emulsification.* (a) Shake a few drops of *neutral* olive oil with 5 cc. of water. A temporary emulsion is obtained; allow to stand.

(b) Repeat test (a) but use 5 cc. of water to which has been added a few drops of 0.5% sodium carbonate solution.

(c) Repeat test (b) using *rancid* oil and compare the results.

(d) Shake a few drops of oil with soap solution and with a dilute albumin solution and allow to stand. Explain why emulsions are formed.

(e) Allow a drop of neutral olive oil or melted fresh butter to fall carefully on some 0.25% sodium carbonate solution in a watch glass. The oil should spread out in a clear circular film.

Repeat using rancid oil and compare the results. Explain the difference observed.

(4) *Acrolein test.* Heat in a dry test tube a small amount of fat with twice its bulk of dry potassium bisulfate. Note the odor of acrolein. Explain the reaction and write the equation.

(5) *Sudan III test.* Dip the end of a strip of paper into olive oil and immerse the smeared end in a little Sudan III solution in a small beaker. After 10 minutes remove the paper and wash it in a little alcohol in a beaker. Compare the effect upon the smeared and unsmeared portions of the paper.

(6) *Reaction with bromine.* Dissolve a few drops of olive oil in alcohol, add a little bromine water and shake thoroughly. Is the bromine absorbed? Explain the reaction.

(7) *Hydrolysis (saponification) of fat.* (A) **Olive oil.** Dissolve 2 cc. of olive oil in 4 cc. of ether and add 10 cc. of alcoholic NaOH solution. Cork the tube and let the mixture stand until it solidifies. What is formed? Dissolve a little of the solid in water and add calcium chloride solution. What is the ppt.?

(B) **Lard.** To a horn spoonful of lard in a casserole, add 50 cc. of KOH solution and boil until no fat drops separate when a small amount is transferred to some hot water in a test tube (15-20 minutes). Indicate the equation. Note the frothiness of the soap solution. Add about 25 cc. of water and test the solution as follows:

(a) Add solid sodium chloride in small quantities to about 5 cc. of the solution. What is the ppt. that forms?

(b) To small portions in each of three test tubes add solutions of calcium chloride, copper sulfate and ferric chloride. What is the ppt. in each case?

(c) Acidify the remainder of the soap solution by adding carefully conc. HCl. What is pptd.? Filter through a wet paper. Carefully neutralize the filtrate and evaporate to a syrup on the water bath. Set aside and apply the acrolein and Dunstan tests after performing the tests on glycerol.

(8) **Glycerol.**—(a) *Solubility.* Test the solubility of glycerol in water, alcohol and ether.

(b) *Acrolein test.* Repeat test 4, page 19, using glycerol instead of fat.

(c) *Dunstan test.* Add phenolthalein solution to a 5% borax solution until a permanent pink color is produced. Add, drop by drop, just enough glycerol solution (10% or less) to discharge the color. Boil the solution. The red color returns if excess of glycerol has not been added.

(d) To 5 cc. of copper sulfate solution add a few drops of KOH. Cupric hydroxide is pptd. Add glycerol to the suspension and note its action.

(9) **Palmitic acid.**—(A) *Preparation.* Melt about 10 grams of myrtle wax by heating with 150 cc. of water in a casserole. Add 50 cc. of KOH and boil until saponification is complete, adding water from time to time to keep the volume constant. To the hot soapy mixture add conc. HCl carefully until the mixture is acid (test with litmus). Pour the liquid into a small beaker and set the beaker in cold water in a casserole. Allow to cool without stirring.

After the palmitic acid has solidified, lift out the cake and wash it several times with water to remove the adherent acid. Break into small pieces, dropping them into an Erlenmeyer flask containing about 150 cc. of alcohol. Warm on a water bath until the acid is dissolved. Filter the hot solution through a *dry* paper and allow the filtrate to cool slowly.

Write all the equations.

Filter off the crystals, wash with cold alcohol and dry between folds of filter paper. Pour the filtrate into the waste alcohol bottle. Use the dry palmitic acid in the tests given below.

(B) *Properties.* (a) Make a microscopic examination of the crystals.

(b) Dissolve a little of the palmitic acid in warm alcohol. To 5 cc. of water add a few drops of phenolthalein and 2 drops

of 0.5% sodium carbonate. To the red liquid add the alcoholic palmitic acid solution drop by drop. Why is the color discharged?

(c) Apply the acrolein and Sudan III tests to a little of the palmitic acid. Explain the results.

(d) Shake a little alcoholic solution of palmitic acid with a small quantity of bromine water. Compare test (6), page 19.

(e) Melt the remainder of the palmitic acid by warming cautiously in a small beaker. Add 10% *NaOH* drop by drop to the warm oil, stirring repeatedly and continuing the warming, until the mixture is *slightly* alkaline. Pour some of the hot liquid into a test tube and allow it to cool. What has been formed? To the remaining liquid add a little alcohol and stir thoroughly. Allow to cool in the beaker. Compare the results.

(10) **Detergent action of soap.** To two drops of a conc. soap solution in a test tube add a drop of phenolthalein. What is the reaction? Fill the tube with water and mix. Explain the result. To what is the cleansing effect of soap due?

TYPICAL SUBSTANCES IN THE BENZENE SERIES OF ORGANIC COMPOUNDS (CYCLIC OR AROMATIC SERIES).

Benzene.—Ignite a few drops of benzene in an evaporating dish and compare the flame with that of burning alcohol. Explain the difference.

Nitrobenzene.—Make a mixture of 2 cc. of conc. sulfuric acid and 1 cc. of conc. nitric acid. Add a drop or two of benzene and warm. Note the odor of the nitrobenzene which is formed. Write the equation. Why is it necessary to carry out the reaction in the presence of conc. sulfuric acid?

Aniline.—To 2 cc. of nitrobenzene in a small beaker add an equal volume of glacial acetic acid. Sprinkle in several small amounts of zinc dust. After the reaction has subsided, warm very cautiously for two minutes, add about 10 cc. of water and decant the clear liquid. Add NaOH solution until the initial ppt. redissolves. Cool. Explain all the reactions.

Shake the cold alkaline solution with 10 cc. of ether, pour off the ether into an evaporating dish and allow it to evaporate. Add about 10 cc. of water to the residue, shake and use the solution in the following tests:

(1) To some of the solution add a little bleaching powder solution. Note the color. Allow to stand, then add ammonium hydroxide solution. Note the change of color.

(2) Add a few drops of potassium dichromate solution to a second portion of the solution.

(3) Add a little bromine water to some of the solution. Tribromaniline is pptd. What is its formula?

Acetanilide.—To 5 cc. of aniline add an equal volume of acetic anhydride and heat the mixture slowly until it just begins to boil. Cool, pour into about 20 cc. of cold water and filter off the crystals which separate. Recrystallize by dis-

solving in boiling water and allowing the solution to cool. Make a microscopic examination of the recrystallized product. Write the equation.

To a small amount of the crystals in a test tube, add about 3 cc. of conc. HCl and boil for a minute. Dilute with 15 cc. of water. Has the acetanilide been decomposed?

Diazobenzene.—To about 2 cc. of aniline in a test tube add slowly about 4 cc. of conc. HCl. What is formed? Cool the mixture thoroughly by immersing the tube in cold water in a beaker. Add to the cold solution, drop by drop, about 2 cc. of 20% sodium nitrite solution. Diazobenzene chloride is formed. Write the equation.

Warm half the solution carefully and note the odor. Nitrogen is evolved. Write the equation.

To the other half of the solution add an equal volume of a solution of phenol in NaOH. Sodium hydroxyazobenzene is formed; write the equation. Filter and boil a small piece of white cotton cloth in the filtrate. Wash the cloth thoroughly in running water and then boil it for a few minutes in water. Is the compound a dye?

Phenol.—(1) To a solution of phenol add a few drops of ferric chloride solution. Acidify with dilute HCl.

(2) Add excess of bromine water to a dilute phenol solution. Tribromphenol is formed. What is its formula?

(3) To about 2 cc. of phenol solution add an equal volume of Millon reagent and warm. Note the color produced. This reaction is given by all substances containing the *oxyphenyl* (C_6H_4OH—) group.

(4) To a phenol solution add a little ammonium hydroxide and bleaching powder solution until a blue color is produced. Acidify with HCl. Is there a change of color?

Benzoic acid.—(1) Grind in a mortar a small amount of benzoic acid with four times its bulk of soda lime. Transfer to a dry test tube and heat. Note the odor. Write the equation.

(2) Add a little Millon reagent to some dry benzoic acid in a test tube and warm. Explain the result.

(3) Shake a little benzoic acid with water in a test tube and add KOH until solution is complete. Acidify with HCl. The benzoic acid is pptd. in crystalline form. Heat to boiling, then cool.

Salicylic acid.—(1) Grind in a mortar a small amount of salicylic acid with four times its bulk of soda lime. Transfer to a dry test tube and heat. Identify the substance formed by its odor. Write the equation.

(2) To a solution of salicylic acid add ferric chloride solution. Compare with the similar test on phenol.

(3) Add Millon reagent to a salicylic acid solution. Compare with test (2) on benzoic acid and explain the difference in result.

(4) To a small amount of salicylic acid in a dry test tube add about 1 cc. of methyl alcohol and an equal volume of conc. sulfuric acid. Warm carefully. Cool and pour into about 25 cc. of water in a small beaker. Note the odor. Write the equation.

COMPLEX HETERO-CYCLIC COMPOUNDS.

Uric acid (2, 6, 8, trioxypurine).—Write the structural formula for uric acid.

(1) *Solubility.* Test the solubility of uric acid in cold water, hot water, sodium carbonate solution and KOH. Use *very* small particles in each test.

(2) *Murexide test.* To some dry uric acid in an evaporating dish add 2 drops of conc. nitric acid. Evaporate on a water bath until the nitric acid is completely removed. Touch the edge of the residue with a glass rod which has been dipped in very dilute ammonium hydroxide (10 drops in a test tube of water). Bring in contact with the residue also a drop of KOH. Note the colors that develop.

(3) *Schiff's test.* Make a solution of uric acid in sodium carbonate and pour a little on a filter paper which has been moistened with silver nitrate solution. Explain the result.

(4) *Effect on Fehling's solution.* Prepare a conc. solution of uric acid by boiling a few crystals with sodium carbonate solution [keep part of the solution for tests (5) and (6)]. Dilute 1 cc. of Fehling's solution with 4 cc. of water and, after heating to boiling, add 1 cc. of the sodium urate solution a few drops at a time, boiling after each addition. Continue the boiling for several minutes. Does reduction occur?

(5) *Effect on Benedict's solution.* To 5 cc. of Benedict's solution add a little sodium urate solution and boil. Compare the result with that of test (4).

(6) *Effect on Nylander's solution.* Boil 5 cc. of sodium urate solution to which have been added 10 drops of Nylander's solution. Compare with tests (4) and (5).

Compare the results of tests (3-6) with those obtained in tests (3), (5), (6) and (8), pages 12 and 13.

(7) *Folin's test.* Dissolve a fragment of uric acid in 10 cc. of saturated sodium carbonate solution and add 10 drops of Folin's uric acid reagent. Note the color produced.

(8) Dissolve a little uric acid in half a test tube of sodium carbonate solution, add a few drops of conc. HCl and allow to stand. Make a microscopic examination of the crystals of uric acid which separate. Make drawings of the different forms observed.

COLLOIDS.

The success of the following experiments depends in a large degree upon the use of *perfectly* clean apparatus which should be rinsed with *distilled* water just before use. In the preparation of all solutions and in all dilutions use only *distilled* water.

Suspensoids.—*Colloidal Prussian blue.* To a test tube of water add a single drop of ferric chloride solution and to another test tube full add a single drop of potassium ferrocyanide solution. Mix the two solutions. Does pptn. occur? Does the solution froth when shaken?

Dilute 5 cc. of the solution with an equal volume of water, add 10 cc. of magnesium chloride solution and allow to stand. Explain the result.

Colloidal silver. To 5 cc. of 1% silver nitrate solution in a beaker add 2 drops of ammonium hydroxide and 100 cc. of water. To 100 cc. of water add 1 cc. of tannic acid solution and pour into the alkaline silver solution. A solution of colloidal silver is produced. Colloidal silver is a negatively charged colloid. Retain the solution for use in further experiments.

Into each of five clean test tubes pour 5 cc. of the colloidal silver solution. Keep the first tube for reference; to the second add 10 drops of sodium chloride solution, to the third, 10 drops of dilute HCl; to the fourth, 10 drops of calcium chloride solution; dissolve some sucrose in the fifth tube. Note the order in which coagulation occurs and explain the results.

Colloidal ferric hydroxide. To 100 cc. of boiling water in a beaker add 1 cc. of 33% ferric chloride solution. A reddish solution of colloidal ferric hydroxide results. Ferric hydroxide is a positively charged suspensoid. Retain the solution.

To 5 cc. of colloidal ferric hydroxide solution in each of 4 test tubes add, successively, 10 drops of one of the following solutions: sodium chloride, sodium sulfate, sodium carbonate and calcium chloride. Note the order in which coagulation occurs and explain the results.

Mutual precipitation of suspensoids. To 5 cc. of colloidal ferric hydroxide solution add, drop by drop, colloidal silver solution, shaking after each addition. Both substances are pptd. Why?

Repeat using colloidal arsenious sulfide solution instead of the ferric hydroxide. Explain the result.

Emulsoids.—*Gelatin.* Dissolve half a sheet of gelatin in 30 cc. of hot water in a casserole. To 5 cc. of the warm solution add an equal volume of saturated ammonium sulfate solution. Remove some of the ppt. on a rubber-tipped stirring rod and determine whether it will redissolve in hot water.

To 5 cc. colloidal ferric hydroxide solution add an equal volume of saturated sulfate solution and allow to stand. Filter off the ppt. and determine whether it will redissolve in water. Compare the solubility of the pptd. suspensoid with that of the pptd. emulsoid.

Emulsoids are reversible colloids, suspensoids are irreversible.

Formation of a hydrogel. Shake up 5 cc. of the warm gelatin solution in a test tube. Does it froth? Compare with the colloidal solution of Prussian blue. Cool the solution by immersing the tube in a beaker of cold water. The fluid hydrosol is converted into a hydrogel.

Protective action of colloids. Add 15 cc. of water to 5 cc. of the gelatin solution in a test tube. To the gelatin solution and to 20 cc. of water in another test tube add equal amounts (1 cc.) of silver nitrate solution. Shake thoroughly, then add to each tube 10 drops of dilute HCl. Shake and allow to stand. Keep the tubes until the next laboratory exercise; examine again, compare and explain the results. What is the practical bearing of the protective action of colloids?

PROTEINS.*

COLOR REACTIONS.

(1) *Biuret reaction.* To 5 cc. of dilute egg albumin solution (1 part of egg white and 10 parts of water) in a porcelain dish (or beaker resting on a sheet of white paper) add an equal volume of KOH and then, drop by drop, a very dilute solution of copper sulfate (5 drops in a test tube full of water) until a pinkish-violet color appears. Compare with test (3), page 11.

Repeat the test, using 5 cc. of "Biuret reagent", and compare the color with that produced by adding 5 cc. of the reagent to 5 cc. of water (control test). For routine work the biuret reagent should always be used in preference to the separate addition of the alkali and copper sulfate. Why?

(2) *Xanthoproteic reaction.* To a little egg albumin solution in a test tube add about a third of its volume of conc. nitric acid. Boil and note the color produced. *Cool* and pour carefully down the side of the tube ammonium hydroxide solution. Note the change in color at the junction of the two liquids. This test shows the presence of a *benzene* nucleus.

(3) *Millon's reaction.* To a little egg albumin solution add half its volume of Millon reagent and boil the mixture. Compare with test (3), page 23. What chemical group is shown to be present in the protein by this reaction?

(4) *Hopkins-Cole reaction.* To about 2 cc. of albumin solution add an equal volume of glyoxylic acid (reduced oxalic acid, formula?) and mix. Pour carefully down the side of the tube about 2 cc. of conc. sulfuric acid. Note the color of the ring formed at the junction of the two liquids. This test shows the presence of the *tryptophan* nucleus.

(5) *Molisch's reaction.* Apply the Molisch test to egg albumin solution.

(6) *Sulfur reaction.* To about 5 cc. of albumin solution add an equal volume of KOH and a single drop of lead acetate

*Not all proteins respond to all the tests given. Variations make it possible to distinguish the different classes.

solution. Boil for some time over a small flame. Why does the solution assume a brownish or black color?

(7) *Bardach's reaction.* To about 5 cc. of albumin solution add 2-3 drops of very dilute acetone sclution (3-4 drops in a test tube full of water). Add sufficient Lugol's solution to give a permanent reddish-brown color and then ammonium hydroxide until the solution is decolorized. Allow to stand until crystals appear. Make a microscopic examination of the crystals and compare with iodoform crystals (page 4).

PRECIPITATION REACTIONS OF PROTEINS.

(1) *Alcohol.* Into each of three test tubes pour about 5 cc. of alcohol. Add to one a drop of 0.2% HCl, to the second a drop of KOH and allow the third to remain neutral. Add to each about 1 cc. of albumin solution and compare the precipitating effects.

Filter *immediately* the neutral solution, transfer the ppt. to some water in a test tube, shake, filter and apply the biuret test. Is the ppt. again soluble in water?

Repeat the test, allowing the pptd. protein to remain in contact with the alcohol for *half an hour* before filtering. Has the alcohol affected the solubility of the albumin?

(2) *Strong mineral acids.* Pour 5 cc. of conc. nitric acid into a test tube and by means of a pipette allow some albumin solution to flow slowly down the side of the inclined tube. Describe the ppt. which is formed at the junction of the two liquids. (*Heller's ring test*).

(3) *Organic acids.* Add a few drops of glacial acetic acid to a little albumin solution. Is the protein pptd.?

(4) *Salts of heavy metals.* To 2-3 cc. of albumin solution add silver nitrate solution, *drop by drop*, until an excess is present. Note whether the ppt. is soluble in excess of the precipitant.

Repeat using solutions of mercuric chloride, ferric chloride, lead acetate and copper sulfate.

(5) *Alkaloidal reagents.* Acidify the albumin solution with HCl and add a few drops of phosphotungstic acid. Note the character of the ppt. Repeat the test without acidifying with HCl. Does pptn. occur?

Repeat adding solutions of picric acid, tannic acid, potassio-mercuric iodide and trichloracetic acid to acidified albumin solution.

Acidify 5 cc. of albumin solution with 2-3 drops of glacial acetic acid and add potassium ferrocyanide drop by drop. This is a very delicate test. (*Acetic acid-potassium ferrocyanide test*).

(6) *Saturation with neutral salts.* (a) Add powdered ammonium sulfate to about 10 cc. of albumin solution in a beaker until the solution is saturated. Filter and test the filtrate for protein by the biuret test (add a piece of solid NaOH before adding the biuret reagent. Why?). What has been the action of the ammonium sulfate?

(b) Saturate about 20 cc. of albumin solution with sodium chloride and filter. Test a portion of the filtrate for protein by applying the biuret test.

Acidify the remainder of the solution by adding 2-3 drops of glacial acetic acid. What occurs? Filter and test the filtrate for protein. Compare the action of ammonium sulfate and sodium chloride.

(c) Repeat test (b), using magnesium sulfate instead of sodium chloride, and compare the results with those obtained in tests (a) and (b).

COAGULATION OF PROTEINS.

(1) *Heat coagulation.* Heat about 10 cc. of albumin solution to boiling in a test tube, noting carefully what occurs. Divide the solution into two portions, acidify one portion by adding a drop of dilute acetic acid and boil again. Compare with the unacidified portion.

Filter off the coagulum and determine whether it is soluble in water.

Heat in boiling water for several minutes a dry test tube containing a small amount of *dry* egg albumin. Remove the tube and determine whether the albumin is soluble in water. Explain the result.

(2) *Coagulation temperature. Very cautiously neutralize* to litmus 25 cc. of albumin solution, using very dilute acetic acid (5 drops of 10% acid in a test tube full of water). Heat the solution at 40° for 10 minutes and filter through a wet fluted paper. Pour 5 cc. of the *neutral* solution into each of five test tubes and treat as follows:

(a) 5 cc. of solution.
(b) " " +1 drop of 10%HCl.
(c) " " +1 drop of 10% acetic acid.
(d) " " +1 drop of 10% sodium carbonate.
(e) " " +2 drops of 10% sodium chloride.

Fasten the tubes together by means of a rubber band and suspend them by a clamp attached to one of the tubes in such a way that the ends of the tubes are about two inches above the bottom of a beaker two-thirds full of cold water. Place a thermometer in the clamped tube and heat the water in the beaker with a *small* flame, stirring constantly. Watch the tubes closely and record the time at which the faintest turbidity is detected. What is the order in which the solutions coagulate? Gradually raise the temperature to the boiling point and compare the appearance of the solutions. Draw conclusions from the results.

HYDROLYSIS OF PROTEIN.

To 25 cc. of conc. HCl in an Erlenmeyer flask add a piece of sheet gelatin about two inches square. Cover the mouth of the flask with a small watch glass and keep the mixture just boiling. At five minute intervals remove about 1 cc., neutralize with KOH and apply the biuret test. Record the time when the test gives negative results. What has occurred? When the mixture no longer gives the biuret test, dilute 1 cc. to 10 cc. with water and carefully neutralize the solution to phenolthalein with KOH. Apply the following test:

Ninhydrin test. To 10 cc. of the *neutral* solution add 5 drops of a 0.1% solution of ninhydrin (triketo-hydrindene-hydrate). Boil for one minute. Allow to stand and note the color ultimately developed. Ninhydrin reacts with substances containing an amino group in the alpha position. What substances having this structure are present in the hydrolysed protein solution?

SIMPLE PROTEINS.

Albumins. Egg albumin.—(1) Determine the solubility of powdered egg albumin in water, 0.2% HCl, 0.5% sodium carbonate and 10% sodium chloride.

(2) Place a small amount of egg albumin in a dry test tube, suspend in the tube a piece of wet red litmus paper and lay across the top of the tube a piece of filter paper moistened with lead acetate solution. Heat carefully and explain all the phenomena observed.

The reactions of albumin solution have been fully studied in the preceding tests.

Globulins. Edestin.—Grind in a mortar 3 horn spoonfuls of ground hemp seed with a little sand and enough 5% sodium chloride to yield a pasty mass. Transfer to a beaker containing about 150 cc. of 5% sodium chloride solution which has been heated to 60-65°. Place the beaker in a larger beaker containing water at 65° and maintain the temperature as near as possible to 60° for half an hour. Filter the *hot* solution through a paper which has been moistened with warm salt solution. Heat the filtrate again carefully to 60°, add an equal volume of warm water (60°) and about 1 cc. of alcoholic thymol solution (preservative). Cover the beaker with a watch glass and set aside in warm water so that the extract may cool slowly until the next laboratory exercise.

Pour off most of the supernatant liquid and set it aside for use later. Make a microscopic examination of the sediment. Filter the remaining mixture, flush the ppt. from the paper into a beaker, add about 300 cc. of water and stir thoroughly. Allow to settle, decant the supernatant wash

water and filter off the solid matter. Scrape the ppt. off the paper with a spatula and dry on a watch glass at room temperature.

(1) Determine the solubility of the dried edestin (powdered) in the usual solvents (page 32).

(2) Apply the biuret, xanthoproteic, Millon and Hopkins-Cole tests to the edestin solution.

(3) Heat some of the solution to boiling. Is the globulin coagulated?

(4) Saturate some of the solution with sodium chloride. Compare with test (6, b), page 30.

(5) Pour 10 cc. of the solution into about 20-30 volumes of water. Explain the result.

CONJUGATED PROTEINS.

Nucleoprotein.—Grind thoroughly in a mortar with sand a cake of compressed yeast, adding also 5 cc. of ether and 10 cc. of water. Flush the mixture into a bottle with 0.4% NaOH solution and add enough of the NaOH to make the total volume about 100 cc. Add about 5 cc. of toluol. Shake the mixture frequently and leave the bottle on the top of the table so that it can be shaken occasionally during a period of at least 24 hours. Pour off carefully the clear supernatant liquid without disturbing the slimy yeast mass. Filter if necessary.

Precipitate the nucleoprotein by adding 10%HCl, *drop by drop*, until there is complete separation of flakes in a "*water clear*" liquid. Carefully avoid adding excess of acid. Allow to settle, pour off as much of the supernatant liquid as possible and filter the remainder through a wet fluted paper.

(1) Determine the solubility of the moist nucleoprotein in the usual solvents (page 32).

(2) Apply the xanthoproteic and Millon tests to small portions of the ppt.

(3) Test a portion of the ppt. for phosphorus by heating with fusion mixture and applying the usual test.

(4) Transfer the remainder of the nucleoprotein to an Erlenmeyer flask, add about 25 cc. of 5% sulfuric acid and boil for about an hour, adding water from time to time to keep the volume constant. Cool and neutralize with ammonium hydroxide.

(a) Apply Benedict's test to a portion of the liquid.

(b) Apply the test for phosphate.

(c) Render the remaining liquid alkaline with ammonium hydroxide, filter if necessary and add 10 cc. of ammoniacal silver nitrate solution. Allow to stand. A brown flocculent ppt. of silver compounds of the purine bases should separate.

Outline in tabular form the decomposition products of nucleoprotein.

DERIVED PROTEINS.

Metaproteins.—Acid metaprotein has been prepared by heating egg white for some time with 10 volumes of 0.2% HCl. Neutralize about 25 cc. of the solution with dilute KOH (5 cc. in a test tube full of water) after adding a few drops of litmus solution. Filter and wash the ppt. with water.

(1) Determine the solubility of the moist metaprotein in the usual solvents (page 32).

(2) Dissolve a portion in 0.2% HCl and heat to boiling. Is the protein coagulated? Cool and add carefully 0.5% sodium carbonate solution until a ppt. appears. Is the ppt. soluble in excess of alkali?

(3) Suspend a little metaprotein in water and boil the suspension. Add 0.2% HCl. Does the ppt. dissolve? Compare with test (2) and explain what has occurred.

(4) Dissolve a portion in 0.5% sodium carbonate solution and heat to boiling. Does coagulation occur? Cool and add 0.2% HCl until a ppt. appears. Is the ppt. soluble in excess of acid? Compare with test (2).

Draw conclusions from the results of tests (2-4).

Proteoses and peptones.—Dilute a 5% solution of Witte's peptone (mixture of proteoses and peptones) with four volumes of water for the following tests:

(1) Apply the biuret, Millon, xanthoproteic and Hopkins-Cole tests to the solution. Note particularly the color produced in the biuret test.

(2) Heat some of the solution to boiling and add a drop of acetic acid. Does coagulation occur?

(3) Add a few drops of conc. nitric acid. Heat to boiling, then cool. What occurs?

(4) Add a drop of glacial acetic acid and a few drops of potassium ferrocyanide solution. Heat to boiling and cool.

(5) Is the protein pptd. by solutions of copper sulfate, picric acid and tannic acid?

Separation of proteoses and peptones. To about 20 cc. of 5% Witte's peptone solution add an equal volume of saturated ammonium sulfate solution. **Primary proteoses** are pptd. Stir vigorously with a rubber-tipped glass rod. Collect as much of the sticky mass as possible on the glass rod, dissolve it in about 5 cc. of hot water, cool and apply tests (3) and (4) to the solution.

Filter the solution from which the primary proteoses have been removed. Add a drop of dilute sulfuric acid and saturate the solution with ammonium sulfate (powder finely in a mortar), stirring until an excess of salt is present. **Secondary proteoses** are pptd. Collect the ppt. on a glass rod or filter if necessary, dissolve in hot water, cool and repeat tests (3) and (4). Compare with the results on the primary proteose solution.

Peptone is present in the solution from which the proteoses have been removed. Filter some of the solution. To 5 cc. add a small piece of solid NaOH (why?) and apply the biuret test.

Detection of proteins in unknown mixtures.—From the preceding tests work out a scheme for the detection of metaproteins, albumins, globulins, proteoses and peptone in a mixture and apply it in the analysis of at least two unknown solutions. To facilitate the preparation of a scheme record the results of the tests in tabular form.

ACTION OF ENZYMES.

AMYLASES.

Salivary amylase (ptyalin).—To about 15 cc. of starch paste in a test tube add a few drops of saliva. At intervals of a minute remove a few drops of the solution on a glass rod to a microscope slide, resting on a piece of white paper. Add a drop of iodine solution to the drop on the slide. When the iodine test becomes negative, test the solution by the Benedict test. Explain what has occurred and compare with experiment (4), page 15.

PROTEASES.

Gastric protease (pepsin).—To about 5 cc. of a 0.2% HCl solution of pepsin add a single small shred of fibrin and immerse the tube in water at 40°. Examine from time to time, noting the changes in the appearance of the fibrin.

Pancreatic protease (trypsin).—Into each of four tubes pour 10 cc. of a faintly alkaline solution of casein. Heat to 40° in warm water. Add pancreatic enzyme solution to the tubes as follows: to the first tube, 2 drops; to the second, 0.5cc.; to the third, 1 cc., and to the fourth, 2 cc. Heat at 40° for 15 minutes. Add to each tube a few drops of 50% alcohol containing 1% acetic acid. If digestion is complete no ppt. will appear. How does the concentration of the enzyme affect the results?

LIPASES.

Pancreatic lipase (steapsin).—Into each of three test tubes pour about 5 cc. of a specially prepared emulsion of olive oil (commercial olive oil, neutralized to phenolthalein with NaOH; explain why an emulsion is formed) and to each tube add a drop (or two if necessary) of 0.5% sodium carbonate to give a decided pink color to the shaken mixture. To the first tube add 2 cc. of water (control); to the second, 2 cc. of enzyme solution; to the third, 2 cc. of enzyme solution which has been boiled. Shake thoroughly, place in the test tube rack and, after half an hour, explain any changes which have occurred.

INVERTASES.

Sucrase (from yeast).—Into each of two test tubes pour about 5 cc. of 1% sucrose solution. To one add 1 cc. of invertase solution and to the other 1 cc. of *boiled* invertase solution. To 5 cc. of starch paste in a third tube add 1 cc. of the invertase solution. Allow to stand at room temperature for half an hour. Test each solution for reducing sugar by applying Benedict's test. Explain the results.

ZYMASES.

Zymase (from yeast).—Review experiment (12), page 13.

OXIDASES.

Oxygenase.—Add 10 drops of tincture of guaiacum to a test tube full of water. To 5 cc. of the emulsion add a small cube of potato. The color which develops on standing is due to the presence of a direct oxidizing enzyme in the potato.

Peroxidase.—To 5 cc. of the guaiacum emulsion add a small piece of fresh meat. No blue color develops (absence of oxygenase). Add about 1 cc. of hydrogen peroxide, shake and note the color on standing. Peroxidase is an indirect oxidizing enzyme which acts only in the presence of peroxides.

Repeat the test, using a piece of meat which has been boiled for a few minutes. Explain the result.

Catalase.—Drop a small amount of pulped liver tissue into 10 cc. of water in a test tube and add hydrogen peroxide. Oxygen is liberated from the peroxide through the action of the enzyme, catalase.

AMINASES.

Urease.—Measure out with a pipette 5 cc. of a 2% solution of urea and run it into an Erlenmeyer flask. Add 5 cc. of water and 2 cc. of urease solution, carefully shake the mixture and cover the flask with a small watch glass. Allow the mixture to stand for 30 minutes. Titrate with tenth normal HCl, with methyl red (1 drop) as an indicator. Calculate the weight of urea that has been hydrolysed. Write the equation representing the hydrolysis of urea.

Obtain some urea solution containing an unknown amount of urea and determine the quantity present.

MILK.

(1) Compare the microscopic appearance of whole milk and skimmed milk.

(2) Test the reaction of fresh milk with wet blue and red litmus strips.

(3) Boil about 10 cc. of milk in a beaker. A film forms. Remove the film and apply to it the Millon test. Boil again. Does a film form the second time?

(4) Add a few drops of KOH to a little milk in a test tube and heat. Explain the development of color.

(5) Add 3 drops of milk and a few drops of tincture of guaiacum to 5 cc. of water in a test tube. Mix thoroughly, allow to stand, and compare the color with that of 3 drops of milk in 5 cc. of water. To what is the development of color due?

Repeat using *boiled* milk and compare the results.

(6) Set aside a test tube full of milk and, after it has stood for several days, note any changes in the reaction, appearance and consistence of the milk. Explain the changes observed.

Separation of the constituents of milk.

Caseinogen.—Dilute 50 cc. of milk with three volumes of water. Warm to about 40°. Add dilute acetic acid, drop by drop, until a flocculent ppt. forms and the surrounding liquid is clear. Avoid excess of acid. The fat is mechanically carried down by the pptd. caseinogen. Allow to settle, decant the supernatant liquid and filter the remaining mixture. *Keep* the solution from which the caseinogen and fat have been removed.

Wash the ppt. on the paper once or twice with water. Remove the excess of moisture by pressing between folds of filter paper. Transfer the ppt. to a small dry beaker and cover it with about 25 cc. of alcohol. Stir for a few minutes. Filter again (pour the filtrate into the waste alcohol bottle). Return the ppt. to the beaker, add about 25 cc. of ether and heat for

10 minutes (Caution! Avoid flames) in water at 35°, stirring continuously. Filter into a dry beaker and set aside the filtrate in the locker for spontaneous evaporation of the ether. Transfer the ppt. to an evaporating dish and, after the ether has evaporated, powder the caseinogen in a mortar. Use the powder in the following tests:

(1) Determine the solubility of caseinogen in water, 0.2% HCl, 10% HCl, 0.5% sodium carbonate, and 10% sodium chloride.

(2) Apply the biuret, Millon, xanthoproteic and Hopkins-Cole tests to small portions of the powder.

(3) The presence of sulfur and phosphorus has been previously shown (page 2).

(4) Rub up a little of the powder with 10 cc. of 0.5% sodium carbonate in a mortar. Soluble sodium caseinogenate is formed. Saturate the solution with magnesium sulfate. Is the protein pptd.? Compare with test (6, c), page 30.

(5) The action of rennin on caseinogen will be shown on page 51.

Fat.—Notice the residue of fat which remains in the beaker after the evaporation of the ether. Mix with it a little dry potassium bisulfate, transfer to a dry test tube and heat (acrolein test, page 19).

Lactalbumin and lactoglobulin.—Heat the solution remaining after the removal of the caseinogen to boiling in a casserole and add 2% sodium carbonate solution, drop by drop, until the coagulum separates in flaky form. Do not allow the solution to become alkaline (why?); if this happens, acidify slightly with acetic acid. Evaporate the solution to half its original volume. Filter. Apply Millon's test to the ppt.

Lactose.—To portions of the filtrate apply the Molisch, Benedict and Barfoed tests.

Evaporate about 20 cc. of the liquid with 10 cc. of conc. nitric acid in an evaporating dish on a water bath until the volume has been reduced to 6-8 cc. Transfer to a test tube,

washing out the evaporating dish with a little water. Allow to stand. Note the character of the ppt. of **mucic acid** which should separate. Explain the reaction by which it has been formed. Will any other carbohydrate give this reaction?

Inorganic salts.—Test portions of the filtrate for the presence of chloride, phosphate, sulfate and calcium.

To a portion of the liquid add ammonium hydroxide until excess is present and heat. What is the ppt. which forms?

BUTTER.

Dissolve a small amount of butter in about 10 cc. of alcohol in an Erlenmeyer flask, add 5 cc. of water and about 5 grams of solid KOH. Heat gently on a water bath for about 15 minutes. Pour the mixture into an evaporating dish, add about 100 cc. of water and boil until the odor of alcohol can no longer be detected. Cool and acidify the cold soapy mixture *under a hood* with dilute sulfuric acid. Note the odor of the volatile fatty acids.

Allow to cool and note whether the fatty acids are solid or oily. Dissolve some of the fatty acids in alcohol and shake the solution with a little bromine water. Explain the result. Draw conclusions regarding the constitution of the fats present in butter.

EGGS.

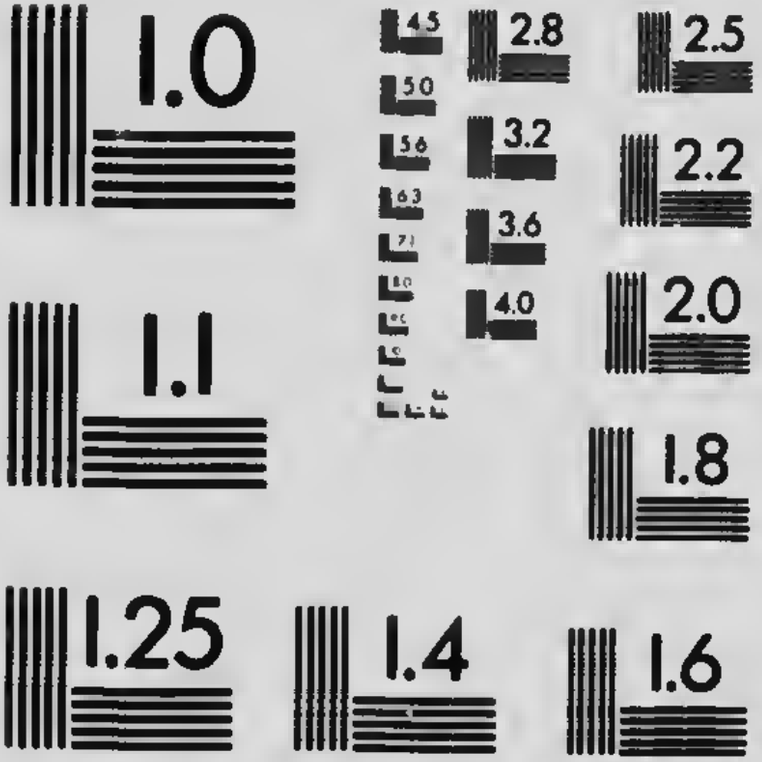
Egg albumin.—The properties of egg albumin have been studied fully on pages 28-31.

Ovomucoid.—Carefully separate the white from the yolk of an egg. Transfer the yolk to a glass-stoppered bottle, add 100 cc. of alcohol, shake vigorously and set the bottle aside in the locker. The yolk will be used in later experiments.

Beat the white with a horn spoon to tear the membranes and pour it slowly in a narrow stream into 100 cc. of boiling water in a casserole. Boil the mixture slowly, stirring constantly to prevent burning, and add to the boiling liquid acetic acid, drop by drop, until the coagulated albumins and globulins separate in flocculent form in a yellowish, water clear liquid.

MICROCOPY RESOLUTION TEST CHART

(ANSI and ISO TEST CHART No. 2)



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Filter; if sufficient acid has been added the liquid will filter rapidly, otherwise it will appear milky. Test the filtrate, which contains ovomucoid, as follows:

(1) Pour half of the solution into an Erlenmeyer flask, add an equal volume of dilute HCl and boil for at least an hour. Fifter if necessary. Neutralize (adding at first a small piece of solid NaOH to prevent dilution). To the neutral solution apply the Molisch and Benedict tests and the test for sulfate. Compare with tests (3) and (4). Explain the changes which have taken place?

(2) Apply the biuret and Millon tests to small amounts of the solution.

(3) Apply the Molisch and Benedict tests.

(4) Test for the presence of sulfate.

(5) Is the protein pptd. by mercuric chloride solution? by tannic acid solution?

Separation of the lipins in egg yolk.—Pour into the glass-stoppered bottle containing the egg yolk in alcohol about 50 cc. of ether (avoid flames!) and shake the mixture at intervals for about 15 minutes. Filter into a casserole through a fluted filter paper which has been moistened with alcohol. Transfer the ppt. to an evaporating dish (return the filter to the funnel), add about 25 cc. of alcohol and heat to boiling on the *steam* bath for about 5 minutes. Cool and filter through the original paper into the casserole containing the first extract. Evaporate the alcohol-ether extract to dryness on the *steam* bath.

The egg yolk residue consists chiefly of **vitellin**, a phosphoprotein. It has been rendered largely insoluble (denatured) by long contact with alcohol. Heat a fragment with KOH solution and apply the biuret test.

Lecithins.—Cool the casserole and to the residue, from which the alcohol and ether have been removed, add about 10 cc. of ether. Stir until solution is complete and transfer the clear solution to a dry beaker, washing the casserole with a very small amount of ether. Add three volumes of acetone.

The lecithin is pptd. in buttery particles which adhere to the stirring rod and sides of the beaker. Filter through a dry paper into a casserole, retaining the lecithin particles in the beaker. Evaporate the filtrate to dryness on the *steam* bath.

Determine the chemical constitution of lecithin by applying the following tests:

(1) To a small particle apply the acrolein test.

(2) Place about a third of the lecithin in an evaporating dish, add about 25 cc. of alcoholic NaOH solution and evaporate slowly to dryness on the *water* bath. Dissolve the residue in about 25 cc. of water and identify the substance present by the following reactions:

(a) Does the residue yield a frothy solution with water?

(b) What is the nature of the ppt. formed on the addition of calcium chloride solution?

(c) Saturate the remainder of the solution with solid sodium chloride. What is the ppt.? Compare test (B, a), page 19.

(3) Apply to a portion of the lecithin the test for organic phosphorus.

(4) Boil the remainder of the lecithin with 10% KOH. Note the fishy odor of the trimethylamine (formula?) liberated by the decomposition of the choline.

Indicate the formula for a typical lecithin containing (1) a glyceryl radicle; (2) two fatty acid radicles attached to the glyceryl radicle; (3) one phosphoric acid radical united with the glyceryl radicle, and (4) a choline (trimethylhydroxyethyl ammonium hydroxide) radicle combined with the phosphoric acid radicle.

Fat.—Determine whether the residue remaining after the evaporation of the acetone-ether mixture contains fat by applying the acrolein and Sudan III (page 19) tests.

To the residue in the casserole add about 50 cc. of alcoholic NaOH solution and evaporate slowly to dryness on the water bath. Cool the casserole and extract the dry residue

with a little ether, stirring thoroughly with a glass rod. Filter through a small *dry* paper into a dry beaker. Extract the residue with a second portion of ether, adding the second ether filtrate to the first. Set the beaker aside, uncovered, in the locker for the spontaneous evaporation of the ether.

Identify the substance present in the residue by adding water and applying tests (2, a, b and c) on the preceding page.

Cholesterol.—A crystalline residue of cholesterol remains after the evaporation of the ether. The color is due to the presence of **lipochrome,** the yellow coloring matter in egg yolk, which has not been particularly modified by the saponification process.

Dissolve a little of the cholesterol in a very small amount of hot alcohol, allow to cool and make a microscopic examination of the recrystallized product. Draw the crystals.

Examine under the microscope crystals of cholesterol which have been treated with iodine in the presence of conc. sulfuric acid.

Salkowski test. Dissolve some cholesterol in about 3 cc. of chloroform in a *dry* test tube, add an equal volume of conc. sulfuric acid and mix by pouring from one tube to another repeatedly. The chloroform, stratifying on top, is colored red and the acid layer shows a greenish fluorescence.

Liebermann-Burchard test. Dissolve a small amount of cholesterol in 2 cc. of chloroform in a *dry* test tube, add 10 drops of acetic anhydride and 2-3 drops of conc. sulfuric acid and shake. Note the succession of colors produced.

WHEAT FLOUR.

Gluten.—To a casserole half full of wheat flour add (a little at a time) water to make a *stiff* dough. Allow to stand for 15 minutes. Pour about 150 cc. of water into the casserole and knead the mass thoroughly. Pour off the liquid and note the sediment of starch on standing. Filter some of the liquid and test the filtrate for the presence of protein, carbohydrate,

chloride and phosphate. Make a microscopic examination of the starch grains and compare with potato starch (page 14).

Continue to knead the sticky gluten mass in frequent changes of water until no more starch is liberated and the wash water does not have a milky appearance. The yellowish gluten mass which remains consists of two proteins, gliadin and glutelin. Tear the gluten into *very* small pieces and drop them into a flask containing a mixture of 80 cc. of alcohol and 20 cc. of water. Leave the flask on the top of the desk so that it can be shaken frequently.

Gliadin.—Long-continued extraction with 70% alcohol has dissolved the gliadin. Filter and evaporate the filtrate to dryness on the water bath. Pulverize the gliadin and use it in the following tests:

Determine the solubility of the gliadin powder in water, 0.2% HCl, 0.5% sodium carbonate, and 50% and 95% alcohol.

Apply the biuret, Millon and xanthoproteic tests to small portions of the powder.

Glutelin.—The residue after the 70% alcoholic extraction is glutelin.

Determine the solubility of the glutelin in water, 0.2% HCl, 0.4% KOH and 10% sodium chloride.

Apply the Millon test to a fragment of the glutelin.

Shake up several pieces of glutelin with 0.4% KOH in a test tube. Pour off the extract and add 0.2% HCl, drop by drop. Is the protein pptd.? Does it dissolve in excess of acid?

BREAD.

Separate carefully the crust from the crumb of a piece of bread, pound each separately in a mortar with a little water, and allow to extract in beakers for 15 minutes. Filter and test each extract with the iodine and Benedict tests. Which portion contains the more reducing sugar? Determine whether the extracts contain protein and explain the results of the tests.

POTATO.

Scrape the surface of a potato with a knife, collecting the scrapings in a beaker. Add 15-20 cc. of water, stir and strain through a piece of cheese cloth on a funnel. Note the residue of insoluble cellulose shreds. Allow the filtrate to stand until the starch has sedimented. Pour off the supernatant liquid and apply to it the Millon and Benedict tests. Note that the liquid quickly assumes a brownish color, brought about by oxidations in the potato extract due to the presence of *oxidases* (page 37).

Wash the starch by stirring several times with water, allowing it to settle each time before pouring off the water. Heat a little of the purified starch with 10 cc. of water, cool and apply the iodine and Benedict tests.

Upon what does the food value of potato largely depend?

NUTS.

To a small amount of nut meal in a dry test tube add about 5 cc. of ether and shake. Pour off the ether into a dry beaker and repeat the extraction with a second portion of ether. Allow the ether to evaporate and note the residue. Apply to it the Sudan III test.

Grind up the fat-free meal in a mortar with about 15 cc. of 10% sodium chloride solution. Filter, divide the filtrate into three portions and test as follows:

(1) Pour one portion into about 100 cc. of water to which has been added a drop of dilute acetic acid.

(2) Saturate a second portion with solid sodium chloride. Compare test (4), page 33.

(3) Slightly acidify the third portion with acetic acid and boil. Apply Millon's test to the coagulum.

To what class of proteins does the substance belong?

MEATS.

Meat will be studied as muscle tissue (page 63).

THE CHEMISTRY OF DIGESTION.

SALIVARY DIGESTION.

Examination of saliva.—Stimulate the flow of saliva by chewing a piece of soft paraffin and collect the saliva in a clean beaker.

(1) Make a careful microscopic examination of a drop of unfiltered saliva and describe what is seen.

(2) Determine with wet red and blue litmus strips the reaction of saliva.

To about 3 cc. of saliva on a watch glass add 1 drop of phenolthalein solution and then 0.1% sodium carbonate solution, drop by drop. Explain the result. Is it correct to state simply that saliva is acid or alkaline in reaction?

(3) Apply to *filtered* saliva (filter through a wet fluted filter paper) the biuret and heat coagulation tests.

(4) Apply to filtered saliva the Molisch Benedict tests and explain the results.

(5) Test for the presence of chloride, phosphate, sulfate and calcium.

(6) To about 3 cc. of saliva in a porcelain crucible add 2 drops of dilute HCl and a few drops of ferric chloride solution. A reddish coloration indicates the presence of **sulfocyanate** in the saliva. Add a drop of mercuric chloride solution. Why does the color disappear?

(7) To about 3 cc. of saliva in a crucible add 2 drops of 10% sulfuric acid, 2 drops of dilute potassium iodide solution, and a drop of starch paste. If **nitrite** is present, nitrous acid is formed, liberating iodine from the potassium iodide which gives *blue* starch iodide.

(8) To a small amount of filtered saliva add a few drops of dilute acetic acid. Note the pptn. of **mucin.** Filter and test the filtrate with Millon's reagent. Is protein present?

Collect about 25 cc. of filtered saliva and pour it into 100 cc. of alcohol. Mucin is pptd. Stir thoroughly and allow

the mixture to stand for at least 24 hours. Pour off most of the supernatant alcohol (transfer to the waste alcohol bottle) and filter the remaining mixture through a small dry paper.

(a) Dissolve a small portion of the ppt. in KOH and apply the biuret test.

(b) Suspend a little in water and apply the Molisch test.

(c) Boil the remainder of the mucin in a flask with about 20 cc. of 10% HCl. Add water from time to time to keep the volume constant and boil until the liquid assumes a brown color. Cool, make slightly alkaline with solid (why?) NaOH and apply the Benedict test. Compare with test (1), page 41, and test (4), page 34.

Examination of salivary amylase, ptyalin.—Prepare dilute saliva by rinsing out the mouth thoroughly (1-2 minutes) with several 20 cc. portions of warm water (40°), collecting the washings in a clean beaker. Filter.

The activity of ptyalin. Prepare a series of test tubes each containing about 1 cc. of very dilute iodine solution (a few drops in a test tube full of water).

Measure 5 cc. of 1% starch paste into a test tube, add 5 drops of distilled water and place in water at 40°. Warm also 5 cc. of dilute saliva and when the mixtures have reached the same temperature pour them together and record the time. At frequent intervals remove a little of the digestive mixture with a pipette and drop it into one of the test tubes containing the iodine solution. As digestion proceeds the starch is successively converted into soluble starch, erythrodextrin, achroo-dextrin, malto-dextrin and maltose, and the blue color with iodine becomes violet, reddish-violet and red, and disappears when the erythrodextrin is converted into achroo-dextrin. Record the time at which the "achromic point" is reached, that is, the time when no color is produced with iodine. The activity of the ptyalin is measured by the time taken to reach the achromic point. Compare your results with those obtained by others.

Repeat the experiment, using 5 drops of 10% sodium chloride solution instead of distilled water. Is there any difference in the time required to reach the achromic point?

Influence of temperature. Into each of 4 test tubes pour 5 cc. of starch paste. Immerse one in cold water, keep the second at room temperature and place the third in water at 40°. When the desired temperatures have been reached, pour into each tube 5 cc. of diluted saliva. To the fourth tube add 5 cc. of *boiled* saliva and keep it at room temperature. Determine the order in which the achromic point is reached. Test the contents of the fourth tube for reducing sugar and explain the result.

Influence of reaction. Into each of 5 test tubes pour 5 cc. of starch paste and add to the tubes, successively, 1, 2, 3, 4 and 5 drops of 0.4% HCl. Add to each tube 5 cc. of dilute saliva and determine the effect of the acid on the activity of the ptyalin by noting the time required to reach the achromic point.

Repeat the experiment, using 4 tubes of starch paste containing, successively, 5 drops of 0.4% acetic acid, 5 drops of 0.4% combined HCl, 5 drops of 0.4% KOH and 5 drops of 0.5% sodium carbonate. Slightly acidify the alkaline mixtures before applying the iodine test (why?).

Draw conclusions from the results obtained.

Influence of dilution. To 9 cc. of thin starch paste in a test tube add exactly 1 cc. of undiluted saliva. Shake thoroughly and transfer 1 cc. of the mixture to a second tube containing 9 cc. of starch paste (dilution of saliva, 1 to 100). After shaking, transfer 1 cc. of the mixture in the second tube to 9 cc. of starch in a third tube. Continue the process until a dilution of saliva of 1 part in 1,000,000 (6 tubes) has been reached. After standing for 20-30 minutes, test the contents of each tube by applying the iodine test. In how great dilution is the saliva still active? Compare your results with those obtained by others.

Effect of acid on ptyalin. To about 5 cc. of saliva in a beaker add an equal volume of 0.2% HCl and allow the mixture

to stand for 15 minutes. Make *slightly* alkaline with 0.5% sodium carbonate, add a few cc. of starch paste and heat at 40° for 15 minutes. Test with iodine. Does the acid merely inhibit salivary digestion or destroy the ptyalin?

Specificity of action. Heat about 5 cc. of a solution of pure cane sugar with an equal volume of dilute saliva at 40° for 30 minutes. Test for reducing sugar by applying the Benedict test. Does ptyalin digest sucrose?

Digestion of raw starch. Into each of two test tubes place a small amount of dry raw starch. To one add about 5 cc. of water (control) and to the other 5 cc. of dilute saliva. Shake and allow to stand for half an hour, shaking occasionally. Filter and test each filtrate by Benedict's test. Explain the results.

Excretion of a foreign substance.—Take a 0.2 gram dose of potassium iodide in a gelatin capsule and immediately drink about 50 cc. of water. Record the exact time of dosage. Flush the mouth thoroughly. Test immediately for the presence of iodide in the saliva by allowing a few drops to fall directly from the mouth into a crucible containing 1 cc. of sodium nitrite solution, 1 cc. of 10% sulfuric acid and a few drops of thin starch paste. Repeat the test at two-minute intervals until a positive result is obtained (production of a blue color). The test solution should be renewed occasionally. Record the exact time at which the iodide appears in the saliva. The urine may also be tested. Compare test (7), page 46.

GASTRIC DIGESTION.

Action of gastric protease, pepsin.

Conditions essential for peptic action. Prepare five tubes containing the following mixtures:

Tubes:	1	2	3	4	
Water	5 cc.	5 cc.	. .	. .	. .
Pepsin (0.2%)	5 cc.	. .	5 cc.	5 cc.	5 cc. (boiled)
HCl (0.4%)	. .	5 cc.	5 cc.	. .	5 cc.
Na_2CO_3 (0.5%) . . .	. .	. .	. .	5 cc.	. .

To each tube add a single small shred of fibrin and heat in water at 40° for at least 30 minutes. Note carefully the changes in the appearance of the fibrin in each tube. Draw conclusions from the results.

Detection of pepsin. Pour about 5 cc. of pepsin-HCl solution into each of two test tubes. Boil one of the solutions thoroughly, then cool. Add to each tube a few pieces of carmine fibrin and incubate at 40° for 15 minutes. Shake and compare the color of the solutions.

Influence of temperature. Prepare three test tubes each containing about 5 cc. of pepsin-HCl solution. Immerse one tube in cold water, keep the second at room temperature and incubate the third at 40°. When the desired temperatures have been reached, drop a shred of fibrin into each tube. Note from time to time the progress of digestion. Compare with the similar experiment on ptyalin (page 48). Is the influence of temperature more pronounced in one case than in the other?

Influence of the concentration of HCl. Arrange on a test tube stand three pairs of test tubes, (a), (b) and (c). Add to each pair 5 cc. of HCl in the following concentrations: (a), 4%; (b), 0.4%; (c), 0.04%. To one tube of each pair add 5 cc. of pepsin solution and to the other 5 cc. of water (control series). Place a fibrin shred in each tube, incubate at 40° for 30 minutes and compare the digestive effects. Note also the effect of acid concentration on the swelling of fibrin.

Digestion in the presence of various acids. Prepare six test tubes each containing 5 cc. of one of the following acids: (each 0.1 *N*) HCl, sulfuric, phosphoric, acetic, lactic and oxalic. To each tube add 5 cc. of pepsin solution and a shred of fibrin. After incubating for 30 minutes compare the digestive effects. Is the fibrin swollen in tubes in which there has been no digestion?

Action of alkali on pepsin. To 5 cc. of pepsin solution add an equal volume of 0.5% sodium carbonate solution and heat the mixture at 40° for 30 minutes. Slightly acidify by adding 10% HCl, drop by drop, then add an equal volume of 0.4% HCl

and a shred of fibrin and heat at 40°. Has the pepsin been destroyed by the alkali?

Pepsinogen (zymogen of pepsin). Into eacl of three test tubes pour about 2 cc. of a glycerol extract of pig's stomach. To the first tube add 2 cc. of water; to the second, 2 cc. of 0.4% HCl, and to the third, 2 cc. of 0.5% sodium carbonate solution. To each add a shred of fibrin and incubate for 15 minutes. In which tubes has digestion occurred and why?

To the third tube add about 6 cc. of 0.4% HCl and incubate again. Is the fibrin now digested? Indicate how to distinguish between pepsin and pepsinogen.

Action of gastric rennin.—Prepare four test tubes each containing 10 drops of rennin solution. Add to each tube, successively, 10 drops of one of the following solutions: (1) water; (2) 0.4% HCl; (3) ammonium oxalate; (4) 0.5% sodium carbonate. In a fifth tube place 10 drops each of water and 0.4% HCl. Add to each tube about 5 cc. of milk, shake thoroughly and heat at 40° for 15 minutes. Do not shake the tubes after putting them in the warm water. Compare and explain the results.

To tube (3) add a little calcium chloride solution. What is pptd.?

Action of gastric juice (artificial) on cane sugar.—Into each of three test tubes pour 5 cc. of cane sugar solution. Add to the first, 5 cc. of 0.4% HCl; to the second, 5 cc. of 0.4% HCl-0.2% pepsin solution (artificial gastric juice); add to the third, 5 cc. of the same solution after boiling it thoroughly. Heat the tubes at 40° for half an hour. After approximate neutralization, add an equal volume of Benedict solution to each solution and boil about 5 cc. of each mixture. Determine by observing the different grades of reduction the relative degrees of inversion of the cane sugar which have occurred in the mixtures. To what is the inversion of the sucrose due?

The acidity of the gastric juice.—Determine the reactions of the solutions given below to the following indicators:

(a) Indicators of acidity in general: phenolthalein.

(b) Indicators of all acidity except that due to acid combined with protein; alizarin, congo red.

(c) Indicators of free (hydrochloric) acid: Töpfer's reagent, Günzberg's reagent.

Prepare a series of five test tubes containing (1) 2 cc. of 0.1% HCl; (2) 2 cc. of 0.1% combined HCl (HCl + peptone); (3) 2 cc. of 0.3% lactic acid; (4) 2 cc. of 0.3% lactic acid containing 0.1% HCl; (5) 2 cc. of 0.5% sodium dihydrogen phosphate solution. To each solution add 1-2 drops of phenolthalein solution and compare the results.

Wash out the tubes and repeat the experiment, using, successively, congo red, alizarin and Töpfer's reagent.

Test with Günzberg's reagent by evaporating 1-2 drops of the indicator solution to dryness on a water bath in an evaporating dish. Wet the end of a glass rod with the solution to be tested, draw it over the dried reagent and warm again. A purplish-red color indicates free acid.

Record the results in tabular form.

Test the solutions containing lactic acid with Uffelmann's reagent (test (2), page 10). Does HCl interfere with the reaction?

The products of peptic digestion of protein.—Into 200 cc. of "artificial gastric juice" place (1) minced, hard-boiled egg-white; (2) hashed lean meat; (3) a slice of bread; or (4) half a shredded wheat biscuit. Cover with a watch glass, mark the beaker for identification and leave it on the top of the desk. The contents will be transferred to an incubator and kept there until digestion is complete.

Carefully pour off the supernatant liquid without disturbing any insoluble matter that may be in the bottom of the beaker. Filter if not clear. Use small amounts of the solution in the following tests:

(1) Apply the biuret test.

(2) Test for free acid with Töpfer and Günzberg reagents.

(3) To about 3 cc. add bile, drop by drop, until 1 cc. has been added. Let the mixture stand for some time.

(4) Make 5 cc. alkaline with KOH, then slightly acidify with acetic acid and add a few drops of bromine water. A violet color results if free tryptophan is present.

Metaprotein.—Carefully neutralize the remainder of the solution, using 10% KOH at first, then 1% as the neutral point is approached. If a ppt. appears, filter and ascertain whether it consists of metaprotein.

Proteoses.—To 100 cc. of the filtrate add a single drop of dilute sulfuric acid and saturate with powdered ammonium sulfate. Collect the sticky proteose ppt. on a glass rod or horn spoon, dissolve the ppt. in a little warm water and apply the proteose tests (tests (3) and (4), page 35).

Peptone.—Filter a small amount of the liquid and to the filtrate apply the biuret test. Is peptone present? (Is there any substance in the solution that interferes with the biuret test?)

Comparative digestibility of various proteins.—Into 8 test tubes place, successively, *very* small (equal) masses of the following substances: raw meat, coagulated egg-white, dried egg-white (uncoagulated), gelatin, shredded wheat, milk (2 cc.), fibrin and hair. Add to each tube 5 cc. of 0.1% pepsin-0.2% HCl solution and place the tubes in water at 40°. Note the relative rates of digestion. Are any only partially digested or entirely indigestible?

PANCREATIC DIGESTION.

Action of pancreatic protease, trypsin.—Prepare five test tubes each containing 5 cc. of pancreatic extract. Add to each tube, successively, 5 cc. of one of the following solutions: (1), water; (2), 0.4% HCl; (3) 0.5% sodium carbonate; (4), 0.5% disodium hydrogen phosphate; (5), 0.4% lactic acid. Into a sixth tube pour 5 cc. of 0.5% sodium carbonate solution and 5 cc. of *boiled* pancreatic extract. Drop a single shred of fibrin

into each tube, incubate at 40° and note at intervals the progress of digestion. Does swelling precede the digestion of the fibrin as it does in peptic digestion?

Action of pancreatic amylase, amylopsin.—Prepare six tubes containing the solutions used in the preceding experiment. To each add about 2 cc. of starch paste, shake and heat for 30 minutes at 40°. Apply the iodine and Benedict tests to the contents of each tube. Slightly acidify the solutions before applying the iodine test (why?). Compare the results with those of the preceding experiment.

Action of pancreatic lipase, steapsin.—To 10 cc. of milk in each of four test tubes add 5 drops of 0.5% sodium carbonate solution. To tubes (1) and (2), add sufficient conc. blue litmus solution to impart a deep blue color; to tube (3) add 2 drops of phenolthalein solution. Now to tube (1) add about 3 cc. of *boiled* pancreatic extract and to the other three tubes about 3 cc. of unboiled extract. Incubate at 40° and explain the results. Examine tube (4) especially for evidence of any clotting of the milk.

The products of tryptic digestion of protein.—Examine a mixture resulting from the tryptic digestion of casein for 8-10 days at 40° as follows:

Filter and use small amounts of the filtrate in the following tests:

(1) Apply the biuret test.

(2) To about 3 cc. add bile, drop by drop, until 1 cc. has been added. Compare test (3), page 53.

(3) Slightly acidify 5 cc. with acetic acid and add a few drops of bromine water. Compare test (4), page 53. *Tryptophan test.*

(4) To 5 cc. of the solution add 10 drops of conc. sulfuric acid and 10 cc. of a 10% solution of mercuric sulfate in 5% sulfuric acid. Shake and allow to stand for 5 minutes. A yellow mercury compound of **tryptophan** is pptd.

Filter (reserve the filtrate) and wash the ppt. on the paper thoroughly with several small amounts of water. Allow each portion to run through completely before adding the next. Transfer some of the ppt. to a test tube and apply the Hopkins-Cole test. Why do most proteins give positive results with this test? Apply also the xanthoproteic and Millon tests to portions of the ppt. Explain the results.

Divide the filtrate into three portions and apply the Hopkins-Cole, Millon and xanthoproteic tests. Compare with the tests on the ppt. and explain the differences observed.

(5) Nearly neutralize the remainder of the solution with 10% HCl and exactly neutralize with 0.4% HCl. Is any *metaprotein* pptd.? Filter, if necessary, faintly acidify with acetic acid and evaporate to a thin syrup, first by boiling gently and finally on a water bath. To the warm syrup add alcohol with constant stirring as long as a ppt. forms. Collect the sticky ppt. on a glass rod or horn spoon, dissolve in a little warm water and determine whether *proteoses* and *peptones* are present by applying the usual methods of separation and identification. (Page 35).

Filter through a *dry* paper and evaporate the filtrate to 5-10 cc. on a water bath. Transfer to a beaker and set the mixture aside for at least 24 hours for crystallization. Make a microscopic examination of the crystals. Note the long sheaves of needles of **tyrosine** and the small rosettes of **leucine**. Transfer some of the crystalline mass to a test tube, add about 3 cc. of Mörner's reagent and very gradually raise the temperature to the boiling point. In the presence of tyrosine a green color results.

BILE.

Qualitative examination of bile.—(1) Determine the reaction of bile to litmus and phenolthalein.

(2) Dilute bile with about 5 volumes of water and apply the biuret test.

(3) Apply the Molisch and Benedict tests to diluted bile.

(4) Test for the presence of chloride, sulfate, phosphate, calcium and iron in diluted bile.

(5) Add acetic acid, drop by drop, to undiluted bile. *Nucleoprotein* is pptd. and, in the presence of bile salts, is not soluble in excess of acid.

(6) Evaporate 10-15 cc. of bile to dryness on a water bath. Extract the residue with several small portions of ether. Allow the ether to evaporate spontaneously, dissolve the residue in about 2 cc. of chloroform and apply the Liebermann-Burchard test for *cholesterol.*

(7) Shake a few cc. of bile with a little ether. Is any pigment extracted by the ether? Add a few drops of HCl and shake again. The pigment is liberated from its salt combination by the acid and dissolves in the ether.

Bile pigments.—(1) *Gmelin's test.* Pour about [illegible] of yellow conc. nitric acid into a test tube. Pour very carefully down the side of the tube about 3 cc. of diluted bile so that the bile and acid do not mix. At the point of contact note the production of rings of various colors as the pigment is gradually oxidized by the acid.

Filter about 5 cc. of diluted bile several times through a small paper. When it has drained completely, unfold the paper and allow a drop of the yellow conc. nitric acid to fall in the center of the paper. Note the ring of colors produced as the acid diffuses.

(2) *Salkowski-Schippers' test.* To 35 cc. of water in a graduated cylinder add 5 cc. of bile, 4 cc. of sodium carbonate solution and 6 cc. of calcium chloride solution. Shake thoroughly. Filter and wash the ppt. with water. Transfer some of the ppt. to a test tube, add about 5 cc. of alcohol, sufficient conc. HCl to dissolve the ppt. and a *single* drop of ferric chloride solution. Warm in a water bath and note the color produced.

(3) *Huppert's test.* Shake about 5 cc. of bile with an equal volume of milk of lime. Filter and proceed as in experiment (2).

(4) *Krokiewicz's test.* Make a mixture in a test tube of 5 drops of 1% sulphanilic acid solution and 5 drops of 1% sodium nitrite solution. Add 10 drops of bile and note the color produced. Add a few drops of HCl and dilute with water. Is there a change of color?

Bile salts.—(1) *Pettenkofer's test.* To about 5 cc. of bile add 3 drops of 0.1% furol solution. Pour carefully down the side of the tube about 2 cc. of conc. sulfuric acid and note the color produced at the junction of the two liquids.

Repeat the test, using 3 drops of a 5% sucrose solution instead of the furol (compare test (1), page 12).

Repeat the test with bile greatly diluted with water.

(2) *Hay's test.* To 50 cc. of water in a graduated cylinder add a few drops of bile. Sprinkle a little flowers of sulfur on the surface.

Try the experiment also with pure water, alcohol and dilute soap solution (see that there is no froth on the surface of the soap solution). The results are due to differences in surface tension.

Determine how small a concentration of bile will lower the surface tension of water sufficiently to allow the sulfur to sink.

(3) *Oliver's test.* Acidify 5 cc. of diluted bile with acetic acid. Filter, if not clear, and add to the filtrate an equal volume of Witte's peptone solution. Does pptn. occur? A compound of protein with bile acids is formed. Compare test (3), page 53.

Direct digestive action of bile.— Prepare three test tubes containing, successively, 5, 10 and 20 drops of bile. Add about 5 cc. of water and 1 cc. of starch paste to each tube and incubate at 40° for about an hour. Apply the iodine and Benedict tests to the contents of each tube. Does bile contain amylase?

Influence of bile on digestion.— *Peptic digestion.* Into each of two test tubes pour about 5 cc. of pepsin-HCl solution. To one add 2 cc. of water (control) and to the other 2 cc. of bile and incubate at 40° after placing a shred of fibrin in each tube. In which tube is digestion the more rapid? Explain the result.

Tryptic digestion. To 5 cc. of pancreatic extract (or trypsin solution) in each of two test tubes add about 1 cc. of 0.5% sodium carbonate solution. Add to one tube 2 cc. of water (control) and to the other 2 cc. of bile. To each add a shred of fibrin and heat at 40°. Compare the result with that of the preceding experiment.

Lipolytic digestion. To 10 cc. of milk in each of two tubes add 10 drops of 0.5% sodium carbonate solution and 2 drops of phenolthalein solution. To tube (1) add 2 cc. of enzyme solution and 2 cc. of water; to tube (2) add 2 cc. of enzyme solution and 2 cc. of bile. Incubate as usual. Does the bile influence the activity of the lipolytic enzyme?

Defibrinated blood.—(1) Make a microscopic examination of a drop of defibrinated blood. Describe the objects observed.

(2) Obtain some freshly drawn blood by pricking the finger with a sterile needle. Allow a drop to fall on a microscope slide, cover *quickly* with a cover slip and examine *immediately* under the microscope while the clotting process is in progress.

(3) *Hemolysis (laking of blood).* Pour about 2 cc. of blood into a *dry* test tube. Gradually rotate the tub so that the blood forms a thin film on the inner surface of the tube. Is it possible to see letters through the film of blood?

Add water slowly, drop by drop, shaking after each addition. Note the change in color to deeper shades of red. Does the mixture finally become sufficiently transparent to see letters through it?

Put a cover slip on a drop of blood on a slide and place three drops of water on the slide at the edge of the cover slip. Examine the blood under the microscope along the line where the two liquids mix under the cover slip.

Prepare eight test tubes containing, successively, 5 cc. of solution, as follows: (a) 0.9% sodium chloride (normal saline); (b) 0.4% sodium chloride; (c) 10% sodium chloride; (d) 0.9% sodium chloride shaken thoroughly with 2 cc. of ether; (e) 2% urea in water; (f) 2% urea in normal saline; (g) 0.2% bile salts in normal saline; (h) water. Add about 10 drops of blood to the contents of each tube and mix by inverting the tube. In which tubes does hemolysis occur? Explain the effect of each solution upon the corpuscles.

(4) *Hemagglutination.* Extract a level horn spoonful of bean (Scarlett runner) meal with about 5 cc. of 0.9% sodium chloride solution for 10 minutes. Filter. Into each of three test tubes pour 1 cc. of diluted defibrinated *rabbit* blood. To the first tube add 3 drops of the filtered bean extract; to the second tube, 1 drop; keep the third tube for a control. Mix thoroughly by inverting the tubes. Rapid agglutination occurs

and the corpuscles are pptd. Allow to stand for half an hour. Can the clear serum be poured off without disturbing the pptd. corpuscles?

Boil the remainder of the bean extract, filter off the coagulum and add 3 drops of the boiled extract to the contents of the third (control) tube. Has the hemagglutinin been destroyed by boiling?

The saline solution extracts from the bean a protein substance which has the property of causing the clumping of the red blood corpuscles.

(5) *Reaction.* Wet a red and a blue strip of litmus paper and lay the wet strips on a microscope slide. Allow a drop of blood to fall on each strip. After five minutes wash off the blood with water. What is the reaction of blood?

(6) *Chemical constituents.* Dilute about 15 cc. of blood in a casserole with 100 cc. of water and heat to boiling, stirring constantly. Add dilute acetic acid, drop by drop, until the brown coagulum separates in flocculent form in a water-clear liquid. Of what does the coagulum consist? Filter. The filtrate should be clear and colorless; if brown, the experiment should be repeated. Evaporate the filtrate to about 25 cc., filter if any ppt. appears and make the following tests on the filtrate:

(a) Apply the biuret test.

(b) Apply the Benedict test.

(c) Test for the presence of chloride, phosphate, calcium and iron.

(d) Evaporate the remainder nearly to dryness in a beaker on a water bath. Does the residue contain cubical crystals of sodium chloride?

(7) *Iron test.* The presence of iron in blood has been shown in the experiment on page 2.

To about 5 cc. of blood add about 2 cc. each of dilute HCl and potassium ferrocyanide solution. Shake vigorously and examine the froth for any blue coloration. Does blood contain iron in the ionized condition?

(8) *Guaiacum test.* Add a drop of blood to about 5 cc. of water in a test tube. Add about 1 cc. of hydrogen peroxide and float on the surface of the mixture a little tincture of guaiacum. Note the color which develops.

Repeat the test, boiling the blood solution and cooling before adding the peroxide and guaiacum. Does the result indicate that the reaction is due to enzyme action?

Add a drop of blood to 500 cc. of water and repeat the test with 5 cc. of the very dilute solution.

(9) *Benzidine test.* To a test tube full of water add a drop of blood and mix thoroughly. To 2 cc. of benzidine solution in a clean test tube add 1 cc. of the very dilute blood solution and then 10 drops of 3% hydrogen peroxide. Note the color developed.

Repeat the test with a solution made by adding a single drop of blood to 1 liter of water. Under carefully controlled conditions a positive reaction can be obtained with 1 cc. of blood solution of a concentration of 1 to 5 million.

(10) *Catalase action.* To about 1 cc. of blood in a test tube add 2 cc. of hydrogen peroxide without shaking. What is the cause of the effervescence?

(11) *Preparation of hemin crystals (Nippe's method).* Place a *very small* drop of blood on a microscope slide and spread with a glass rod so that it forms a thin film. Evaporate slowly high above a small flame taking special care not to burn the blood. To the dry film add 2 drops of a 0.1% solution of potassium chloride in glacial acetic acid. Put a cover glass in place and heat gently until the mixture is boiling. Allow another drop of the acid to run under the cover slip and again heat to boiling. Add another drop of acid (to replace that which has evaporated), cool and examine under the high power of the microscope. What are the steps involved in the formation of hemin crystals from hemoglobin? This is an absolute test for blood but does not differentiate between human blood and that of other species.

Blood serum.—Heat about 15 cc. of serum to boiling and add acetic acid, drop by drop, until coagulation is complete. Test the coagulum by applying the Millon test. Filter and apply the following tests to the protein-free filtrate: Benedict, chloride, phosphate and iron. What substances are present in whole blood which are not present in blood serum?

Fibrin.—Apply the biuret, Millon and xanthoproteic tests to small pieces of fibrin.

Note the effect of the following solutions on small shreds of fibrin: (a) water; (b) 0.2% HCl; (c) 0.5% sodium carbonate; (d) 10% sodium chloride.

Examination of blood stains.—If the stain is on a cloth, cut out the stained portion and extract half of it thoroughly with a very small amount of water.

If the extract is decidedly brown, evaporate a drop on a microscope slide and prepare hemin crystals (page 61). If the color is not pronounced, evaporate successively several drops of the extract.

Apply the guaiacum and benzidine tests to portions of the extract.

Extract the remainder of the stain with a few drops of .9% sodium chloride and examine under the microscope for the presence of corpuscles.

LIVER.

Ferratin.—Grind in a mortar with sand about 50 grams of hashed liver. Transfer to about 200 cc. of water in a casserole, mix and determine the reaction of the mixture to litmus. Warm gradually, stirring constantly, over a low flame. Finally boil for about a minute. Filter the hot extract (discard the coagulum). Cool the filtrate and add, drop by drop, 10% tartaric acid solution until a brownish-yellow flocculent ppt. of ferratin is produced. Avoid excess of acid. Filter and test the filtrate for the presence of reducing sugar and inorganic iron.

(a) Determine the solubility of the ferratin in water, 0.2% HCl, 0.5% sodium carbonate and 10% sodium chloride.

(b) To a slightly ammoniacal solution of ferratin add a little ammonium sulfide solution. Explain the production of the brownish-black color that develops on standing.

(c) Suspend a little ferratin in water and add a few drops of potassium ferrocyanide solution. Is a blue color produced? Add a few drops of HCl to another portion and warm. Cool and add a few drops of potassium ferrocyanide solution. Explain the result.

Glycogen.—Glycogen can be obtained in considerable amounts from the liver of a rabbit which has been well fed on a diet rich in carbohydrates. The liver is removed immediately after death, minced in a hashing machine and dropped into boiling water in order to destroy a glycogenolytic enzyme (an amylase) which rapidly converts the glycogen into glucose after the death of the animal. The properties of glycogen will be studied later.

MUSCLE.

(1) **Presence of magnesium and phosphate.**—Obtain a fragment of hashed meat from which most of the blood has been removed by pressing. Spread it in a very thin layer on a microscope slide. Invert the slide over a small beaker con-

taining about 15 cc. of ammonium hydroxide, cover with a watch glass and expose the muscle film to the ammonia fumes for about 15 minutes. Protect with a cover slip and examine under the microscope. Note the prismatic or feathery crystals of *ammonio-magnesium phosphate* abundantly distributed throughout the muscle tissue.

(2) **Qualities of beef extract.**—Apply the following tests to a 2% solution of commercial beef extract:

(a) Determine the reaction of the extract to litmus. Explain the result.

(b) Apply the biuret, xanthoproteic and heat coagulation tests.

(c) Apply the Molisch and Benedict tests.

(d) Test for the presence of chloride, phosphate, sulfate, calcium and iron.

(e) Are any fat globules present?

(f) Fresh meat extract contains **creatine** but a commercial extract also usually contains **creatinine** formed by the action of the acid in the extract on the creatine during evaporation. Test as follows:

(*i*) To 10 cc. of extract add 15 cc. of saturated picric acid solution and 10 cc. of 10% NaOH. After standing *five* minutes dilute to 200 cc. If creatinine is present an orange color develops. Fill a test tube with the mixture and set it aside for comparison with (*ii*) and (*iii*).

(*ii*) Add 10 cc. of 10% NaOH to 10 cc. of meat extract and heat in a boiling water bath for 30 minutes. Cool, add 15 cc. of picric acid solution and, after five minutes, dilute to 200 cc. Set aside a test tube full of the mixture for comparison with (*i*) and (*iii*).

(*iii*) To 10 cc. of meat extract add 5 cc. of dilute HCl and heat in a boiling water bath for about two hours, keeping the volume constant. Neutralize the mixture, add 10 cc. of NaOH and 15 cc. of picric acid and, after standing for five minutes,

dilute to 200 cc. Fill a test tube with the mixture and compare the colors of the solutions in the three tubes. Explain the results.

Write the structural formulas for creatine and creatinine.

(g) Meat extract also contains **purine bases**, principally **xanthine** (2, 6-dioxypurine) and **hypoxanthine** (6-oxypurine). Write the structural formulas for xanthine and hypoxanthine, and compare with uric acid, page 24.

Do the results of these tests indicate that beef extract is a "concentrated food"?

(3) **Myosin.**—Stir up about 25 grams of hashed beef in several changes of water until it has been washed free from blood. Transfer to a bottle, add about 150 cc. of 10% sodium chloride solution and leave the bottle on the top of the desk so that it can be shaken frequently. After extraction, for at least 24 hours, strain carefully through cheese cloth. Test the reaction of the extract to litmus. Pour 5 cc. of the extract into 20 volumes of water; why does pptn. occur? Saturate the extract with sodium chloride and filter off the myosin on several wet, fluted filter papers. The mixture will filter very slowly and the myosin which collects on the sides of the papers may be removed and tested before filtration is complete.

(a) Determine the solubility of the myosin in water, 0.2% HCl and 0.5% sodium carbonate. To what class of proteins does myosin belong?

(b) Apply the biuret and Millon tests.

(c) Suspend a little myosin in about 5 cc. of water in a test tube and heat to boiling. Does the heated myosin dissolve on the addition of an equal volume of 10% sodium chloride solution?

Test the *filtrate* from the myosin ppt. as follows:

(a) Apply the biuret test. Is protein present?

(b) Heat about 50 cc. of the filtrate to boiling in a beaker and acidify slightly with acetic acid. Is coagulable protein present? Filter, and use portions of the filtrate for tests (c) and (d).

(c) Test for proteoses with picric acid.

(d) If proteoses are present, saturate about 10 cc. of the solution with ammonium sulfate, filter and test for peptone by applying the biuret test.

(4) **Glycogen.**—Grind thoroughly in a mortar with sand a few oysters or scallops. Transfer to a casserole, add about 100 cc. of water and heat to boiling. Faintly acidify with acetic acid and boil for about 2 minutes. Filter and use the filtrate in the following tests:

(a) Note the opalescence of the solution which is characteristic.

(b) Add iodine solution, drop by drop, until the wine-red color is permanent. Heat to boiling and allow to cool. Does the color with iodine resemble that of any other carbohydrate studied?

(c) Does the solution reduce Benedict's solution?

(d) To about 10 cc. of the solution add 10 drops of HCl and heat for 20 minutes in a boiling water bath. Does the opalescence disappear? Neutralize and apply the Benedict test. What has been formed?

(e) To about 10 cc. of the solution add a little saliva and after 30 minutes test with Benedict's solution. Is glycogen digested by ptyalin?

(e) Pour about 5 cc. of the solution into 15 cc. of alcohol. Is the glycogen pptd.?

BRAIN.

Macerate thoroughly a small amount of brain tissue in a mortar with a little sand. Transfer to a glass-stoppered bottle, add about 15 cc. of alcohol and, after shaking, 25 cc. of ether. Set aside, tightly stoppered, for several days. Filter the extract (avoid flames!) into a casserole and evaporate the filtrate to dryness on the *steam* bath.

Brain lecithins.—Dissolve the residue in about 10 cc. of ether and filter if necessary. To the filtrate add about 30 cc.

of acetone; lecithins are pptd. Do they resemble in appearance egg-yolk lecithin? Filter into a dry beaker. Apply to the ppt. tests (1) and (4), page 42.

Brain cholesterols.—Evaporate the ether-acetone filtrate nearly to dryness on the *steam* bath. To the crystalline residue apply the Salkowski and Liebermann-Burchard tests for cholesterol (page 43). Apply also the Sudan III test to some of the residue. Is fat present?

Brain tissue contains, beside lecithins, two other phosphorized fats, **protagon** and **kephalin.** Boiling alcohol extracts a group of substances called **cerebrosides** containing nitrogen but no phosphorous. One of the principal proteins is **neurokeratin.** Nervous tissue is characterized by its high content of lipoid substances.

EPIDERMAL TISSUE.

Hair (keratin).—Apply to hair the Millon, xanthoproteic, Hopkins-Cole and sulfur reactions.

On account of its insolubility it does not respond to the biuret test.

Horn.—Repeat the tests in the preceding experiment, using horn shavings instead of hair.

CONNECTIVE TISSUE.

Tendon.—The principal protein present in tendon is the scleroprotein, **collagen.** There is also present a gluco-protein, **tendo-mucoid,** which can be extracted from tendon by half-saturated lime water and yields a reducing substance on hydrolysis.

When collagen is boiled with water or dilute acids it is hydrolysed with the formation of **gelatin.** Perform the following tests on gelatin:

(1) Determine the solubility of gelatin in hot and cold water, 0.2% HCl, 0.5% sodium carbonate and 10% sodium chloride.

(2) Apply the biuret, xanthoproteic, Millon, Hopkins-Cole and sulfur reactions to a solution of gelatin in hot water. The last three tests should give negative results. What does this indicate regarding the amino-acid complexes present in gelatin?

(3) Heat a little gelatin solution to boiling. Does it coagulate?

(4) Ascertain whether gelatin resembles proteoses by applying tests (3), (4) and (5), page 35, to small amounts of gelatin solution.

Ligament.—Yellow elastic tissue is composed largely of **elastin**, a scleroprotein which, like keratin and collagen, is an insoluble substance. It also contains collagen and mucoid, but in much smaller amounts than tendon.

Adipose tissue.—Place a piece of adipose tissue about the size of a pea in a dry beaker and add a few cc. of ether. Crush the tissue with a spatula and stir until the fat is dissolved. Filter through a dry paper into a dry beaker and allow the ether to evaporate spontaneously. Apply the acrolein test to the residue.

To the minute fragment of the tissue which failed to dissolve in the ether apply the Millon test. Does it consist of protein?

Bone tissue.—Place a thin cross section of ox rib in a small beaker and add 100 cc. of water and 10 cc. of conc. nitric acid. Set the beaker aside for 24 hours or longer. Observe that the rigidity has been lost and that the piece is soft and pliable. Filter the acid extract and use it in experiment (3).

(1) Wash the piece of ossein with water and dry with filter paper. Cut out the marrow and extract it for some time in a covered dry beaker with a little ether. Pour the ethereal extract into a dry watch glass and let the ether evaporate. Does the residue contain **fat**?

(2) Cut the **ossein** into small pieces and boil it in a covered casserole with 50 cc. of water containing a few drops

of acetic acid until the ossein is completely dissolved. At first replace the water which evaporates but finally concentrate the liquid to about 25 cc. Pour into a beaker and allow to stand for several hours. Has gelatin been formed?

Ossein is practically the same substance which is called collagen in other connective tissues.

(3) The dilute nitric acid has dissolved the inorganic constituents of bone. Add ammonium hydroxide to the extract until the solution is decidedly alkaline. Phosphates are pptd. Filter and test the filtrate for the presence of chloride, sulfate, phosphate and calcium. Pour over the ppt. on the paper several small amounts of acetic acid and test the second filtrate for calcium and phosphate. Pour over the filter paper about 5 cc. of warm dilute HCl and test the third filtrate for iron and phosphate.

THE CHEMISTRY OF NORMAL URINE.

Collect a 24 hour specimen of urine, measure the volume and bring about 500 cc. to the laboratory. In collecting the sample empty the bladder in the morning (8 A.M.), discarding the urine passed, and save all the urine from that time up to and including that passed the next morning at the same hour. It should be thoroughly mixed before measuring.

Specific gravity.—Determine the specific gravity of the urine with a urinometer. Correct for temperature as follows: add one unit for every 3 degrees above 15°; and subtract one unit for every 3 degrees below 15°.

Total solids.—Calculate the total solids in grams per liter of urine by multiplying the last two units of the specific gravity at 15° by 2.6 (Long's coefficient). Calculate the weight of solids in the 24 hour specimen.

Reaction.—*Qualitative.* Determine the reaction of urine with wet red and blue litmus strips.

Quantitative. Measure 25 cc. of urine with a pipette into an Erlenmeyer flask, add 15 grams of powdered potassium oxalate and shake for 2 minutes. Add 2 drops of phenolthalein and titrate the mixture immediately with 0.1 *N* NaOH until a faint but permanent pink color remains after shaking. Calculate the acidity of the 24 hour specimen in terms of HCl. Keep the solution for the determination of ammonia.

Estimation of ammonia.—Add 2 drops of phenolthalein to 10 cc. of formaldehyde (1 part of formalin and 2 parts of water) in a beaker and neutralize with NaOH. Add this neutralized solution to the neutral urine obtained in the preceding experiment. Why do the two neutral solutions become acid? Write the equation. Titrate again with NaOH and calculate the amount of ammonia in the solution. Express the result in grams of ammonia per 24 hours.

Set aside about 100 cc. of the urine in an uncovered beaker without preservative and at a succeeding laboratory period

repeat the determinations of acidity and ammonia. Account for the difference in results and write the equation for the decomposition which has occurred.

Again set aside the remainder of the partially decomposed urine in a covered beaker for subsequent examination.

INORGANIC CONSTITUENTS.

Chlorides.—Acidify 5 cc. of urine with nitric acid and add silver nitrate solution.

Sulfates.—Acidify 5 cc. of urine with HCl and add barium chloride solution.

Phosphates.—(1) To about 25 cc. of urine add ammonium hydroxide until distinctly alkaline. Filter off the ppt. of earthy phosphates and wash with water. Dissolve the ppt. by pouring dilute HCl through the paper. Test the filtrate for the presence of phosphate and **calcium** and **magnesium.**

(2) Heat to boiling about 25 cc. of urine in a small beaker. Unless the urine is strongly acid, a ppt. of *earthy phosphates* will appear, due to the conversion of soluble di-calcium (or magnesium) phosphate to insoluble tri-calcium phosphate. The turbidity suggests the presence of coagulable protein in the urine. Heat half the turbid mixture again to boiling and add a drop or two of acetic acid. Compare with the unacidified portion. Was the turbidity due to coagulation of protein?

(3) To about 10 cc. of urine add ammonium hydroxide until alkaline. Filter off the *earthy phosphates* and to the filtrate add magnesia mixture. White ammonium magnesium phosphate is ppt̤d. due to the presence of *alkaline phosphates.* Judging fro ᴖ the amounts of ppt., which form of phosphates is present in the larger amount?

(4) Examine the sediment in the sample of urine which has been undergoing alkaline fermentation for several days for the presence of "coffin-lid crystals" of ammonium magnesium phosphate. Note also the "thorn-apple forms" of acid ammonium urate. What is the reaction of the decompcsed urine to litmus?

ORGANIC CONSTITUENTS.

Urea.—Evaporate about 75 cc. of urine to dryness on a water bath in an evaporating dish. Turn out the flame and add about 20 cc. of acetone to the residue. Rub up thoroughly with a glass rod until the acetone is boiling. Pour off the acetone extract into a clean dry watch glass. Crystals of urea separate on cooling. Allow the acetone to evaporate and identify the urea by applying tests (3), (4), and (7), page 11.

Uric acid.—(1) To 200 cc. of clear urine add 10 cc. of conc. HCl, stir thoroughly and set aside the mixture for at least 24 hours. Pigmented crystals of uric acid will separate as a granular deposit on the sides and bottom of the beaker. Examine some of the crystals under the microscope. Carefully pour off the liquid and apply the murexide test to the crystals, test (2), page 24.

(2) Saturate 100 cc. of urine with ammonium chloride (about 30 grams necessary) and add 1 cc. of ammonium hydroxide. Allow to stand for 15 minutes. Filter off the ppt. of ammonium urate and use portions of it in the following tests:

(a) Apply the murexide test.

(b) Apply the Folin test, page 25. A modification of this reaction is used in the quantitative determination of uric acid.

(c) Suspend the remainder of the ppt. in about 10 cc. of water in a beaker, add about 2 cc. of conc. sulfuric acid and a few drops of potassium permanganate solution. C n the amount of uric acid pre- quantitatively determined by this method?

Creatinine.—*Jaffe's test.* To about 5 cc. of urine add a small amount of picric acid solution and make the mixture alkaline with KOH. Compare est (f). page 64, and test (10), page 13.

Weyl's test. To 5 cc. of urine add a few drops of a freshly prepared solution of sodium nitroprusside and make alkaline

with NaOH. What color develops? Is it permanent? Compare test (2), page 8.

Salkowski's test. Add an excess of acetic acid to the solution obtained in the preceding test and heat in a boiling water bath. Is there a change of color? A ppt. of Prussian blue may separate.

Hippuric acid.—Dissolve about 2 grams of ammonium benzoate in half a glass full of water and take the solution before retiring at night. Collect the urine passed the following morning and bring the specimen to the laboratory for the isolation of hippuric acid, which has been formed in the body by the conjugation of glycocoll and benzoic acid. Write the equation.

To 50 cc. of the urine add 25 grams of solid ammonium sulfate and 1 cc. of conc. sulfuric acid. Stir until solution is complete and allow the mixture to stand for 15 minutes. Filter off the pigmented crystals of hippuric acid and dry them between folds of filter paper. Use the crystals in the following tests:

(a) Dissolve a small amount in 5 cc. of hot water and make a microscopic examination of the crystals which separate on cooling.

(b) Place a few crystals in a dry test tube and heat. They melt and on further heating the liquid assumes a red color and a sublimate of benzoic acid appears.

(c) Add 1 cc. of conc. nitric acid to a few crystals in a small evaporating dish and evaporate to dryness on a water bath. Transfer the residue to a dry test tube and heat. Note the odor of nitrobenzene (page 22).

(d) Boil the remaining crystals with about 5 cc. of conc. HCl for several minutes. Allow the crystals of benzoic acid to settle and pour off the supernatant liquid into a beaker. Add ammonium hydroxide until the solution is slightly alkaline and boil until the excess of ammonia is removed. Add a few drops of copper sulfate solution and note the color of the copper salt of glycocoll. Write the equation for the hydrolysis of hippuric acid.

Ethereal or organic sulfates.—To about 20 cc. of urine in a small beaker add 3 cc. of acetic acid and 5 cc. of barium chloride solution. *Inorganic* sulfates are pptd. Filter the solution until a clear filtrate is obtained (pour repeatedly through the same filter if necessary). To the clear filtrate add about 3 cc. of conc. HCl and boil for several minutes. Explain the further pptn. of barium sulfate.

Neutral sulfur compounds.—To about 10 cc. of urine in a test tube add a piece of granulated zinc and sufficient HCl to cause a gentle evolution of gas. Suspend in the mouth of the tube a piece of filter paper wet with lead acetate solution. What gas is produced? Explain its formation.

Indican.—To 10 cc. of urine in a test tube add an equal volume of Obermayer's reagent and 2 cc. of chloroform. Pour the mixture from one test tube to another until there is no further evolution of gas, then thoroughly shake it for about a minute. In the presence of indican (potassium indoxyl sulfate) the chloroform is colored blue. Explain the reaction.

To 10 cc. of urine add 1 cc. of lead acetate solution and filter. Repeat the preceding test with the decolorized urine and compare results.

Amylase.—Prepare three test tubes containing, successively, 5, 10 and 20 drops of urine. Add to the first tube 15 drops and to the second 10 drops of water. Pour 2 cc. of 0.1% starch solution into each tube and heat at 40°. At intervals apply the iodine test. Continue testing with iodine until the achromic point is reached in the third tube. Has digestion of the starch occurred in the other tubes?

CERTAIN CONSTITUENTS OF ABNORMAL URINE.

Protein.—Fill a test tube two-thirds full of clear urine, hold it by the bottom and heat the upper half of the tube with a small flame until the urine boils. Compare the heated and unheated portions of urine. A turbidity indicates albumin or phosphates (page 71). Add 3-5 drops of dilute acetic acid and explain any reaction which may occur.

Repeat the test with urine known to contain albumin and compare the results.

Apply *Heller's ring test* (test (2), page 29) to a sample of normal urine and to a sample containing albumin and compare the results.

Glucose.—*Fehling's test.* Dilute about 2 cc. of Fehling's solution with 4 cc. of water and boil. There should be no discoloration of the solution. To the hot solution add urine (free from protein), a few drops at a time, boiling after each addition, until about 2 cc. have been added. Does reduction occur? Does any normal urinary constituent have a reducing action on Fehling's solution?

Benedict's test. To 5 cc. of Benedict's solution add *eight drops* (not more) of urine (free from protein), and boil for one minute. Allow to cool spontaneously. *After cooling,* if glucose is present, the entire solution will be filled with a ppt. which may be red, yellow or green, depending on the amount of glucose in the urine. In the absence of glucose there may be a slight turbidity due to pptd. urates. The test may be applied with success in artificial light since amount rather than color of ppt. is the basis of the test.

Repeat the Fehling and Benedict tests, using urines containing 0.1% and 0.05% of glucose and compare the delicacy of the tests.

Quantitative determination of glucose by Benedict's method. Dilute 10 cc. of urine to 100 cc. with water (unless the sugar content is known to be low) and fill a burette with the diluted urine. Pipette 25 cc. of Benedict's quantitative solution into a 150 cc. Erlenmeyer flask (or porcelain evaporating dish), add 25 cc. of water, a horn spoonful of anhydrous sodium carbonate and a little powdered pumice stone. Heat the mixture over a free flame until the carbonate is dissolved. Run the diluted urine into the boiling solution rather rapidly until a chalk-white ppt. forms and then a few drops at a time until the last trace of blue color disappears. Keep the solution

boiling vigorously during the entire titration. As the end point is approached, add the urine, two drops at a time, and boil the solution for 30 seconds after each addition. The volume of diluted urine necessary to effect the reduction contains 50 mg. of glucose. A sample of a 24-hour specimen of urine should always be used for the quantitative determination and the amount of glucose eliminated in 24 hours calculated.

Bile.—*Gmelin's test.* Filter 5 cc. of urine repeatedly through a small filter and, when it has drained completely, unfold the filter and allow a drop of yellow conc. nitric acid to fall in the center. Compare test (1), page 56.

Salkowski-Schippers' test. Perform the test according to the method described on page 56, using 10 cc. of urine and 25 cc. of water.

Hay's test. Sprinkle some flowers of sulfur on the surface of the urine. Compare test (2), page 57.

Oliver's test. Acidify 5 cc. of urine with acetic acid and filter if not clear. Add an equal volume of 1% Witte's peptone solution acidified with acetic acid. A white ppt. indicates the presence of bile salts. Compare test (3), page 57.

Repeat the tests with urine known to contain bile.

Blood.—*Benzidine test.* To 2 cc. of benzidine solution in a clean test tube add 1 cc. of urine and then 10 drops of 3% hydrogen peroxide. Compare test (9), page 61. Perform a control test substituting water for the urine.

Repeat the test with urine known to contain blood.

Acetone and aceto-acetic acid.—*Rothera's test.* Saturate 10 cc. of urine with solid ammonium sulfate, add 3 drops of 5% sodium nitroprusside solution (freshly prepared) and about 3 cc. of conc. ammonium hydroxide. Mix and allow to stand for 30 minutes. The development of a reddish-purple, permanganate color indicates the presence of acetone and aceto-acetic acid.

Legal's test. To about 5 cc. of urine add a few drops of sodium nitroprusside solution (freshly prepared) and make

the mixture alkaline with KOH. Compare Weyl's test, page 72. Acidify with acetic acid and, if **acetone** is present, the red color will be intensified, otherwise the color will change to yellow. Compare test (2), page 8.

Gerhardt's test. To about 5 cc. of urine add ferric chloride solution, drop by drop, as long as pptn. occurs. Filter off the ferric phosphate. To the filtrate add more ferric chloride. A Bordeaux-red color indicates the *possible* presence of **aceto-acetic acid.**

Boil about 5 cc. of the urine for about three minutes, cool, and repeat the test. Boiling converts the aceto-acetic acid into acetone and the test should be negative. A positive result indicates the presence of other substances (e.g. various drugs) which give the reaction.

Write the formula for aceto-acetic acid and an equation showing its conversion into acetone.

Repeat the tests with urine known to contain acetone and aceto-acetic acid.

SPECIAL REAGENTS.

Barfoed's solution. Dissolve 4.5 grams of neutral crystalline cupric acetate in 100 cc. of water and add 1.2 cc. of 50% acetic acid.

Benedict's qualitative solution. Dissolve with the aid of heat 170 grams of sodium citrate and 100 grams of anhydrous sodium carbonate in about 600 cc. of distilled water. Filter through a folded paper and make up to about 850 cc. Dissolve 17.3 grams of crystallized copper sulfate in 100 cc. of distilled water and pour the solution, slowly, with constant stirring, into the carbonate citrate solution. After cooling, make up the solution to 1 liter.

Benedict's quantitative solution. Dissolve with the aid of heat 200 grams of sodium citrate, 125 grams of potassium sulfocyanate and 100 grams of anhydrous sodium carbonate in about 600 cc. of water, and filter if necessary. Dissolve 18 grams of crystallized copper sulfate in 100 cc. of water and pour the solution, with constant stirring, into the other liquid. Add 5 cc. of a 5% solution of potassium ferrocyanide. When the solution is cold, dilute to exactly 1 liter.

The copper sulfate is the only constituent of the solution which must be weighed with exactness. Twenty-five cc. of the solution is completely reduced by 50 mg. of glucose.

Benzidine solution. Heat 4.5 cc. of glacial acetic acid in an Erlenmeyer flask to 50° and add 0.5 gram of benzidine. Heat the flask for 8-10 minutes in water maintained at 50°. To the solution add 19 cc. of water distilled from glass. The reagent should be freshly prepared at least once a week.

Bial's reagent. Dissolve 1.5 grams of orcin in 500 cc. of conc. HCl and add 25 drops of 10% ferric chloride solution.

Biuret reagent. Pour 25 cc. of 3% copper sulfate solution, slowly with constant stirring, into 1 liter of 10% potassium hydroxide solution.

Casein solution. Dissolve 0.4 gram of casein in 40 cc. of 0.1 *N* sodium hydroxide solution, add 130 cc. of distilled water and 30 cc. of 0.1 *N* hydrochloric acid solution.

Combined hydrochloric acid. Add Witte's peptone (or meat peptone) to hydrochloric acid of the desired concentration until it no longer gives a test for free acid with Töpfer's reagent.

Fehling's solution. Copper portion.—Dissolve exactly 34.65 grams of crystalline copper sulfate in 500 cc. of water. Alkaline portion.—Dissolve 175 grams of Rochelle salt (sodio-potassium tartrate) and 60 grams of sodium hydroxide in water and make up to 500 cc. Preserve separately and mix in equal volumes just before using. The mixed reagent readily decomposes soon after its preparation.

Folin's uric acid reagent. Boil for 2 hours 100 grams of sodium tungstate, 80 cc. of ortho-phosphoric acid (85%) and 750 cc. of water in a flask with a reflux condenser. Cool and dilute to 1 liter.

Fusion mixture. Grind thoroughly in a mortar one formula weight of sodium carbonate with two formula weights of potassium nitrate.

Glyoxylic (reduced oxalic) acid. Cover with distilled water 10 grams of powdered magnesium in a large flask. Add slowly 250 cc. of saturated oxalic acid solution, cooling in running water during the addition of the acid. Shake, filter and wash the ppt. twice with water. Acidify the filtrate with acetic acid and make up to 1 liter.

Günzberg's reagent. Dissolve 2 grams of phloroglucin and 1 gram of vanillin in 100 cc. of 95% alcohol.

Iodine solution. Dissolve 10 grams of iodine in 1 liter of 2% potassium iodide solution.

Lugol's solution. Dissolve 4 grams of iodine in 100 cc. of a 6% potassium iodide solution.

Millon's reagent. Dissolve (without heating) 30 cc. (400 grams) of mercury in 570 cc. (800 grams) of conc. nitric acid. Dilute the solution with 2 volumes of water. The solution contains mercurous and mercuric nitrates, excess of nitric acid and a small amount of nitrous acid.

Mörner's reagent. Mix 1 volume of formalin and 45 volumes of water and add 55 volumes of conc. sulfuric acid.

Nylander's solution. Dissolve 4 grams of Rochelle salt in 100 cc. of 10% sodium hydroxide solution. Add 2 grams of bismuth subnitrate and heat on a water bath. Cool and filter when the solution is saturated with the bismuth compound. Cool and filter.

Obermayer's reagent. Dissolve 3 grams of ferric chloride in 1 liter of conc. hydrochloric acid.

Olive oil emulsion. Add to commercial olive oil (which contains free oleic acid) in a flask 1 drop of 1% alcoholic phenolphthalein solution for every 10 cc. of oil. Add 0.1 *N* sodium hydroxide solution from a burette, shaking until the mixture is neutral.

Schweitzer's reagent. To 200 cc. of 5% copper sulfate solution containing 10 grams of ammonium chloride, add potassium hydroxide solution until pptn. is complete. Filter and wash the ppt. free from alkali with water. Dissolve 3 grams of the moist ppt. in 1 liter of concentrated ammonium hydroxide.

Seliwanoff's reagent. Dissolve 0.05 gram of resorcin in 100 cc. of dilute (1:2) hydrochloric acid.

Sodium hypobromite solution. Dissolve 100 grams of sodium hydroxide in 250 cc. of water. Preserve in a rubber-stoppered bottle and, when needed for use, add 1 cc. of bromine to 15 cc. of the conc. hydroxide solution. The hypobromite solution should always be freshly prepared.

Tincture of guaiacum. Dissolve 1 gram of guaiac resin in 60 cc. of 95% alcohol.

Töpfer's reagent. Dissolve 0.5 gram of dimethylaminoazo-benzol in 100 cc. of 95% alcohol.

Uffelmann's reagent. Add ferric chloride solution to a 1% phenol solution until the solution assumes an amethyst-blue color.

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