CIHM
Microfiche
Series
(Monographs)

ICMH
Collection de
microfiches
(monographies)

Canadian Institute for Historical Microreproductions / Institut canadien de microreproductions historiques

Technical and Bibliographic Notes / Notes techniques et bibliographiques

The Institute has attempted to obtain the best original copy available for filming. Features of this copy which may be bibliographically unique, which may alter any of the images in the reproduction, or which may significantly change the usual method of filming are checked below.

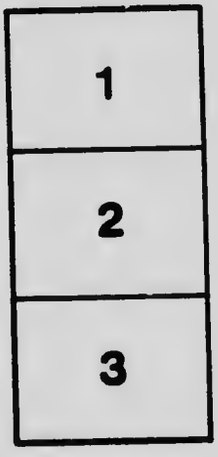
- [x] Coloured covers / Couverture de couleur
- [ ] Covers damaged / Couverture endommagée
- [ ] Covers restored and/or laminated / Couverture restaurée et/ou pelliculée
- [ ] Cover title missing / Le titre de couverture manque
- [ ] Coloured maps / Cartes géographiques en couleur
- [ ] Coloured ink (i.e. other than blue or black) / Encre de couleur (i.e. autre que bleue ou noire)
- [ ] Coloured plates and/or illustrations / Planches et/ou illustrations en couleur
- [ ] Bound with other material / Relié avec d'autres documents
- [ ] Only edition available / Seule édition disponible
- [ ] Tight binding may cause shadows or distortion along interior margin / La reliure serrée peut causer de l'ombre ou de la distorsion le long de la marge intérieure.
- [ ] Blank leaves added during restorations may appear within the text. Whenever possible, these have been omitted from filming / Il se peut que certaines pages blanches ajoutées lors d'une restauration apparaissent dans le texte, mais, lorsque cela était possible, ces pages n'ont pas été filmées.
- [ ] Additional comments / Commentaires supplémentaires:

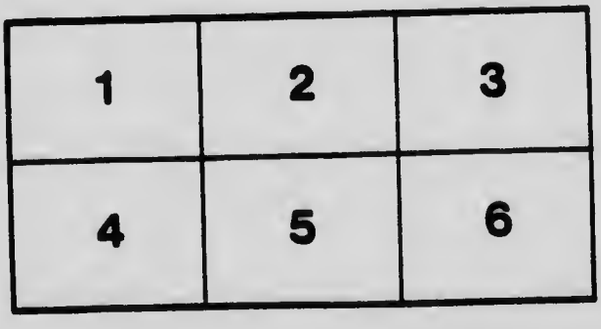
L'Institut a microfilmé le meilleur exemplaire qu'il lui a été possible de se procurer. Les détails de cet exemplaire qui sont peut-être uniques du point de vue bibliographique, qui peuvent modifier une image reproduite, ou qui peuvent exiger une modification dans la méthode normale de filmage sont indiqués ci-dessous.

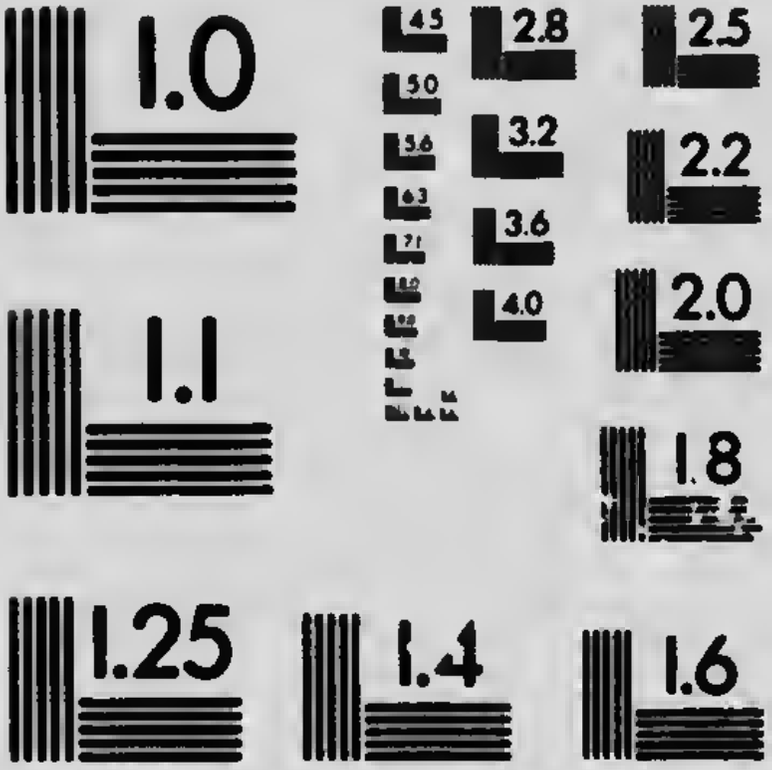
- [ ] Coloured pages / Pages de couleur
- [ ] Pages damaged / Pages endommagées
- [ ] Pages restored and/or laminated / Pages restaurées et/ou pelliculées
- [x] Pages discoloured, stained or foxed / Pages décolorées, tachetées ou piquées
- [ ] Pages detached / Pages détachées
- [x] Showthrough / Transparence
- [ ] Quality of print varies / Qualité inégale de l'impression
- [ ] Includes supplementary material / Comprend du matériel supplémentaire
- [ ] Pages wholly or partially obscured by errata slips, tissues, etc., have been refilmed to ensure the best possible image / Les pages totalement ou partiellement obscurcies par un feuillet d'errata, une pelure, etc., ont été filmées à nouveau de façon à obtenir la meilleure image possible.
- [ ] Opposing pages with varying colouration or discolourations are filmed twice to ensure the best possible image / Les pages s'opposant ayant des colorations variables ou des décolorations sont filmées deux fois afin d'obtenir la meilleure image possible.

This item is filmed at the reduction ratio checked below / Ce document est filmé au taux de réduction indiqué ci-dessous.

10x	12x	14x	16x	18x	20x	22x	24x	26x	28x	30x	32x
							✓				







To Professor Miller
with Dr Ruttan's
kindest regards

SYNOPSES OF LABORATORY COURSES

IN

I. ORGANIC AND BIOLOGICAL CHEMISTRY
II. CHEMISTRY OF HUMAN PHYSIOLOGY
III. CLINICAL CHEMISTRY
IV. ADVANCED BIOLOGICAL CHEMISTRY

GIVEN AT

McGILL UNIVERSITY, MONTREAL

BY

R. F. RUTTAN, B.A., M.D., D.Sc.

AND

V. J. HARDING, D.Sc.

SECOND EDITION

RENOUF PUBLISHING COMPANY
25 McGILL COLLEGE AVENUE
MONTREAL

2326

INTRODUCTION

THESE Synopses of Laboratory Courses in Chemistry directed to Medicine are here, for convenience, printed from the manifolded sheets formerly distributed to the students of the second and third years attending the Medical Faculty of McGill University.

By giving in the second year a course of lectures and laboratory work (Part I) on the application of Organic and Physical Chemistry to the study of life, followed in the third year by a course on the Chemistry of Human Physiology (Part II), it has been found that a better grasp of value of chemistry to pathology and medicine has been attained by the students than when the two courses are merged into one on General Physiological Chemistry. Part III, a simple short course on the application of elementary chemical tests to diagnosis, is given when the students begin their hospital work. Part IV is not given to all students. Only those wishing to prepare for research studies in metabolism and who have had special training in chemistry are admitted.

The writers have freely selected suitable experiments in these courses from many of the recent text

books on Biological and Physiological Chemistry. They are especially indebted to the excellent manuals of Drs. Plimmer, Hawk, and Cole, to which many references are made.

McGill University,
Faculty of Medicine,
September 1914.

CONTENTS

PART I

ORGANIC AND BIOLOGICAL CHEMISTRY

SYNOPSES OF THE COURSE OF TWENTY-SIX LABORATORY PERIODS IN ORGANIC AND BIOLOGICAL CHEMISTRY GIVEN DURING THE WINTER TERM OF THE SECOND YEAR IN MEDICINE.

PART II

CHEMISTRY OF HUMAN PHYSIOLOGY

A Laboratory Course of about Twenty-Five Periods given during the first term of the Third Year.

PART III

CLINICAL CHEMISTRY

Synopses of the Course of Twelve Laboratory Periods in Clinical Chemistry given at the end of the Winter Term of the Third Year.

PART IV

ADVANCED CLINICAL AND BIOLOGICAL CHEMISTRY

SYNOPSES OF AN OPTIONAL COURSE OF TWELVE OR FOURTEEN LABORATORY PERIODS IN ADVANCED CLINICAL AND BIOLOGICAL CHEMISTRY GIVEN AT THE END OF THE THIRD YEAR.

Synopses of Laboratory Courses

PART I

ORGANIC AND BIOLOGICAL CHEMISTRY

SYNOPSES OF THE COURSE OF TWENTY-SIX LABORATORY PERIODS IN ORGANIC AND BIOLOGICAL CHEMISTRY GIVEN DURING THE WINTER TERM OF THE SECOND YEAR IN MEDICINE

THIS short course of laboratory work is designed to supplement the regular course of lectures in Organic and Biological Chemistry.

Having regard to the limited time available, only those experiments have been selected which are easily and quickly performed and which are essential to the study of the chemical problems of Human Physiology and Pathology.

The whole course in Biological Chemistry is intended as a foundation for the more specialised courses in Chemical Physiology and Pathology given during the third year.

Constant reference to the reactions and tests studied in this course will be found in the synopses of the courses in Parts II and III, and a sound knowledge of them will be taken for granted.

R. F. R.

SYNOPSIS I

Detection of Elements in Organic Substances

Nitrogen.—(A) Triturate in mortar a portion of albumin size of a pea with about three times its volume of soda lime, and when well mixed add five times the volume of soda lime and mix thoroughly. Take half of this in a small dry test tube, heat and note the odour of gas ; test for ammonia with litmus.

(*Note.*—One mixture enough for two students.)

(B) Just fill the hollow of a small dry test tube with dry albumin, add a piece of metallic sodium size of a pea which has been pressed and dried with filter paper. Heat gently at first, using a test tube holder, then strongly until end of test tube is red hot and fumes cease to come off. Have 10–15 c.c. of water in a small beaker, drop gently the hot test tube in the water. It will break to pieces and the substance will dissolve in the water. Stir up well with the remains of the test tube, take care there are no pieces of sodium left in the test tube. The nitrogen and the carbon combine with the sodium to form sodium cyanide. Filter from the carbon and glass. If decomposition has been complete, the filtrate will be nearly colourless. Take a portion in a test tube and test for cyanide. Add a drop of a ferrous sol. and a drop of a ferric sol., boil, and when cool acidify with HCl ; a green to a blue colour or ppte. of Prussian blue is obtained. Write equations.

Sulphur.—The sulphur in albumin will be changed to sodium sulphide in preceding reactions. Take a second portion of the cool filtrate, add a drop of freshly-made sodium nitroprusside sol.; a violet colour shows presence of alkaline sulphide. Compare with colour obtained by adding a drop of this reagent to a test tube of water to which one drop of an alkaline sulphide has been added. To a third portion of the filtrate add a drop or two of lead acetate, acidify with acetic acid; a brown to a black colour or ppte. shows traces of lead sulphide.

Phosphorus (work in groups of four).—Mix thoroughly in a mortar a portion of caseinogen size of a small pea with enough fusion mixture (bottle marked flux) to nearly fill small crucible. Heat until fused and residue is white. (Flux is 1 sod. carbonate and 2 sod. nitrate.) The phosphorus is oxidised to phosphoric acid, and the sulphur to sulphuric acid. Detect sod. phosphate by dissolving the mass, after cooling, in hot water by placing the crucible and contents in a 100 c.c. beaker with about 20 c.c. of hot water and making acid with nitric acid; take a portion of the sol. in a t.t., add an equal vol. of ammon. molybdate, a few drops of nitric acid and boil; a canary yellow colour with opalescence or ppte. shows presence of phosphoric acid. Detect the sulphate of sodium by adding barium chloride to another portion after filtering; a ppte. or opalescence shows presence of sulphate.

The Halogen Elements (Beilstein's Test).—A piece of copper oxide wire wrapped in platinum wire is held in colourless Bunsen flame until flame is colourless. After cooling, a particle of the solid or a drop of the solution to be tested is placed on the oxide and again held in the flame; a bluish-green flame of the volatile copper halogen compound is obtained. Try a particle of chloral hydrate and a few grains of iodoform.

Iron (work in pairs).—Test blood sol. with potas. ferro-cyanide. Place a few particles of dry blood in a crucible, heat, at first slowly, then till red hot for some time; cool, add one drop of nitric acid, dissolve in 2 c.c. HCl, filter and add a few drops of potas. ferro-cyanide to filtrate; note Prussian blue.

SYNOPSIS II

Alcohols and Aldehydes

1. *Alcohols.*—Apply the following general reactions to methyl and ethyl alcohol and to glycerol, writing equations for each and explaining the reactions.

(*a*) To a test tube one-third filled with water add a few drops of the alcohol, about the same volume of potassium bichromate solution, and a few drops of sulphuric acid. Heat and note change in colour and odour of the aldehyde.

(*b*) To some solid sodium acetate in a test tube add a few drops of methyl or ethyl alcohol, and just

cover with sulphuric acid. Warm gently What is the odour? Write equations.

(The esters of glycerol are non-volatile, and hence have no odour.)

SPECIAL REACTIONS FOR THESE ALCOHOLS

(*a*) To a few drops of ethyl alcohol in a half test tube full of water add a few drops of strong iodine solution, Lugol's sol. and potas. hydrate until nearly decolourised. Observe there is no ppte. until it is warmed gently to about 40 or 50°. Note ppt'd. iodoform. Write equations. This reaction detects 1 part alcohol in 2000 water. Examine, and draw crystals of iodoform under microscopes 1 and 2.

(*b*) To 1 c.c. of glycerol in a dry test tube add half its volume of dry potass. sulphate. Heat to charring, and note odour of acrolein. Write equations.

2. *Aldehydes.*—PREPARATIONS :—

(1) Draw apparatus, and explain the method of preparing acetic aldehyde in quantity as demonstrated to the class.

(2) Fit a dry hard glass test tube with cork and delivery tube as instructed. Introduce dry calcium formate to depth of 2–3 ctm. Heat to dull redness, keeping the end of the delivery tube dipping under 2–3 c.c. of water in another test tube. Remove receiving test tube and contents. Label for future use.

(3) Clean a dry hard glass test tube, fit it with the same delivery tube, and repeat (*b*), using a mixture of

dry calcium acetate and calcium formate. Keep receiving test tube and contents as before. What aldehyde is formed in each case? Write equation.

PROPERTIES :—

Apply the reactions *a*, *b*, *c*, and *d*, to each of the following aldehydes, viz. formic (formaline), acetic, chloral, and glucose. Study each separately. Write equations, and explain what occurs. Note and explain special behaviour of chloral, with (*b*) and (*d*), also of formic aldehyde with (*d*).

(*a*) Add a few drops of the aldehyde and a few drops of an ammoniacal solution of silver oxide to half a test tube of water, warm it nearly to boiling point. What has happened to (*a*) the silver oxide, (*b*) the aldehyde?

(*b*) Dilute 1 c.c. of Fehling's solution with four volumes of water in a test tube, add a few drops of the aldehyde solution and boil. What is the ppte.? What has become of the aldehyde? Fehling's solution is a strongly alkaline solution of cupric hydroxide.

(*c*) To 1 c.c. of the dilute fuchsin solution (rosaniline) add solution of sulphurous acid until colour just disappears; add the aldehyde solution, and shake. A violet-red colour shows presence of aldehyde.

(*d*) Boil a little strong potas. hydrate solution, to which a few c.c. of aldehyde has been added. Note production of "aldehyde resin" in case of acetic aldehyde and glucose. Why not in all?

Apply the reactions (*a*) or (*b*) to the solutions prepared by distilling the calcium salts.

SYNOPSIS III

KETONES AND ORGANIC ACIDS

1. KETONES

Acetone.—Heat in dry hard glass test tube as in preparation of aldehydes, about 1 grm. of dry calcium acetate at first gently, then strongly, collecting distillate in a few c.c. of water in another test tube. Explain reaction and test distillate by iodoform reaction below.

Note odour of solution of acetone. Add two drops of this 1 per cent. solution of acetone to half a test tube of water. Add iodine solution and potas. hydrate, as in alcohol test. Note iodoform ppte. without heating. Give equations. Dilute the 1 per cent. solution of acetone with known volume of water, repeat the test and calculate quantity of acetone detected.

2. ORGANIC ACIDS

The following acids are to be employed in the reactions, viz. Formic, Acetic, Butyric, Stearic, Oleic, Lactic, and Salicylic.

Note appearance of pure specimens of each acid on centre table.

General reactions :—

(*a*) *Solubility in dilute alkaline carbonates.* To two-thirds of a test tube of sodium carbonate solution add about the volume of a pea of the acid, shake up,

warm if necessary, and each dissolves. Use Butyric, Stearic, Oleic, and Salicylic acids. Write equations.

(*b*) *Esterification.*—Add to about 1 c.c. of the acid, a few drops of ethyl alcohol and a few drops of sulphuric acid, shake up, warm slightly, and note odour. Use Acetic, Butyric, and Salicylic acids. These odours are characteristic and may serve as tests for the above acids.

Special reactions :—

A. *Formic Acid.*—(1) Heat about 1 c.c. of formic acid (note odour) with an equal volume of sulphuric acid. Is the gas combustible ? What occurs ?

(2) Add a few drops of ferric chloride to a solution of sodium formate. Boil. What is the ppte. ?

B. *Acetic Acid* (Ferric chloride test).—Prepare a solution of ammonium acetate by neutralising a little acetic acid in a test tube with ammonium hydroxide (adding a slight excess to litmus and boiling). To the cooled solution of ammonium acetate so prepared add one or two drops of ferric chloride. Note blood-red colouration. Boil a portion of solution: what is the ppte. produced ?

Compare this test with that for formic acid. This test is applicable to acetic or formic acids in the form of their neutral solutions only.

C. *Aceto-acetic Acid* (Diacetic acid).—(1) To a few c.c. of the solution provided add a drop or two of ferric chloride. Observe the violet-red colour of the ferric salt.

(2) To a few c.c. of the solution add an equal volume of glacial acetic acid, a few drops of nitro-prusside of sodium, and float on the surface some strong solution of ammonia. Note the violet-red colour at line of junction of the fluids.

(3) Take about 10 c.c. of the solution in a hard glass test tube, acidify with a drop or two of HCl, fit with a delivery tube as in Synopsis II, distil over slowly about 1 c.c. in a receiving test tube containing 2 or 3 c.c. of water. Keep receiving tube cold under tap. Test liquid in receiver for acetone. Is there any aceto-acetic acid in the solution left in the hard glass test tube? Test when cold. Explain what has occurred, giving formulæ.

SYNOPSIS IV

ORGANIC ACIDS (*continued*)

A. *Stearic Acid.*—I. Try solubility in water, alcohol, and ether. Pour a drop of the dilute alcoholic solution (hot) on a watch-glass and set aside to cool. Test reaction of alcoholic solution with litmus. Examine and sketch crystals in your watch-glass under microscope.

II. Place a piece the size of a bean with 20–30 c.c. of a solution of sodium carbonate in evaporating dish on water bath. Heat for half an hour or until dissolved, stirring and adding more water if dry. What indications of reaction? What is formed? Dissolve in 50 c.c. of hot water. Test for soap. (See A, III.)

III. Take 2 c.c. of soap solution provided, in each of three test tubes. To one add hydrochloric acid, drop by drop, until acid. What happens? Shake up with two volumes of ether. What occurs? To a second add an equal volume of calcium sulphate solution, and to the third add an equal volume of lead acetate solution. What is formed in each case?

IV. *Detergent Action of Soap.*—To two drops of strong soap solution add one drop of phenol phthalein. Note effect. Fill test tube with water, and mix. Why does soap solution give a strong alkaline reaction when diluted? Give formula. Explain the cleansing effect of soap.

B. *Oleic Acid.*—Add one drop to a solution of bromine in alcohol. Shake up. Why is the alcohol decolourised? Add a small particle of stearic acid to the alcoholic solution of bromine, and shake again. Is it decolourised? Why?

C. *Lactic Acid.*—I. To 1 c.c. of lactic acid in about 3 c.c. of water add little by little excess of dry zinc carbonate; boil until reaction ceases. Filter hot, and note crystals of zinc lactate in the filtrate when cold.

II. Warm gently about 1 c.c. of lactic acid with about 10 cc. potas. bichromate. Note odour of aldehyde and evolution of carbon dioxide. Is it a reducing agent?

III. Uffelmann's reaction. To a solution of Uffelmann's reagent add one drop of lactic acid. Note change of colour from violet to yellow.

D. *Salicylic Acid.*—I. Mix a mass the size of a bean of dry sodium salicylate with soda-lime in a dry test tube and heat strongly. What is the odour? Give graphic equation.

II. Dissolve a minute fragment of sodium salicylate in a test tube half full of water, and add a drop of ferric chloride. Note the colour. What is the salt?

SYNOPSIS V

Organic Bases

A. *Ammonia.*—I. Add potas. hydrate to a little solid ammonium chloride and warm gently. What happens?

II. To a crystal of ammon. chloride on watch-glass add one drop of water, and to the solution add one drop of platinum chloride solution. Give equation.

B. *Ethylamine.*—I. Note odour of the aqueous solution and reaction on red litmus.

II. Neutralise 1 c.c. in an evaporating dish with a drop or two of HCl, evaporate almost to dryness on water bath. What is left when cold?

III. To a fragment of residue in B II on watch-glass add a drop of platinum chloride. What occurs?

IV. Acidify 2 c.c. of ethylamine solution with acetic acid, and add a few drops of a solution of sodium nitrite. What gas is evolved? Give equation.

C. *Aniline.*—I. Note odour and colour. To a few drops on a watch-glass add two drops of strong HCl (end shelf). What occurs?

II. To about one gramme of hydrochloride add potassium hydrate. What occurs?

III. Repeat A II, using aniline hydrochloride. Give equation.

IV. To a drop or two of aniline in test tube add a minute fragment of solid potas. hydrate, 1 c.c. of alcohol and a few drops of chloroform; warm. Note odour of isocyanide. Write equation. Empty and rinse out test tube at once.

D. *Pyridine.*—I. Note odour and action of vapour on litmus.

II. To 1 c.c. in test tube add 2 c.c. of strong HCl (end shelf). What are the fumes?

III. Repeat A II, using a drop or two of D II. Write equation.

IV. Dilute 1 c.c. of the solution of pyridine with 10 c.c. of water. Test 1 c.c. of this dilute solution with one drop of (1) potas. mercuric iodide, (2) phosphotungstic acid, and (3) of tannic acid.

E. *Quinine.*—$C_{20}H_{24}N_2O_2$. I. Dissolve a portion of quinine sulphate size of a bean in 10–20 c.c. water, add one drop sulphuric acid. To 1 c.c. of this add a few drops of potas. hydrate. What occurs? Shake up with 2 c.c. of ether. Explain.

II. Repeat A II, using quinine, with a drop of HCl. Give equation.

III. Repeat D IV, using the solution E I largely diluted.

SYNOPSIS VI

Esters and Fats

(Two Students working together.)

Saponification.—I. Pour 50 c.c. of ethyl acetate in a 400–500 c.c. distilling flask, and add 50 c.c. of water and 50 c.c. of a 50 per cent. sodium hydrate solution provided. Add a few fragments of broken unglazed porcelain. Connect flask with reversed condenser as instructed. Heat slowly on a wire gauze until flask feels quite hot to the hand. If flask is now shaken, gently at first and then more vigorously, the saponification will proceed with little or no further application of heat. Continue shaking and warming until the two layers disappear, and then heat more strongly for about five minutes.

When nearly cold disconnect the reversed condenser and attach the flask for distillation. Distil over about 50–60 c.c. and test for ethyl alcohol by aldehyde and iodoform tests. (Synopsis II.)

Remove the stopper from flask, add a few drops of litmus solution, and add slowly sulphuric acid until strongly acid. Use a long-necked funnel to add to acid.

Distil over about 50 c.c. Test this for acetic acid. (Synopsis III.)

Make drawing of apparatus, and give equations for reactions.

II. Melt in an evaporating dish 5 grammes of

stearin, and add 5 c.c. of the 50 per cent. solution of sodium hydrate and about 25 c.c. of alcohol. Stir well, and boil gently on a water bath for twenty minutes. Shake a drop of the mixture in half a test tube of water; if no solid particles of stearin appear, saponification is complete. If incomplete, add 10 c.c. of alcohol, stir well, and continue heating until saponification is complete. Add about 10 c.c. of water, heat gently to boiling while stirring on wire gauze to expel the alcohol.

Pour the saponified mixture when still warm in a large beaker, add about 50 c.c. of saturated salt solution; allow to cool. Separate the solid soap, and wash with a little cold water.

What is in the aqueous saline solution? Give equation for reaction.

REACTIONS OF FATS

A drop of warm fat or fat acid, or of a dilute ethereal solution on filter paper leaves permanent translucency.

Acrolein Reaction.—A few drops of fat or oil in a test tube with an equal volume of dry acid potas. sulphate, heated at first gently, then more strongly until it begins to char; note odour of acrolein. Repeat, using (1) stearic acid. Explain reaction. (See Synopsis II.)

To a particle of fat in a watch-glass add a drop of the dye called Sudan III; after standing a few

minutes wash with water. Note the permanent stain of the fat.

Examine section under microscope showing staining of fat in heart muscle.

Osmic acid blackened by olein, not by saturated fat acids and their esters. Compare action of a drop of osmic acid upon a drop of olein and a particle of pure stearin.

Shake 2 or 3 c.c. of ether with a few drops of bromine water; try effect of shaking this mixture with a drop or two of olein; repeat, using a minute particle of stearin. Explain. (See Synopsis III D.)

Warm in a test tube, with shaking, a few drops of olein with sodium carbonate solution. Repeat, using oleic acid. Compare effect. Explain. (See Synopsis III, 2 (*a*).)

Write clearly (1) how you would distinguish a fat acid from a fat; (2) how you would detect olein in a mixed fat.

SYNOPSIS VII

Esterification, Fats, and Emulsions

1. *Esterification.*—Effect of temperature upon the rate of reaction.

From burette measure exactly 11.6 c.c. (equal to 9.2 grms.) of absolute alcohol in small bottle, and add 11.4 c.c. (equal to 12 grms.) of glacial acetic acid; shake up. Take 1 c.c. of mixture measured carefully

with dry pipette, and determine the acidity, using phenol phthalein and fifth normal sodium hydrate.

Occupants of alternate benches will suspend their bottles in the water bath at 40° C. after tying down the stoppers as instructed. Watch temperature carefully. At the end of the period (12.30), again determine the amount of acetic acid in 1 c.c. (*N.B.*—The other bottles to be kept on shelf at room temperature.) The bottles from the warm water must be cooled under a tap before opening, being careful to allow no moisture to remain about the stopper or neck of the bottle. Close bottles at once as before, and place in thermostat at 40° C. until next laboratory period. Those who keep their bottles at room temperature will, after the second titration, place theirs in their cupboards until next laboratory period. Compare all titration results with your neighbour, and record results. (*N.B.*—When measuring with the pipette be careful to drop any excess of the mixture back into the bottles, as a record must be kept of the exact volume taken from the mixture.) (See Synopsis XIX.)

2. *Emulsification.*—Put 1 c.c. of a solution of sodium carbonate in watch-glass, add a drop of rancid olein from a rod. Note white rim and opacity about drop. What is it?

Examine at once under 1-inch objective; what causes activity in vicinity of drop? What is the gas?

In a test tube two-thirds full of water, shake up two drops olein.

In another test tube two-thirds full of sodium carbonate solution, shake up two drops of olein.

To a third two-thirds full of sodium carbonate solution add two drops of rancid oil.

To a fourth two-thirds full of albumin solution add two drops of olein.

To a fifth two-thirds full of soap solution add two drops of olein.

To a sixth two-thirds full of gum arabic solution add two drops of olein.

Shake each thoroughly; note and explain condition after standing five minutes in test tube rack.

Examine a drop of milk and a drop of good emulsion under microscope. What are the conditions which bring about emulsification?

3. *Acidity of Rancid Fat. Acid Equivalent of Fats.*—Measure exactly, into a small flask, 10 c.c. of rancid oil from burette (allow time for oil to run down from sides of burette). Add 10 c.c. of alcohol and two drops of phenol phthalein.

Run in from your burette decinormal sodium hydrate solution until permanent pink colour is obtained; shake continually.

Calculate as free oleic acid, the acidity of 100 c.c. of rancid olein.

SYNOPSIS VIII

CARBOHYDRATES

Esterification (continued).—Determine the acidity of the mixture of acetic acid and alcohol left from last period, using 1 c.c. and observing the same care in manipulation. Note the effect of temperature on the rate of reaction by comparing the mixture kept under the different conditions.

Replace the bottles as before for further study at a later period.

A. PENTOSES—ARABINOSE

1 *Orcin Test. Tollen's Reaction.*—To 2 c.c. of arabinose solution add an equal volume of strong HCl and a few grains of solid orcin. Boil for a minute and note colour, reddish blue to green, and a ppte. of green pigment.

Allow to stand, pour off the liquid and dissolve the ppte. in a little amyl alcohol. Note colour and examine with spectroscope. Bands between C and D.

2. *Bial's Test.*—Boil about 5 c.c. of the reagent (500 c.c. HCl, 1.5 grm. orcin, with 25 drops of ferric chloride). Add 2 drops of solution of pentose to the reagent while boiling, and remove at once from the flame. Note green colour without further heating.

3. *Reducing Action.*—To 1 c.c. of Fehling's solution (cupric hydroxide in solution) add 1 c.c. of pentose solution and four volumes of water. Boil and note reduction of the copper solution. Explain the reaction.

B. HEXOSES—GLUCOSE

1. *Molisch's Reaction.*—To a few c.c. of the glucose solution in a test tube add a drop or two of an alcoholic solution of alpha naphthol; underlie this with about 2 c.c. of strong sulphuric acid. Note reddish-violet colour at line of junction.

Repeat the reaction, using a few drops of an alcoholic solution of thymol. Compare colours.

Dilute solution of glucose, 1 to 100, and repeat test.

Repeat Molisch's reaction upon water, to which a few drops of furfurol solution has been added. Explain the reaction.

2. *Moore's Test.*—Boil 2 or 3 c.c. of the solution with equal volume of sodium hydrate solution. What is formed?

3. *Silver Mirror Test.*—Boil a diluted solution of glucose with a few drops of ammoniacal silver oxide. What has occurred? Represent equation. (See Synopsis II 2 (*a*).)

4. *Fehling's Test.*—To about 1 c.c. of Fehling's solution (cupric hydrate in solution) add 1 c.c. of sugar solution; dilute with four volumes of water, and boil. Give reaction in full. (See Synopsis II, 2 (*b*).)

5. *Nylander's Test.*—Add about one-eighth the volume of the solution to be tested of Nylander's solution (bismuth hydrate in solution), and boil; note carefully the gradual change in colour from yellow to black. Why?

Compare delicacy of Nylander's and Fehling's solutions in detecting traces of glucose. Dilute the 2 per cent. solution provided quantitatively, and find least quantity you are able to detect with each reagent.

In using Nylander's solution to detect minute traces, add 1 c.c. of the reagent to 10 c.c. of the solution to be tested.

SYNOPSIS IX

CARBOHYDRATES (*continued*)

1. GLUCOSE (*continued*)

A. Fermentation Test.—Mix about 10 c.c. of the solution with a piece of compressed yeast size of a pea until the yeast is thoroughly distributed in the mixture. Fill a fermentation tube, and stand in a warm place (fermentation cupboard) (75–80° F.—24–26° C.).

Note accumulation of gas (what ?) in closed arm of tube. Place number on tube, and put aside until next day, when liquid will be filtered and tested for ethyl alcohol.

B. Phenylhydrazine Reaction.—In a medium-sized test tube pour some solid phenylhydrazine hydrochloride to depth of half an inch ; add an equal volume of solid sodium acetate, fill test tube two-thirds full of solution to be tested ; carry out the reaction in duplicate, using two test tubes and a 2 per cent. solution in one and a 0.2 per cent. solution in the other. Shake up thoroughly, place tube in boiling

water bath, heat for half an hour; shake up or stir thoroughly two or three times after it becomes hot. Allow to cool slowly, and note appearance when cold.

Examine crystals of phenyl glucosazone under the microscope; sketch crystals from three or four reactions. Represent the formula. Why should sodium acetate be added?

II. LÆVULOSE

Apply to the solution of lævulose (1) Fehling's reduction test, (2) fermentation test, (3) Nylander's test, (4) phenylhydrazine reaction. Give formula for the osazone and examine it under microscope.

5. *Ketose Reaction.*—To a few c.c. of lævulose solution add an equal volume of strong hydrochloric acid and a few crystals of resorcin. Treat a solution of pure glucose in the same way. Place both test tubes in a water bath, heat for a few minutes; compare colours. Boil lævulose mixture over flame; dissolve the brown ppte. in alcohol; note colour of the solution.

III. GLUCOSE: QUANTITATIVE DETERMINATION BY BENEDICT'S METHOD

25 c.c. Benedict's solution are reduced by 0.05 grm. glucose. Measure with pipette 25 c.c. Benedict's solution into a clean flask of about 200 c.c. capacity, add 1–2 grms. (measured) of anhydrous sodium carbonate and a few pieces of broken porcelain.

Dilute the glucose solution 1 in 10 (measure 10 c.c. of the solution by pipette into 100 c.c. cylinder and make up to containing mark with water), and fill burette.

Note.—It is not always necessary to dilute the glucose solution to that particular strength. The most convenient dilution in any particular case can only be found by previous experiment. It is important that at the end of the determination the solution shall not have become too bulky or too concentrated.

Boil the Benedict's solution, and while still boiling run in the sugar solution from the burette, rapidly at first until a heavy chalky ppte. appears and the blue colour is perceptibly paler, and then more slowly. The solution must be kept vigorously boiling throughout the titration. The end point is reached when the Benedict's solution becomes colourless. Note number of c.c. sugar solution required, and calculate the percentage of glucose in the original solution.

SYNOPSIS X

Catalysis and Use of the Polariscope

1. *Catalytic Action of Hydrogen Ions.*—Measure exactly with pipette 1 c.c. of methyl. acetate in small stoppered bottle, add 20 c.c. (using pipette) of dilute acid, half the class using 0.4 per cent. hydrochloric acid and half using 0.2 per cent. HCl. (See instructions on board.)

After adding the acid shake up thoroughly, and determine immediately the acidity of 5 c.c. of the mixture.

Half the class, as designated, will keep their bottles in water bath at 40° C., the other half will keep their bottles at room temperature; bottles to be shaken frequently, and at the end of the period the acidity again determined, using 5 c.c. as before. All bottles to be again replaced as before until next laboratory period.

Compare results with control experiment (demonstrated) consisting of 1 c.c. of methyl acetate and 20 c.c. of distilled water kept at laboratory temperature and at 40° C.

2. Determine the percentage of glucose in the solution of commercial glucose syrup provided, using Benedict's method.

3. *Use of Polariscope.*—Make a reading of the percentage of glucose in the solution of commercial glucose syrup provided directly, using the Reichert clinical saccharimeter. Why does the percentage found by the saccharimeter not agree with that obtained by Benedict's solution?

Compare reading of a solution of lævulose and one of glucose of similar concentration, using Schmidt and Haensch Polariscope.

Write full account of use of the clinical saccharimeter.

4. *Specific Rotation.*—Make a reading, using a large

polariscope of a 5 per cent. solution of a carbohydrate provided in a 5 cm. tube. Calculate its specific rotation.

SYNOPSIS XI

Glucosides and Disaccharides

1. *Catalysis and Hydrolysis* (*continued*).—Determine the free acid in the methyl acetate and hydrochloric acid, using 5 c.c. as before. Record results and conclusions regarding the effects of temperature and concentration of the catalyser on the rate of reaction.

2. *Glucosides—Salicin.* (See lecture notes and text-book on Glucosides.)

Note appearance. It is easily soluble in water. Note the lævorotatory action of the aqueous solution in polariscope. Does it reduce Fehling's solution?

Measure 20 c.c. of the 2 per cent. solution in a small flask; add about half the volume of hydrochloric acid and boil for two minutes. Cool, filter off the insoluble salicylic alcohol (and saliretin); neutralise the filtrate. Examine the solution in the polari-

[illegible]e. Has its action on polarised light changed?

[illegible] the solution for glucose by Fehling's solution.

[illegible]ain fully the hydrolysis of salicin.

Calculate the quantity of glucose present in the 20 c.c. of hydrolised salicin.

Add a few drops of potassium bichromate and a drop or two of sulphuric acid; boil, and note odour

of "Meadow Sweet" (salicylic aldehyde). Explain reaction, giving formula.

3. *Cane Sugar—Saccharose.*—I. Apply Molisch's reaction (after diluting 1–10), the phenylhydrazine reaction, and Fehling's test to solution of cane sugar. Explain why cane sugar differs from glucose and lævulose.

II. To 20 or 30 c.c. of cane sugar solution add 8 or 10 drops of hydrochloric acid, and boil gently for two minutes. When solution is cool make it neutral or faintly alkaline, and repeat test with Fehling's solution or phenylhydrazine. Explain results.

III. Examine a 3 per cent. solution of cane sugar by the polariscope before and after treatment with dilute acid. Explain result. What is invert sugar?

IV. Apply ketose reaction. Why is the reaction positive with saccharose?

SYNOPSIS XII

DISACCHARIDES (*continued*)

I. *Multirotation* (*Mutarotation*).—Demonstrated.

Prepare a 5 p.c. solution of pure glucose in cold water; read its rotation at once, and again at intervals of ten minutes, for three hours. Plot the results in a curve on the board. Note results, and explain.

II. *Maltose and Lactose.*—1. Test very dilute solutions of each with Molisch's reaction.

2. Use 2 p.c. solutions of each, and test with Fehling's solution.

3. Prepare the osazones of glucose, maltose, and lactose in three test tubes, continue the heating for half an hour, and note that the maltosazone and the lactosazone are not precipitated while the mixture is hot.

Examine each osazone under the microscope. Sketch and compare with drawing.

4. Try fermentation test with solutions of lactose and maltose.

5. Apply Barfœd's test to maltose, lactose, and glucose, 2 p.c. solutions; 5–6 c.c. of reagent in test tube (copper acetate in dilute acetic acid), add 5 or 6 drops of sugar solution. Heat in boiling water about five minutes, place in rack to cool. Note which shows reduction. This test distinguished monosaccharides from disaccharides, especially lactose from glucose.

6. Determine by Benedict's solution the percentage of lactose in solution, 25 c.c. Benedict's=0.068 gramme of lactose.

7. Boil 10 c.c. of 10 p.c. solution of lactose with 6 c.c. of nitric acid (reagent) for a few minutes. Pour into evaporating dish. Evaporate on water bath to about 3 c.c., stirring frequently; add about 2 c.c. water, cool; crystals of mucic acid appear after an hour, or if left standing until next period. Explain reaction.

SYNOPSIS XIII

POLYSACCHARIDES

A. *Starch.*—1. Examine the slides of the starch granules under microscopes 1, 2, and 3. Draw the three varieties; compare with illustrations; note effect of polarised light upon potato and wheat starch (microscope 4).

2. Starch Paste. Heat to boiling in a beaker 100 c.c. of water; rub up in small evaporating dish one gramme of starch with a little cold water. While water is boiling run in the milky mixture a little at a time, stirring thoroughly; continue boiling for a minute after the starch has been all added.

(*a*) Examine a drop microscopically.

(*b*) Apply Molisch's reaction to starch solution greatly diluted.

(*c*) Test the starch solution with Fehling's solution.

(*d*) Add one drop of the starch solution to half a test tube of water and one drop of dilute iodine solution; heat in water bath; cool again; boil for half a minute; again cool. To another very dilute solution of starch add a few drops of potas. hydrate and then a drop of iodine. Make acid with a few drops of hydrochloric acid. Note effects in each case and draw conclusions.

(*e*) Add a few drops of tannic acid to a few c.c. in a test tube. What occurs?

(*f*) Take 50 c.c. of the starch paste in small flask;

add about 2 c.c. of dilute (reagent) hydrochloric acid and bring the mixture to the boiling point. Draw up about 1 c.c. (rough pipette), cool, dilute with water, divide into two, apply iodine test to one and Fehling's solution to the other, after making alkaline. Repeat at intervals of five minutes and number the test tubes. Keep the solution just boiling. Place the test tubes in order, and make a record of the changes up to half an hour. Note roughly how the reduction increases. After half an hour neutralise and try phenylhydrazine test. What sugar is present? Explain the changes that have occurred.

(*g*) Diffusibility of starch solution (demonstrated). Test the fluid outside the dialyser for starch and chlorine ions. Dialyser contains a starch paste and sodium chloride solution; it was placed in distilled water.

B. *Inulin.*—Try Molisch's, Fehling's, and Iodine tests. Hydrolyse solution as in A (*f*). What is formed by hydrolysis of Inulin? Apply Ketose reaction to the hydrolysed solution.

C. *Dextrine.*—Apply to the 1 p.c. solution of dextrine provided—

I. The Iodine reaction.

II. Fehling's solution and Barfœd's reagent.

III. Tannic acid.

IV. Hydrolyse the dextrine solution, using 25 c.c. and 2 c.c. of hydrochloric acid. Compare with starch reactions as above. Apply tests every two minutes as in the starch hydrolysis.

Keep careful notes of results. Hydrolysis will probably be complete in fifteen minutes. What sugar is present?

SYNOPSIS XIV

POLYSACCHARIDES (*continued*)

A. *Glycogen.*—1. To 1 or 2 c.c. of glycogen solution add a drop of sodium chloride solution, and a drop or two of dilute iodine solution. Repeat with dextrine solution and compare colours.

2. Try Molisch's reaction.

3. Test 1 or 2 c.c. with a few drops of Fehling's solution. Does it reduce?

4. Boil 1 or 2 c.c. with a few drops of HCl for a minute, make alkaline and test with Fehling's solution again. Note and explain result.

B. *Lichenin* (Iceland moss).

Dissolve a [illegible]-like piece of lichenin about two inches long, after rubbing up, in a test tube of water by boiling; allow to cool. What has occurred? Test a portion with Fehling's solution. Try Molisch's reaction.

Take in small flask about 40 c.c. of the solution provided, add 2 c.c. of dilute HCl, and keep boiling for ten minutes, adding a little water to keep the volume nearly constant. Cool, make alkaline, again test for reducing sugar with Fehling's solution.

C. *Cellulose.*—1. Test solubility of absorbent cotton

or a piece of filter paper in alcohol, boiling water, dilute hydrochloric acid, and dilute potassium hydrate.

2. Dissolve a sheet of filter paper in about 5 c.c. of cold strong sulphuric acid in a flask; dilute to about 200–300 c.c., boil for an hour, adding water if necessary. Cool, make a portion of it just alkaline with potas. hydrate, using particles of the solid caustic potash at first, then use the solution. Test for glucose.

3. Add a drop of dilute iodine to some pure cotton fibre.

4. Mix 5 c.c. strong nitric acid with 10 c.c. strong sulphuric in small beaker; when cold immerse with a rod some absorbent cotton, stir it about slowly with rod for about a minute. Take out, wash several times with fresh water to get rid of all acid, then once with alcohol; squeeze thoroughly, then spread out to dry at warm temperature. What has been formed? When dry dissolve half in smallest quantity of a mixture of 3 volumes of ether and 1 of alcohol. Evaporate a little of the solution on your hand; what is left? What is collodion? Try the inflammability of the other portion as compared with the original cotton.

5. Heat some absorbent cotton in a test tube with Schweitzer's reagent (copper hydrate dissolved in ammonia); when completely dissolved acidify with acetic acid; note amorphous ppte. of cellulose.

6. Try Molisch's reaction on a strip of filter paper in a test tube.

TABULATED RESULTS OF STUDY OF CARBOHYDRATES

Fill out the following chart at home with reactions of the various carbohydrates, using + and − to signify a reaction or absence of reaction. Take information from your laboratory notes.

	Arabinose	*Glucose*	*Lævulose*	*Lactose*	*Maltose*	*Saccharose*	*Starch*	*Dextrine*	*Glycogen*	*Inulin*	*Lichinin*	*Cellulose*
Solubility												
Iodine												
Fehling's												
Nylander's												
Barfœd's												
Molisch's												
Osazone												
Rotation												
Fermentation												
Remarks on diffusibility												
Appearance of solution												
Taste, mixed reactions, etc., from notes												

SYNOPSIS XV

COLLOIDS

N.B.—All apparatus used in these experiments must be scrupulously clean and rinsed out with distilled water just before use. Use only distilled water at end of bench for dilution, &c.

SUSPENSOIDS

Colloidal Prussian Blue.—To a test tube full of water add 1 drop of ferric chloride solution; to another 1 drop of potassium ferrocyanide solution. Pour these together in a small beaker; a blue colloidal solution of Prussian blue results with no ppte. Shake up a few c.c. in a test tube, no frothing, absence of viscosity. Dilute with an equal volume of water, and add an equal volume of an electrolyte magnesium chloride solution, shake up place in test tube rack and observe ppte. on standing.

Each student will prepare one of the following suspensoids as directed:—

Colloidal Antimony Sulphide.—Take 50 c.c. of solution of "tartar emetic" (what is it?) on centre table, and add to it 50 c.c. of the dilute ammonium sulphide solution. (The antimony solution is 2 per cent. and the ammonium sulphide 1 of strong solution in 25 of water.) Note the colour and fluorescence after a few minutes. After colour appears fill a clean burette

with a portion of it. This suspensoid carries a negative charge.

To 2 or 3 c.c. in a test tube add an equal volume of dilute hydrochloric acid. Note ppd. sulphide after standing.

Colloidal Silver.—Run in 5 c.c. of 1 per cent. silver nitrate from burette on side bench in a clean flask or beaker, add two drops of NH_4OH, and then dilute with 100 c.c. of water. Add 1 c.c. of tannin solution from burette to 100 c.c. of water, and mix the two solutions. A yellowish-brown solution of colloidal silver is produced. This suspensoid is negatively charged. After standing about ten minutes, put in clean burette and reserve for future use. To a few c.c. in a test tube add some barium chloride. Note pptd. silver on standing.

Colloidal Arsenic Sulphide.—Boil about 2 grammes of arsenious acid with 150 c.c. of water for a few minutes. Cool under tap, and filter. Pass H_2S through the cold solution; it gradually turns yellow, with a greenish fluorescence. Filter any ppte. which may appear after standing a few minutes, and place solution in a clean burette. This suspensoid is negatively charged.

To 2 or 3 c.c. of the colloid solution add an equal volume of sodium chloride solution. Note ppte. of arsenic sulphide.

Colloidal Ferric Hydroxide.—Heat about 100 c.c. of water to boiling in a beaker or flask, and while

boiling add 1 c.c. of 33 per cent. $FeCl_3$ from burette on side bench. The colour at once changes to a brownish red solution of colloidal ferric hydroxide. Allow to cool and place in burette for future use. This suspensoid is positively charged.

To 2 or 3 c.c. of the colloid solution in a test tube add an equal volume of sodium sulphate solution. Observe ppte. of ferric hydroxide on standing.

Note.—Electrolytes precipitate colloids because the ions of opposite electrical sign neutralise the charge on the suspensoid particles, and, being no longer held apart by their electrical charges, collect in large masses which are pptd. Thus, ferric hydroxide is pptd. by the anions, and silver and arsenic sulphide are pptd. by kations.

Precipitation of Suspensoids of Opposite Electrical Sign.—To 5 c.c. of the ferric hydroxide solution add little by little from a burette some of suspensoid of arsenic sulphide, silver or antimony sulphide, which are negatively charged, shake after each addition until a ppte. appears. Allow to stand. Both suspensoids are pptd.

Repeat using the suspensoid of silver with either arsenic or antimony sulphide; no ppte. is formed as they have similar electrical charges.

Dialysis.—(1) Test a few c.c. of the solution surrounding the dialyser containing the suspensoid ferric hydroxide for iron (a drop of HCl and a drop of ferrocyanide of potassium).

(2) Note the colour of the water in which the dialyser containing congo red is placed. Is there any marked colour? Compare with the water about the dialyser containing fuchsine. Which is a colloid?

Ultra-Microscope.—Record the appearance of the suspensoids of silver and gum tragicanth under ultra microscopes 1 and 2, also the ultra particles of ammonium chloride under ultra-microscope 3. Note that particles of the suspensoid silver are invisible using high power, one-twelfth immersion, under microscope 5.

SYNOPSIS XVI

Colloids (*continued*).—Surface Tension

Note the colours of the colloid solution of gold on central table and the "Tyndall effect." To what is this due?

Emulsoids

Add some barium chloride solution to half a test tube full of the suspensoid ferric hydroxide (side bench), shake up, and allow to stand. Shake up half a test tube full of starch solution, which is an emulsoid; the froth shows high viscosity. Add ammonium sulphate in powder little by little, shaking until a ppte. is obtained, allow to stand a few minutes, pour off the supernatant liquid, and add water. Does the ppte. dissolve? Try the solubility of the ppte. of ferric hydroxide. Will that dissolve in water again? Suspensoids are irreversible colloids, emulsoids are

reversible, *i.e.* they will redissolve after pptn. by electrolytes or alcohol.

Hydrosol and Hydrogel.—Pour a few c.c. of a hot solution (5 per cent.) of gelatine in a test tube, shake up, note the viscosity. Cool under the tap; it becomes a jelly, the hydrosol becomes a hydrogel.

Permeability of Hydrogels.—Take two test tubes containing agar-agar in state of hydrogel (one pair provided for each section). Into one pour some suspensoid of Prussian blue, into the other some ammoniacal copper sulphate solution (a crystalloid). Place the tubes in the rack, and at the end of the period empty the liquid from the top of the gel, and wash with a little water. Which has penetrated the hydrogel?

Examine the stratified effect in gels of penetration and precipitation. See tubes on central table (the Liesegang effect).

Protective Effect of Hydrosols.—To a few c.c. of a suspensoid of silver add a little gelatine solution; shake up, and then add barium chloride solution. Is the silver precipitated?

Surface Tension

Fill one test tube with water, and another with soap solution; see that there is no froth on the soap solution. Place in test tube rack, and on each sprinkle a few grains of sulphur in fine powder. Note result. Which has the higher surface tension?

Fill another test tube with dilute solution of bile salts. Does the sulphur easily sink in this? (Hay's test for bile salts in urine.)

ADSORPTION

(1) Test solution of arsenious acid for arsenic (a drop of hydrochloric acid and solution of hydrogen sulphide). To one-third test tube full of the arsenic solution add an equal volume of the strong colloidal ferric hydroxide ("dialysed iron"); shake up thoroughly, filter, and test filtrate for arsenic. Why is the arsenic taken up? (Antidote for arsenic.)

(2) Test the solution of serum albumen for proteins; by acidifying and boiling a little in a test tube, the protein is coagulated.

To 10 c.c. of the protein solution (a hydrosol) add 2 c.c. of the strong colloidal ferric hydroxide. Shake up, and add powdered sodium sulphate little by little, shaking after each addition until a gelatinous ppte. forms. Filter and test the filtrate for protein. Why was a ppte. formed when sodium sulphate was added? Why was the colloid taken up?

SYNOPSIS XVII

AMINO ACIDS

Represent the structural formulæ for Glycine, Tyrosine, Leucine, Tryptophane, Serine, and Cystine.

REACTIONS OF AMINO ACIDS

Glycine and *Glycine solution* provided.

1. *Distinction from Acid Amides.*—Boil about 5 c.c. of glycine solution with an equal volume of potas. hydrate, no ammonia is evolved. Boil 5 c.c. of solution of acetamide with potas. hydrate. Can you detect the ammonia? Give reaction.

2. *Nitrous Acid Reaction.*—To 2 c.c. of the glycine solution add 5 or 10 drops of a solution of sodium nitrite, and make acid with acetic acid or dilute HCl. What gas is evolved? Give equation.

3. *Salts.*—To 2 or 3 c.c. of the glycine solution add a few drops of a dilute solution of copper sulphate; note deepening of the blue colour. For comparison add a few drops of the copper solution to a 2 or 3 c.c. of water.

Write formulæ for the copper salt of glycine and glycine hydrochloride. Note appearance of the copper salt of glycine in specimen bottle.

4. *Ninhydrin Test.*—To 10 c.c. of the solution containing alpha—amino acids—labelled amino acid solution—add 5 drops of a 1 per cent. solution of ninhydrin. Boil for one minute. The solution on standing will pass from pale yellow to deep blue.

Ninhydrin reacts only with substances containing the amino group in the alpha position. Try the reaction, using acetamide solution.

5. *Estimation of Amino Acids.*—Dilute 10 c.c. of

formalin with two volumes of water in a 200 c.c. flask, and add 7 or 8 drops of phenol phthalein solution, add decinormal caustic soda from burette drop by drop until the mixture is just permanently alkaline. Neglect this burette reading. Measure in with a pipette 20 c.c. of the solution of glycine. The mixture becomes colourless. Now note burette reading, and run in the decinormal alkali until the pink colour again appears, and read the number of c.c. of alkali required. If 8 c.c. were used, then 20 c.c. of the glycine solution contained 8 c.c. of decinormal glycine. Calculate the percentage of glycine in the solution. If a mixture of amino acids is present, the results are stated in terms of "Nitrogen as amino acids." Repeat titration, using the solution of peptone provided, and state results in terms of nitrogen as amino acids.

This formaldehyde method is also employed for the estimation of ammonia. (See Urine, Part II.)

SYNOPSIS XVIII

Detection of Unknown Carbohydrate

1. Examine solutions marked A, B, C, and D. Determine the carbohydrate present. For this purpose proceed as follows: If solution is alkaline, acidify faintly with HCl and test with iodine.

(A) Blue colour—Starch.

(B) Reddish colour—Dextrin or Glycogen.

(C) No colour—a Sugar, Inulin, or Lichenin.

B. Note appearance of solution—clear, transparent, is Dextrine; opalescent, Glycogen. A few drops of basic lead acetate gives ppte. with Glycogen, none with Dextrine. Try Tannic acid also.

C. I. Apply Fehling's solution. It is reduced—Dextrose, Lævulose, Maltose, Lactose. Apply Barfœd's test—positive, Dextrose or Lævulose; Maltose, very faint. Apply the Ketose test, using water bath—negative, Maltose or Lactose. Maltose ferments with yeast in one hour; Lactose negative.

II. No reduction with Fehling's solution, Saccharose, Inulin, Lichenin, Agar-agar. Invert it, 5 c.c., two drops of HCl, boil one minute; cool, neutralise; reacts with Fehling's—Saccharose. Does not react with Fehling's or gives very faint reduction, Inulin or Lichenin; original solution opalescent and limpid—Inulin; original solution is viscid or gelatinous—Lichenin or Agar-agar. Work out the three problems on the board.

2. HYDROLYSIS OF ACETANILIDE

A. In a small flask place two-thirds of a test tube of solid acetanilide, add a test tube full of strong sodium hydrate, connect with a condensing tube and distil gently. Collect half a test tube of distillate; describe it. Note reaction with litmus. Test a portion of it with chloroform and alcoholic potash for Amino group. (See Synopsis V, C IV.)

Test for aniline by dissolving 1 or 2 drops in half a test tube of water and adding bleaching powder solu-

tion drop by drop; observe purple colour (see notes on aniline). Add water to residue in distilling flask, and then make a portion of it just neutral with HCl, and test for sodium acetate.

B. Clean flask and tube, and repeat, using a similar quantity of acetanilide but distilling with two test tubes full of dilute (1–3) sulphuric acid. Note odour and reaction of distillate. Neutralise and test for acetic acid. What is left in the retort? Dissolve a little of this residue in a test tube of water, divide into two parts; add a drop of potas. bichromate to one and a drop of bleaching powder solution to the other. Describe the results. What does this show?

Represent reactions A and B graphically.

SYNOPSIS XIX

Reactions of Proteins—Colour Reactions of the Protein Nuclei

Concentration Law.—Determine acidity of the ethyl acetate mixture, from Synopsis VII, using 1 c.c. as before; tabulate the results of the four titrations. Calculate the percentage of acid and alcohol converted at this last estimation.

Calculate the volume of the reaction mixture left; add an equal volume of distilled water, shake up, and replace bottle until next period. Then titrate 2 c.c. of mixture (equal 1 c.c. of original), and state effect of the water. What is the percentage change?

1. BARDACH'S IODINE REACTION FOR ALL PROTEINS

To 5 c.c. of albumin solution add 2 to 3 drops of dilute solution of acetone and solution of iodine until mixture is just reddish-brown; then just decolourise by an excess of ammonium hydrate. Keep test tube in rack until crystals appear; examine under microscope. What do crystals of iodoform look like? Compare with those found.

2. THE BIURET COMPLEX

To equal volumes of egg albumin solution and potas. hydrate add drop by drop a very dilute copper sulphate solution until purple-violet colour appears. Note carefully for future comparison colour obtained with egg albumin.

3. THE PHENYL GROUP

To 2 or 3 c.c. of egg albumin solution add about ten drops of strong nitric acid. Boil; white ppte. turns yellow, dissolves to a yellow solution. Cool solution, and add excess of ammon. hydrate; note change of colour. Xanthoproteic reaction. What common amino acids are identified by this reaction? Give formulæ.

4. THE PHENOL GROUP

Add a few drops of Millon's reagent to 5 c.c. of a solution of phenol; boil, note colour. Repeat, using

thymol or naphthol. What is the formula for thymol? Look for phenol group in egg albumin solution provided. What amino acid is detected by this reaction?

5. TEST FOR TRYPTOPHANE

Mix equal volumes solution of egg albumin and glyoxylic acid solution in test tube; float up the mixture with strong sulphuric acid by use of a pipette. Note violet-red colour at junction. Hopkins-Cole reaction. What is formula for tryptophane?

To solution A containing free tryptophane add in test tube 2 drops of dilute acetic acid and a few drops of bromine water. Note violet colour. Does bromine water give this reaction with proteins?

6. CARBOHYDRATE GROUP

Apply Molisch's reaction. What nucleus in the protein molecule might respond to this test?

7. SULPHUR CONTAINING COMPLEXES

To 4 or 5 c.c. of egg albumin solution add an equal volume of potas. hydrate and 1 or 2 drops of lead acetate; boil the mixture for one minute. What is the reaction? What amino acid would give this reaction?

SYNOPSIS XX

PROTEINS (*continued*)

COAGULATION AND PRECIPITATION

Influence of Concentration.—Titrate 2 c.c. of the diluted mixture of ester alcohol and acid from previous period. Calculate the percentage of acid. Has the reaction changed? Explain.

PROTEINS

1. Dilute sample of egg white solution supplied, with two volumes of water, and note precipitation of the protein by:—

(*a*) Boiling—fill test tube nearly full, hold by lower end and boil upper half; add a drop of acetic acid.

(*b*) A few drops of nitric acid.

(*c*) Acetic and tannic acids.

(*d*) Acetic acid and potassium ferrocyanide.

(*e*) Potassio mercuric iodide.

(*f*) Phospho-tungstic acid.

(*g*) Picric acid.

2. Add solid ammon. sulphate to about 10 c.c. of albumin solution in a small flask until saturated; shake thoroughly, allow to stand, observe flocculent ppte., filter, test filtrate for protein by boiling. Re-dissolve the ppte. on filter in five volumes water by shaking. Again filter and test for protein.

3. Repeat (2), using sodium chloride instead of ammonium sulphate. What is the difference in results? Why?

4. Repeat (3), adding 2 or 3 drops of glacial acetic after again saturating with sodium chloride. Do you find albumin in filtrate?

5. Place the clear white of one egg diluted with an equal volume of 5 per cent. sodium chloride solution in a dialysing tube. Suspend as directed in large beaker of distilled water. Allow to stand until next period.

6. *Metaproteins* (Acid Albumin).—Treat 5 c.c. of dilute blood serum (1 in 10) supplied, with 0.4 per cent. HCl drop by drop till the solution is clear (usually 5 drops). Boil to coagulation. Cool, and add 2 per cent. sodium carbonate drop by drop. A ppte. is formed soluble in excess.

(Alkali Albumin).—Boil 5 c.c. with two drops of 2 per cent. sodium carbonate. No coagulum is formed. Cool the tube, and add 1 per cent. acetic acid drop by drop. A ppte. of metaprotein is formed solution in excess of acid.

7. Prepare three test tubes containing 5 c.c. each of 95 per cent. alcohol, to one add 1 drop of 2 per cent. hydrochloric acid, to the second 1 drop of potas. hydrate solution, and keep the third neutral. To each add a few drops of the egg albumin solution. What do you learn from this experiment?

SYNOPSIS XXI

PROTEINS (*continued*)

EDESTIN—REACTIONS OF PROTEINS—NUCLEIN—PREPARATION OF GLYCINE

1. *Dialysis.*—Examine the dialysis experiment begun last day in the laboratory. Pour out in a beaker the contents of the dialysing tube. What change has occurred in the appearance of the egg albumin? Why? Test a little of the water in the large beaker for albumin and chlorides.

2. *Preparation of Edestin.*—Take a handful of ground hemp seed which has been washed free of oil, place in large flask with 250 c.c. of 5 per cent. sodium chloride solution; shake up and warm, occasionally stirring, to 60° on water bath. Keep the temperature as near as possible to 60° for half an hour. Filter first through cheese cloth, while hot, into a beaker; then heat filtrate again to 60–65° C., and filter while hot through filter paper in hot funnel, filter paper and funnel to be first moistened and heated with hot 5 per cent. sodium chloride solution. Keep the liquid hot 60–65° C. while filtering.

Mark the beakers or small flasks, add a particle of thymol, warm again to 60° C. and place in incubator to crystallise slowly until next period. Then examine for edestin crystals. Edestin is soluble in hot 5 per cent. sodium chloride solution, and crystallises out on cooling.

3. Test each of the five proteins, viz. serum albumin, hair, peptone, casein, and gelatin in solution or in solid form, for :

I. Biuret reaction.
II. Tyrosine.
III. Tryptophane.
IV. Benzene ring.
V. Sulphur.
VI. Carbohydrate.

Compare intensity of reaction and tabulate results. In applying biuret reaction, heat solids with potas. hydrate, cool, and then add the dilute copper sulphate.

4. *Nuclein.*—Use solution in distilled water provided.

I. Test reaction with litmus paper ; boil about 5 c.c. with 1 or 2 c.c. of nitric acid for a few minutes ; filter if not clear, and add about 1 c.c. of ammon. molybdate. Boil again. What is the ppte. ? What does it show ?

II. Boil another 5 c.c. with 1 to 2 c.c. of dilute, hydrochloric acid for a few minutes, divide into two, cool and add excess of ammonia to one part and a few drops of silver nitrate. What is the precipitate ?

III. Apply the pentose test to another portion of II.

5. *Preparation of Pure Glycocoll (Glycine) from Gelatin.*—Demonstrated.

I. Hydrolysis of gelatin to amino-acids.

II. Esterification of amino-acids.

III. Isolation of ethyl glycocoll hydrochloride. What is its formula ?

IV. Neutralisation of the salt and liberation of ester.

V. Saponification of ester.

See microscopic specimens.

SYNOPSIS XXII

Edestin, Complete Hydrolysis of Proteins, Hippuric Acid

1. Examine the crystals of edestin obtained from hemp seed at last laboratory period, using high power. Test portions of these crystals after dissolving in dilute sodium chloride solution by biuret reaction and Millon's reagent.

Boil some of the pptd. edestin in water. Does it coagulate ? Add hydrochloric acid, and note result.

2. Hydrolysis of a Protein

To 25 c.c. of crude hydrochloric acid in a small flask add a piece of sheet gelatin, about two square inches. Bring to the boil, take about 1 c.c., make strongly alkaline with potas. hydrate, and apply biuret test. Keep the mixture just boiling, and apply biuret test as above every five minutes, and note when it disappears. What has occurred ?

3. HIPPURIC ACID

Write its graphic formula.

(*a*) Examine and draw crystals.

(*b*) *Hydrolysis of Hippuric Acid.*—Boil a portion size of a pea with 10 c.c. of strong hydrochloric acid in test tube for about five minutes. Allow to cool. What are the crystals? Shake up with 5 c.c. of petroleum spirit. Which of the three possible substances present is soluble in this spirit? Allow a portion of the spirit extract (very inflammable) to evaporate spontaneously on watch-glass; what is left? What is in the aqueous solution? Explain fully the reaction. Compare fully with hydrolysis of Acetanilide, Synopsis XVIII.

(*c*) *Synthesis of Hippuric Acid.*—Take about 1 gramme of glycocoll (small measure half full) in a small flask, add 10 c.c. of water, a few drops of litmus solution, and make alkaline with potas. hydrate. Add by rough pipette 1 c.c. of benzoyl chloride (use draught cupboard), and shake up. When it becomes acid add a little more potas. hydrate. Continue shaking and add potas. hydrate until reaction is permanently alkaline and odour of benzoyl chloride has disappeared.

Filter into small beaker and make filtrate acid with HCl. What is the ppte. thrown down? Filter acidified solution; wash the ppte. twice with a little cold water in the filter paper; transfer as completely as possible from filter to evaporating dish, dissolve

in a few c.c. of hot water, and set aside to crystallise. Examine crystals under low power, and compare with hippuric acid crystals. Represent each stage of the reaction by equation.

4. Note the final stages in the demonstration of preparation of glycocoll from ethyl glycine hydrochloride.

SYNOPSIS XXIII

Uric Acid, Creatinine and Indoxyl Sulphate

(A) *Uric Acid.*—Write formula.

1. Examine and draw crystals under microscopes 1, 2, and 3. Note colours.

2. Try solubility of uric acid in water, hydrochloric acid, alcohol, and potas. hydrate. Use a minute particle only, and about 1 c.c. of solvent.

3. To the solution in potas. hydrate add an excess of ammonia solution and a drop of nitrate of silver. Why does it turn black?

4. To a minute particle of uric acid in an evaporating dish add two drops of nitric acid; evaporate to dryness on water bath, and add, after cooling, 1 drop of ammon. hydrate. Note purple colour; Murexide test; Ammonium purpurate. Add a drop of potas. hydrate and warm. Does blue colour persist?

5. Dissolve a few particles in about 5 c.c. of potas. hydrate, divide in two; to one add a drop or two of Nylander's reagent, and heat to boiling. Dilute 1 c.c.

of Fehling's solution to 4 or 5 c.c., add drop by drop the solution of uric acid, boiling after each addition. Which reagent is reduced ?

6. Saturate 5 c.c. of the solution of sodium urate provided with solid ammonium chloride. Note ppte. of ammonium urate. This reaction is employed to determine the quantity of uric acid in urine.

(B) *Creatinine.*—Write formulæ of Creatine and Creatinine.

1. Observe crystals of creatinine-zinc chloride under microscope. Compare with illustration.

2. Picramic acid reaction. To 5 c.c. of urine in a test tube add a few drops of picric acid solution ; make alkaline with potas. hydrate. Note orange-red colour. (Jaffe's test.)

To a few drops of glucose solution in 5 c.c. of water apply the same reaction. Note colour when cold. Boil solution. What occurs ?

3. Take 5 c.c. of urine in test tube, add a few drops of sodium nitroprussiate solution, and make alkaline with potas. hydrate. Note ruby red turning to yellow. (Weyl's test.) To this yellow solution add excess of acetic acid, and heat. A green colour results, which may give blue of Prussian blue. (Salkowski's test.)

(C) *Indoxyl Sulphate.*—Write formula.

Indigo reactions. (1) Mix 5 c.c. of urine with equal volume of strong HCl, add a very little solution of hypochlorite, drop by drop, shaking each time until colour becomes purple red, then add about 2 c.c.

of chloroform, shake thoroughly and allow to stand. Observe blue or slate blue colour of chloroform. (Jaffe's test.) (2) To 5 c.c. of urine add an equal volume of Obermeyer's reagent, shake up and add 2 to 3 c.c. of chloroform, shake thoroughly again. Compare results with Jaffe's test after standing. Reagent is 3 per cent. solution of ferric chloride in strong HCl.

Explain fully the reaction of hydrochloric acid and the oxidising agent. Give formulæ.

SYNOPSIS XXIV

Urea

1. *Synthesis from Carbonyl Chloride and Ammonia.*—Take 20 c.c. of the solution of phosgene gas in toluol in a dried small flask. Pass dried ammonia gas through it in draught cupboard until no further ppte. comes down. What two substances are formed at this stage? Filter off this ppte. and dry it; rub up to powder, place in large test tube, nearly fill with alcohol, and warm in water bath for a few minutes. Filter, evaporate a portion of the filtrate in a porcelain dish nearly to dryness on water bath, and examine a portion of the residue for urea crystals. What are the other crystals present? Represent all reactions by equations.

2. *Synthesis from Ammonium Cyanate.*—Make a solution in 100 c.c. flask of approximately equal

parts of ammonium sulphate and potassium cyanate (measured) in 50 c.c. of water, boil for a short time, and then evaporate in porcelain dish to dryness on the water bath. Extract the dry residue with alcohol, and filter from potassium sulphate. Evaporate off the alcohol from the filtrate on the water bath; urea is left as residue. Test it. Write formulæ.

PROPERTIES OF UREA

1. Note that a crystal of urea is soluble in a drop of water, but insoluble in ether.

2. Boil a mass of urea the size of a pea with 4–5 c.c. potas. hydrate. Note ammonia gas. Amide reaction. Give equation.

3. Place a crystal of urea on a watch-glass, dissolve in one drop of water; place beside it one drop of strong nitric acid. Examine under lower power the crystals of urea nitrate formed when the two drops join. Draw them.

4. Fill the concavity of a dry test tube with urea, warm gently; it melts at 132° C., and then a gas comes off. Note odour and action on red litmus. What reaction has occurred? Continue heating until a portion solidifies. Cool, add a little water; filter. What is the insoluble substance? Give its formula. Try the biuret reaction on the filtrate.

5. To a few c.c. of a solution of urea add 1 c.c. of a solution of sodium nitrite and about 1 c.c. of

acetic acid. To what is the effervescence due? Give equation.

6. To a few c.c. of urea solution add about one c.c. hypobromite solution. What occurs? Give equation. Repeat 5 and 6, using urine.

7. What chemical reaction occurs when ammonium cyanate is heated? Heat a piece of ammonium sulpho-cyanate size of a bean until deposit of sulphur appears. Thio-urea is formed. Give formula. Dissolve in half test tube full of water; to a portion of this add a few drops of sodium nitrite and acetic acid. To another portion add a little hypobromite solution; boil the latter, and test solution for sulphide with lead acetate.

SYNOPSIS XXV

Enzymes

(A.) Lipase

1. *Hydrolysis of Ethyl Butyrate.*—Measure 1 c.c. of ethyl butyrate in a small bottle and with pipette 20 c.c. of distilled water, and then half a capsule of the lipase powder. Shake well. Half the class will keep their bottles at 40° C., and half as directed at room temperature. Shake the mixture frequently, every ten minutes. At end of period titrate 5 c.c. and calculate the percentage of ethyl butyrate hydrolysed (sp. gr. of ethyl butyrate 0.897). Place all bottles in incubator at 40° until next period.

2. *Reversible Action of the Enzyme.*—Mix 10 c.c. of dilute butyric acid solution (0.5 per cent.) with an equal volume of dilute ethyl alcohol (2.5 per cent.); add a capsule of the lipase powder or fresh pancreatic extract. Note odour of mixture. Shake thoroughly. Keep at 40° in water bath, with occasional shaking, for one hour.

Remove from water bath, neutralise with sodium carbonate solution, and note odour of ethyl butyrate.

3. *Hydrolysis of Fats.*—Preparation of an emulsion of fat. Commercial olive oil, to which 0.1 per cent. of oleic acid has been added, is treated with one drop of phenol phthalein for each 10 c.c. of oil well shaken up, and decinormal sodium hydrate added with frequent shaking until the mixture is neutral. Why should this method give a good emulsion? Fill three test tubes half full with this emulsion, and to each add a drop or two of sodium carbonate solution to give a decided pink colour on shaking. Place one in test tube rack. To each of the two others add half a capsule of the lipase powder. Shake up thoroughly and place one in the rack, boil the third for half a minute, cool, and place beside the other two. Mark the 1, 2, and 3; note and explain any change at end of half an hour.

(B.) YEAST ENZYMES

Note.—Yeast cells from brewer's yeast contain zymase, maltase, and invertase, but no lactase.

(See Synopsis XII. 4.) Of these invertase diffuses through the cell wall, and is obtained as follows: Ten grammes of compressed beer yeast is thoroughly washed with water with aid of a filter pump; it is then mixed with 100 c.c. of water and a few drops of toluol to prevent yeast growth. On warming the mixture to 30° for a few hours, and filtering, the filtrate will be found to contain invertase which has diffused from the cells.

INVERTASE

1. Take 5 c.c. of this filtrate in small beaker, and add to it 50 c.c. of a 10 per cent. solution of cane sugar. Warm to 30° for an hour (fermentation cupboard), and test by Fehling's solution for invert sugar.

2. Add 2 or 3 c.c. of this invertase solution to some glucose solution in a fermentation tube. Mark it invertase. Keep the tube at 30°, and observe at end of period whether any action has occurred.

Invertase is an extra cellular enzyme in a yeast mixture.

MALTASE

Rub up a small fragment of yeast size of a pea with 10 c.c. of maltose solution in a test tube. Pour this into another fermentation tube. Mark it Maltase, and keep at 30° for an hour. Note evidence of fermentation. Explain.

ZYMASE

(See Synopsis IX.)

What are the fermentable hexoses?

(Titrate the ethyl butyrate mixture. Make notes regarding the fermentation tubes, removing all tubes and beakers from the fermentation cupboards, and clean them thoroughly before leaving.)

SYNOPSIS XXVI

ENZYME ACTION (*continued*)

1. *Lipase* (continued).—Titrate the ethyl butyrate mixture, using 5 c.c., and again calculate the percentage of the ester that has been hydrolysed.

2. *Urease.*—Measure 10 c.c. of the 1 per cent. solution of urea provided, with pipette into a 100 c.c. flask or bottle, run in 2 c.c. of the solution of urease (alco-urease 15 per cent.) from a central burette. Rotate the mixture, close with a cork and after a half an hour titrate with decinormal hydrochloric acid, using one drop of alizarine as an indicator. Represent the reaction which has occurred by equation, and calculate the weight of urea that has been hydrolysed.

3. *Amylase.*—Dilute 1 c.c. of diastase solution with about 10 c.c. of water, and boil for a few minutes; add about an equal volume of starch solution. Mark this "5," and place the tube with the others as prepared below in the water bath at 40° C.

Place a handful of snow or powdered ice in large beaker half full of water; stir up. Take four clean test tubes, run 5 c.c. of clear starch solution in each, place in beaker containing melting snow and water for five minutes. Mark them 1, 2, 3, and 4; to 1 add 2 drops of solution of diastase; to 2, 5 drops; to 3, ½ c.c.; add to 4 1 c.c. of the solution. Shake up, place in water bath at 40° C. for thirty minutes, then fill nearly to top with water; shake up, and to each add 1 drop of dilute iodine solution and shake again. What changes have occurred? What conclusions do you draw regarding the effect of concentration of the enzyme and effect of boiling the enzyme?

4. *Protease.*—Prepare a series of four tubes, each containing 10 c.c. of the faintly alkaline 0.01 per cent. solution of casein provided. Warm them in water bath at 40° C.; run in to No. 1, 2 drops; No. 2, ½ c.c.; No. 3, 1 c.c.; and No. 4, 2 c.c. of the pancreatic enzyme solution provided; place in water bath at 40° C. for fifteen minutes. Acidify each with a few drops of 1 per cent. solution of acetic acid; casein is pptd. from alkaline solution by dilute acetic acid, and when completely digested gives no ppte. What is the effect of concentration of this enzyme? Compare with effect of hydrogen ions. in hydrolysis of esters. (Synopsis X.)

The unit of tryptic activity is the power of 1 c.c. of ferment to digest 10 c.c. of 0.01 per cent. solution

of casein in fifteen minutes. Calculate the activity of the solution provided.

5. *Oxidases.*—Add 4 or 5 drops of the alcoholic solution of gum guaiacum to a test tube nearly full of water. To the milky emulsion add a slice of potato; it turns blue on standing—direct oxidase. To another similar emulsion add a piece of fresh meat, no reaction. Add about 1 c.c. of hydrogen peroxide, shake up, and note the colour on standing—peroxidase; bubbles appear on surface of tissue—catalase. Boil a small piece of the potato and a fragment of the animal tissue, and repeat above experiments. Why is there no reaction?

PART II

CHEMISTRY OF HUMAN PHYSIOLOGY

A Laboratory Course of about twenty-five periods given during the first term of the third year

This course consists of a series of lectures and laboratory exercises. It comprises a study of the proximate constituents of the commonly occurring food stuffs, the chemical characteristics and actions of the digestive enzymes, the examination of normal fæces and urine, with their application to human metabolism; a thorough study of blood and bile is also made. The aim is to give the student a sound experimental knowledge of the normal human metabolism.

V. J. H.

EXAMINATION OF FOOD STUFFS

(A.) Milk: Chemical Constituents

Reaction.—Test with both red and blue litmus paper.

Fat.—Make microscopic examination of (*a*) Whole cow's milk, (*b*) Skimmed milk, (*c*) Human milk, and (*d*) Colostrum.

Take the specific gravity of whole and skimmed milk. Why has skimmed milk the higher specific gravity?

Shake a little milk in a test tube with an equal volume of ether; the fat globules do not apparently dissolve in the ether and the milk remains opaque. Add a few drops of KOH solution, and shake again. Add KOH to another portion of milk; opacity does not disappear. Explain the difference in the results obtained.

Butter Saponification.—Place a little butter in the bottom of a small flask, and cover with alcohol. Now add 5 grms. of solid KOH and 5 c.c. water, and heat on gently boiling water bath for twenty minutes. (If saponification at end of that time is complete, a small portion in a test tube on dilution with water will remain clear; if not, add 2 grms. KOH, 2 c.c. H_2O, and 10 c.c. alcohol, and boil again for a further ten minutes.) When saponification is complete, pour into evaporating basin, add 100 c.c. H_2C, and evaporate gently until odour of alcohol has disappeared. Cool and acidify the solution with H_2SO_4 dilute. Note smell of butyric acid. Allow to cool. Note if the fatty acids are solid or oily. Take a portion of the fatty acids, and shake in test tube with solution of bromine in chloroform. Note disappearance of colour of bromine. Why is this?

From these experiments what have you learned about the nature and condition of the fats present in milk?

Read Bio. Chem. Notes, Synop. VI.

PROTEIN.—Place 10 c.c. milk in small beaker and

boil gently a few minutes. Note formation of film; remove film, and heat again. Does film now form? Of what substance is this film composed?

Treat 5 c.c. milk with 5 c.c. saturated ammonium sulphate solution (= ½ saturated). The caseinogen is precipitated as the calcium salt. Filter off ppte. When filtered, wash on filter with a little water. The calcium caseinogenate will be found to dissolve giving an opalescent solution. Note that calcium caseinogenate behaves like a globulin. Bio. Chem., Synop. XX.

Dilute 25 c.c. of milk with three times its volume of water, then add dilute acetic acid, drop by drop, shaking after each addition of acid. A flocculent ppte. of caseinogen is formed which mechanically carries down the fat with it. Add the acid until the ppte. is no longer formed and the solution appears quite clear; pptn. is quicker if gently warmed. Carefully avoid excess of acid, which again dissolves the ppte. Filter off the ppte., and wash it with water.

Keep the filtrate (*a*).

Remove excess of moisture from the ppte. of caseinogen by pressing between filter paper. Transfer dried caseinogen to small dry flask, and cover with 20 c.c. alcohol and 20 c.c. ether, and shake up for ten minutes with occasional warming in warm water (temperature about 35° and carefully avoid all flames). Filter again, and put the filtrate into the residue bottle on the centre table. This filtrate contains the butter fat (cream). Dry the thus purified caseinogen

between filter paper, grind in mortar, and apply the following protein tests :—

(1) Biuret.

(2) Xanthoproteic.

(3) Millon's.

(4) Hopkins-Cole.

Cp. Bio. Chem., Synop. XIX. Also test for organic sulphur and phosphorus by following test :—

Place a little caseinogen in crucible, and add two to three times the bulk of flux (Na_2CO_3 and KNO_3). Heat, gently at first and then more strongly, until a clear colourless liquid is produced. When cool, dissolve residue in a little water, acidify with HNO_3 (dilute), boil and filter. Divide into two portions. To one portion add $BaCl_2$; a faint white ppte. or cloudiness indicates a sulphate = presence of *organic* sulphur. To second portion add excess of ammonium molybdate, and warm gently; yellow ppte. or colouration indicates phosphate = presence of *organic* phosphorus.

Why do these tests for sulphate and phosphate mean the presence of *organic* sulphur and phosphorus in the original compound ?

Take the filtrate (*a*) from the caseinogen precipitation, and evaporate it to about one half its bulk. Coagulation of lactalbumin and lactoglobulin takes place. Filter off the ppte. and test the ppte. for protein by biuret, Millon's, and Hopkins-Cole reactions.

CARBOHYDRATE.—The filtrate from the coagulable proteins should be concentrated to about half its bulk.

(1) Test a portion for carbohydrate by Fehling's solution. (2) Grind up a small piece of yeast with about 20 c.c. of the liquid (first making neutral with NaOH), fill fermentation tube and place in incubator for one hour. Note if fermentation takes place. (3) Evaporate 10 c.c. of liquid with 5 c.c. HNO_3 (conc.) slowly on water bath in draught cupboard to about one-third the bulk, add 10 c.c. of water, and note the appearance of any crystals. If so, examine under microscope (cp. Bio. Chem., Synop. XII. 11. 7).

SALTS.—Test small portions of liquid, which must be quite clear, for :—

(1) Phosphates.—Add HNO_3 ammonium molybdate, and warm gently.

(2) Chlorides.—Add HNO_3 and $AgNO_3$.

(3) Sulphates.—Add HNO_3 and $BaCl_2$.

(4) Calcium.—Add ammon. oxalate.

CLOTTING OF MILK.—(The action of rennin). Prepare four test tubes :—

	i.	ii.	iii.	iv.
Milk	5 c.c.	5 c.c.	5 c.c.	5 c.c.
Rennet	2 c.c.	2 c.c. (boiled)	2 c.c.	2 c.c.
Pot. oxal.	—	—	1 c.c.	1 c.c.
Ca. Chloride . . .	—	—	—	1 c.c.

Add the milk last, and place in water-bath at 40° until No. 1 coagulates. Note in which tubes clotting takes place, and explain all the results obtained. To

III., at end of experiment, boil and add a little calcium chloride solution, and explain fully the result.

ENZYMES IN MILK

PEROXIDASE.—Add a few drops of tincture of guaiacum to fresh milk in a test tube, and then 2 or 3 of hydrogen peroxide solution, and allow to stand. Repeat, using boiled milk. (Cp. Pasteurised milk.)

CATALASE.—Fill a fermentation tube with hydrogen peroxide solution, and then introduce about 2 c.c. of milk. Note bubbles of oxygen, collecting, on standing. Repeat, using boiled milk.

What is the difference between peroxidases and catalases? (Bio. Chem., Synop. XXVI.)

In examining the following foodstuffs, only the important points will be taken. Students are advised to consult Halliburton's *Handbook of Physiology*, pp. 464–85, or Howell's *Text-book of Physiology*, pp. 726–9, for the quantitative composition of the more commonly occurring foodstuffs.

(B.) EGGS

Egg-white.—Read up notes on Bio. Chem., Synop. XX and XXI, where the albumin solution employed was a solution of egg-white in dilute sodium chloride.

Egg-yolk.—Egg-yolks have been extracted with ether, the ethereal extract has been distilled to remove the ether. The oil remaining from the ethereal

extract contains the fats. The residue from the extraction contains the protein.

Demonstration of Soxhlet extraction of fat from dried egg-yolk.

Fats.—Examination of the oil extracted from egg-yolk by means of ether.

To 2 c.c. of oil add 10 c.c. of acetone; note the ppte. of lecithin. Filter off the lecithin. Filtrate must be put into residue bottle on [illegible] table. Lecithin on filter paper should be dissolved in a little warm alcohol, and a clear solution examined as follows:—

(1) It gives a white emulsion on pouring into water.

(2) Boil a small quantity from which the alcohol has been evaporated, with solid KOH; trimethylamine (from choline), is evolved and can be recognised by the fishy smell. Fatty acids are pptd. on acidifying with H_2SO_4 (dilute).

(3) Evaporate the remainder of your alcoholic solution to dryness in a basin, and test residue for organic phosphorus.

Summarise the properties of the lipoid lecithin.

Proteins.—Examination of insoluble residue from ethereal extracts of egg-yolk = lecitho-vitellin.

Grind up a small amount of the lecitho-vitellin with 10 per cent. NaCl solution for about 10 minutes, and filter. Filtrate will be found to contain the lecitho-vitellin, and should be examined as follows:—

(*a*) Faintly acidify a portion with acetic acid, and boil. Note coagulation.

(*b*) Pour a portion into a large excess of water faintly acidified with acetic acid; note precipitation.

(*c*) Apply Biuret, Xanthoproteic, Millon's, and Hopkins-Cole reactions to portions.

Boil a small amount of lecitho-vitellin with alcohol for a few minutes; filter. The filtrate contains lecithin, and should be tested by tests (1) and (2). The residue is vitellin, a phospho-protein.

Note how lecitho-vitellin (a protein derived from a phospho-protein), behaves as a globulin, and compare its properties with those of calcium caseinogenate.

The inorganic constituents of eggs are similar to the salts in milk. See **Milk**.

(C.) Cereals

Wheat Flour. *Carbohydrates.*—Knead some wheat flour with water to form a dough, and allow to stand for a short time; then wrap up a portion in muslin, and knead it in a basin of water. Note the deposit of starch grains. Examine them under the microscope, and compare with those of potato. Boil some with water, cool, and test with iodine.

Proteins.—Completely remove the starch by kneading in a stream of water until no more grains pass through. A yellow gelatinous mass remains behind. This is termed gluten, and it contains the two proteins

of wheat (gliadin and glutelin). Prove this by the protein colour tests.

Carbohydrates of Bread.—Separately soak some crust and crumb of bread in cold water. Filter each and test each for starch and reducing sugar. Which portion contains the more reducing sugar?

CORN MEAL.—Repeat the experiment with wheat flour, using corn meal. Note the differences in the results. The protein does not form the gelatinous mass, as in the case of wheat flour, although both classes of protein are present (gliadins and glutelins).

Fat.—Determination of fat by Soxhlet extraction, in corn meal and wheat flour.

(D.) NUTS

Fat.—Determination of fat in nuts by Soxhlet apparatus.

Protein.—Extract about 1 grm. of fat-free nut meal with 10 per cent. NaCl solution by grinding in mortar. Filter, and test filtrate:—

(i.) Pour into excess of water faintly acidified with dilute acetic acid. Note pptn. of protein. (ii.) Faintly acidify with acetic acid, and boil. Note coagulation of protein. (iii.) Apply the usual colour tests.

Note that the protein is a globulin, and compare with experiments on edestin. (Bio. Chem., Synop. XXI.)

(E.) Potato

Carbohydrate.—Scrape a piece of uncooked potato, add a little water to scrapings and filter; examine the insoluble portion for starch cells. Test the filtrate for traces of proteins and soluble carbohydrates. Fats are practically absent.

(F.) Meats

See Muscle and digestion of proteins.

Summarise your knowledge of the food values of the various classes of foodstuffs studied.

EXPERIMENTS ON DIGESTION

Salivary Digestion

Examination of Saliva.—Examine a drop of unfiltered saliva microscopically. Note any epithelial scales and salivary corpuscles.

Test reaction to litmus. Add HNO_3 to portion, boil, filter, and test for *phosphates*. To what compound is the alkalinity of saliva due?

To a small quantity of saliva add a few drops of $Fe Cl_3$; a red colouration indicates sulphocyanide To show that the red colour is not due to iron phosphates, add a drop of $HgCl_2$, when the red colouration is discharged.

Mucin.—Add a few drops of dilute acetic acid to saliva. Note pptn. of mucin. Pour 10 c.c. of saliva

into 50 c.c. of alcohol, stirring constantly (first rinsing the mouth with water before collecting the saliva); mucin is pptd. Pour off the supernatant liquid, collect ppte. on a filter, wash while still in filter, with alcohol, and dry between filter paper. Prove that mucin is a protein by the colour tests (Biuret, Millon's, Hopkins-Cole, &c.). Prove that it is a gluco-protein by boiling a little with 1 or 2 c.c. of HCl (conc.), neutralising with solid KOH, and applying Fehling's test. What reducing substance has been formed?

Examination of Enzyme Ptyalin.—Collect 5 c.c. of saliva, dilute to 15 c.c., and filter. In order to obtain comparable results, the saliva should always be collected in this way. If it is necessary to stimulate the salivary flow this may be accomplished by sucking 2 or 3 glass beads, or by chewing small pieces of paraffin wax.

Action of Ptyalin on Starch.—The filtered saliva and the starch paste should be tested for the presence of reducing sugars before carrying out these experiments. The tests should be negative.

To 10 c.c. of starch paste add 5 c.c. of diluted filtered saliva, stir thoroughly, and place on water bath at 40° C. At intervals of a minute take out a small proportion, and test for the presence of starch by the iodine test, and reducing sugars by the Fehling's test.

The starch passes through the following changes, which should be recognised experimentally; starch, soluble starch, erythrodextrin, achroo-dextrin, maltose.

Confirm the presence of maltose at the end of the experiment by preparing the osazone and making the microscopical examination. (Cp. Bio. Chem., Synop. XII and XIII.)

Destruction of Ptyalin by Acid.—To 4 c.c. of saliva add 4 c.c. of 0.4 per cent. HCl, and allow to stand for ten minutes. Then just make faintly alkaline with Na_2CO_3 and see if the saliva now has any action on starch solution by incubating for fifteen minutes with 1 c.c. of starch solution at 40°.

GASTRIC DIGESTION

Action of Pepsin on Fibrin

Prepare five tubes as follows :—

	i.	ii.	iii.	iv.	v.
Water	10 c.c.	5 c.c.	5	7 c.c.	5
Pepsin	2 c.c.	2 c.c.	2 c.c. (boiled)	—	2 c.c.
HCl (0.4 per cent.)	—	5 c.c.	5 c.c.	5 c.c.	—
Na_2CO_3 (0.2 per cent.	—	—	—	—	5 c.c.

To each add a small piece of fibrin, and place in water bath at 40°. Note in which tubes the fibrin is digested.

Destruction of Pepsin by Alkali

To 5 c.c. of pepsin solution add 5 c.c. Na_2CO_3 solution (0.2 per cent.) and put in water bath at 40° for half hour. Neutralise with 0.4 per cent. HCl, and

add 5 c.c. of acid in excess. Add a small piece of fibrin, and keep at 40°. See if digestion occurs.

Products of Action of Pepsin

Digest about 2 grms. fibrin with 5 c.c. pepsin solution and 5 c.c. of 0.4 per cent. HCl, for at least half hour. Test for presence of free HCl by Gunzberg's reagent.

At end of digestion neutralise solution with dilute alkali, using litmus. Filter off the *metaprotein*. Boil the filtrate, and whilst boiling faintly acidify with acetic acid. This destroys the enzyme. Filter.

Test portion of filtrate for proteoses with nitric acid. A ppte. is formed which dissolves on warming and reappears on cooling.

Saturate remainder of filtrate with ammonium sulphate, and filter off proteoses. Test for peptone in filtrate by Biuret reaction.

Acidify a portion of the filtrate with acetic acid, and add a few drops of bromine water. A violet colouration would indicate *free* tryptophane.

Note on Gunzberg's reagent.—Place 1 to 2 drops of reagent on porcelain dish, and carefully evaporate to dryness over a low flame (be careful not to char). Insert a glass rod into the liquid to be tested for free HCl, and draw the moist end over the dried reagent. A purplish red colour on warming indicates free HCl.

Digestion in Presence of Various Acids

Prepare a series of tubes each containing 5 c.c. of the following acids of 0.4 per cent. strength: HCl, Lactic, Butyric, Acetic.

To each add 1 c.c. of glycerin extract of pig's stomach and a small piece of fibrin. Shake well, and place in water bath at 40°. In which tube does the digestion proceed most rapidly?

Pepsinogen

To each of three tubes add 1 c.c. glycerin extract of stomach, and then add to (i.) a small piece of fibrin, to (ii.) a small piece of fibrin and 2 c.c. 0.4 per cent. HCl, to (iii.) a small piece of fibrin and 2 c.c. 0.2 per cent. Na_2CO_3, and incubate at 40° for about ten minutes.

At end of experiment, add to No. (iii.) 4 c.c. of 0.4 per cent. HCl, and incubate again.

Note that digestion only occurs in No. (ii.), but that it can be made to take place in No. (iii.) on acidification. How does this distinguish pepsin from its zymogen?

Clotting of Milk by Gastric Juice

Place 5 c.c. of milk in each of four test tubes, and add to (i.) 1 c.c. of 0.4 per cent. HCl, to (ii.) 1 c.c. 0.4 per cent. HCl and 1 c.c. extract of stomach, to (iii.) 1 c.c. 0.4 per cent. HCl and 1 c.c. extract of stomach (boiled), and to (iv.) 1 c.c. 0.2 per cent. Na_2CO_3 and

1 c.c. extract of stomach. Incubate at 40°. In which tubes does clotting take place ?

From these experiments, and the previous ones on milk, write out a full account of the clotting of milk during gastric digestion.

Inversion of Sucrose

Test the extract of stomach and the sucrose solution for the presence of reducing sugars by Fehling's. If negative, prepare the following series of tubes :

(i.) 1 c.c. extract of stomach and 2 c.c. 0.4 per cent. HCl.

(ii.) 1 c.c. extract of stomach and 2 c.c. 0.4 per cent. HCl and 2 c.c. sucrose.

(iii.) 1 c.c. extract of stomach (boiled) and 2 c.c. 0.4 per cent. HCl and 2 c.c. sucrose.

Incubate at 40° for about 15 min., and then test each by Fehling's solution.

To what is the inversion of the sugar due ? What products are formed ? (Bio. Chem., Synop. XI.)

Antiseptic Action of Gastric Juice

Demonstration.—Comparison with pancreatic digestion.

Repeat the action of pepsin on fibrin to determine the products of digestion (see above). Use larger quantities of fibrin, and add HCl (0.4 per cent.) from time to time, testing by the use of the Gunzberg's reagent. Always have free HCl present.

Action of Pepsin on Various Proteins

Test the digestive action of pepsin (taking 4 c.c. strong pepsin from burette) on the following substances: Fibrin, boiled fibrin, coagulated egg-white, milk, hair.

BILE

Inorganic Constituents

Test the reaction of fresh ox bile to litmus. Test for the presence of phosphates, chlorides, sulphates.

Protein

Acidify a portion of bile with dilute acetic acid, taking care not to add excess. A ppte. of nucleo-protein is obtained. Filter off, wash on the filter with a little water, and apply the protein colour tests to the precipitate.

Bile Pigments

Gmelin's test: To about 5 c.c. of HNO_3 (conc.) add 3 c.c. of diluted bile, carefully, so that the two fluids do not mix. At point of contact note various layers of colours which form, on standing, green, blue, red, yellow. This test may also be performed by filtering the diluted bile, allowing the filter paper to drain thoroughly and placing a drop of dilute fuming nitric acid on the filter paper from the end of a glass rod, when the characteristic layers of colours will be seen surrounding the drop of nitric acid.

Bile Salts

Pettenkofer's Test.—To 5 c.c. of diluted bile add 5 drops of 1 per cent. sucrose solution. Now run a little H_2SO_4 (conc.) down the side of the tube, and note the red ring at the point of contact. Take care that the liquid does not char.

Hay's Test.—Reduction of surface tension. To 100 c.c. of water add 1 c.c. of bile; sprinkle a little flowers of sulphur on the surface. Note that the sulphur sinks. Compare the action of sulphur on pure water.

Cholesterol

Boil a small amount of powdered gall-stones with a little alcohol, and filter while still hot into a test tube (use dry, clean, apparatus). On cooling, the filtrate will deposit crystals of cholesterol. Note their crystalline form under the microscope. Filter off the cholesterol.

Salkowski's reaction: dissolve some of the cholesterol in chloroform in a dry test tube, and gently shake it with an equal volume of H_2SO_4 (conc.). The $CHCl_3$ solution turns red, and the subjacent acid acquires a greenish fluorescence.

Influence of Bile on Gastric Digestion

Prepare two tubes each containing 1 c.c. pepsin and 2 c.c. of 0.4 per cent. HCl, and then to one add 1 c.c. of

bile. Add a piece of fibrin to each, and incubate at 40°. In which tube is digestion the more rapid, and why?

Test the solutions A, B, and C for the presence of bile.

PANCREATIC DIGESTION

The powder or solution given out contains the enzymes usually found in pancreatic juice and is highly active. Only a very small amount is required in an experiment.

Trypsin

To 5 tubes add a little pancreatic powder, or solution, and then add to (1) 5 c.c. of 0.2 per cent. Na_2CO_3; (2) 5 c.c. of 0.2 per cent. Na_2CO_3, boil; (3) 5 c.c. of 0.4 per cent. HCl; (4) 5 c.c. 0.4 per cent. lactic acid, and (5) 5 c.c. of 0.2 per cent. Na_2CO_3, and 1 c.c. of bile. To each add a small piece of fibrin, and incubate at 40°. In which tubes does digestion take place? What is influence of bile? Note that in pancreatic digestion fibrin is eaten away and slowly disintegrates; there is no swelling as in gastric digestion.

Amylopsin

Prepare 5 tubes as in trypsin, only adding 2 c.c. of starch paste instead of fibrin. Test progress of digestion of starch from time to time by means of iodine, taking care that solution is previously made acid. Also test from time to time by means of Fehl-

ing's for presence of reducing sugars. What are products of action of amylopsin on starch? What is influence of bile on activity of amylopsin?

Steapsin and Pancreatic Rennin

Into each of three tubes introduce 10 c.c. of milk, 1 c.c. 0.2 per cent. Na_2CO_3 and two drops phenol phthalein solution. To contents of (1) add small quantity pancreatic powder, to (2) add the same quantity of pancreatic powder and 2 c.c. bile; incubate all three at 40°. Note whether any clotting of milk takes place, and in which tubes lipoclastic action occurs as shown by change in alkalinity. What is the influence of bile upon lipoclastic activity?

Products of Tryptic Digestion

"Casein" has been allowed to undergo tryptic digestion for several days. Each student will receive about 100 c.c. of the digest for examination as follows:

Strain off any undissolved residue through cheese cloth, nearly neutralise solution with dilute HCl, and then exactly neutralise with 0.4 per cent. HCl. A ppte. at this point would indicate "alkali metaprotein." Filter off any ppte., and divide the filtrate into two parts, a $\frac{1}{4}$ and $\frac{3}{4}$ portion.

(*a*) Transfer $\frac{1}{4}$ portion to an evaporating basin; concentrate to almost $\frac{1}{2}$ its volume by boiling; filter if solution is not clear, make separation of proteoses and

peptones and the tests for these bodies, exactly as in the examination of the products of peptic digestion.

(*b*) Place 5 c.c. of the ½ portion in a test tube, and add bromine water, drop by drop; a pink colouration indicates the presence of free tryptophane.

(*c*) Add alcohol to remainder of the ½ portion until no more ppte. forms, stirring continuously with glass rod. What is ppte.? Warm gently, and filter. Ppte. should be tested for presence of proteins. Concentrate filtrate by gentle boiling to a thin syrup, being careful not to char and also to keep the solution slightly acid during concentration. Then set aside to crystallise, and place crystals under microscope. These will consist of tyrosine (long sheaves of needles) and leucine (small rosettes of needles).

INTESTINAL DIGESTION

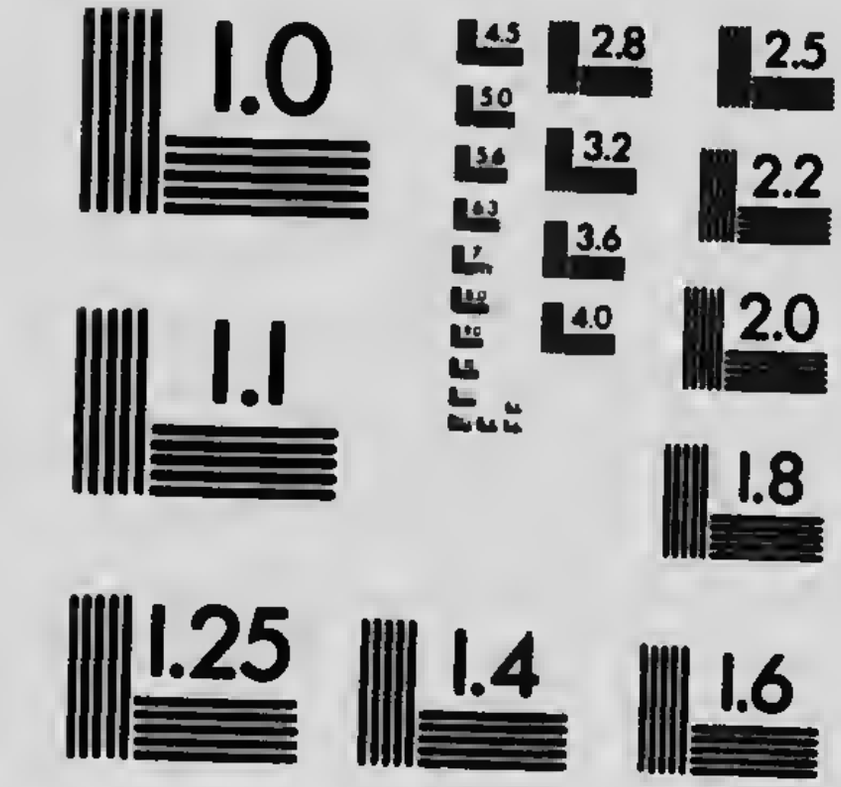
(*Succus Entericus*)

Preparation of Invertase.—Scrape off the mucous membrane of a small piece of small intestine. Add 10 c.c. of distilled water, and allow the extraction to remain an hour. Filter through cheese cloth, and test the filtrate for invertase as directed below.

The liquid given out is an aqueous extract of the small intestine of the pig. It should be examined for the presence of the following enzymes:

MICROCOPY RESOLUTION TEST CHART

(ANSI and ISO TEST CHART No. 2)



APPLIED IMAGE Inc

1653 East Main Street
Rochester, New York 14609 USA
(716) 482 - 0300 - Phone
(716) 288 - 5989 - Fax

Invertase (*Saccharase*)

Incubate 5 c.c. of saccharose solution with 1 c.c. of intestinal extract at 40° for twenty minutes. Prepare also a boiled control, and keep under the same conditions. Then test each with Fehling's solution; see if hydroloysis has occurred. If not, leave at ordinary temperature until next period, adding one or two drops of toluene, and placing a loose stopper of cotton wool in mouth of the tube, and test again.

Maltase

As invertase—only using maltose solution instead of saccharose. Test presence of mono-saccharides by Barfœd's reagent (Bio. Chem., Synop. XII, II 5).

Lactase

As Maltase—only using lactose solution.

Erepsin

Incubate 2 c.c. of 1 per cent. peptone solution with 2 c.c. of intestinal extract at 40° C., until next period. and then test by the Biuret reaction for the presence of peptone. Add one or two drops of toluene to act as preservative, and stopper the tube with cotton wool. Also prepare boiled control.

Test the peptone solutions at the end of the incubation for the presence of free tryptophane.

FÆCES

Make 2 or 3 slides of fresh fæces for examination under microscope, using both low and high powers. Sketch various elements observed.

Note reaction of fresh fæces. Boil a small quantity of fresh fæces with water and a little animal charcoal; filter. Filtrate should be almost colourless. Divide into two portions, test (1) for the presence of starch and sugar; (2). for chlorides, phosphates, sulphates.

Extract small quantity of fresh fæces with 10 per cent NaCl; filter. Acidify filtrate with dilute acetic acid; if ppte. is produced, filter again, and test ppte. for mucin. Test filtrate for proteins.

Extract a small quantity of dried fæces with alcohol, and filter. Evaporate filtrate on water bath to drive off alcohol; dissolve any residue in dilute KOH, and test solution for presence of bile acids.

Rub up a small amount of fæces in a mortar to a paste with a saturated solution of $HgCl_2$ and allow to stand till next period. A red colouration of fæcal particles due to hydrobilirubin should be observed. Examine under microscope. Any green colouration of fæcal particles is due to bilirubin, and is pathological.

DEMONSTRATION:

Determination of percentage of fat.

Determination of percentage of water.

Products of protein putrefaction.

BLOOD

1. Examine a drop of defibrinated ox blood under microscope; compare with a drop of your own blood. What objects do you observe?

2. *Reaction.*—Place a drop of blood on strip of litmus paper, remove quickly with a stream of water; note reaction.

3. Take specific gravity of your blood as follows: (Hammerschlag's method)—Fill an ordinary urinometer cylinder about one half full of a mixture of chloroform and benzene, having a specific gravity approximately of 1060. Allow a drop of the blood from your finger to fall directly into the mixture. Care must be taken not to use too large a drop of blood, and to keep the blood from coming into contact with the walls of the cyclinder.

If the blood drop sinks to the bottom of the vessel, add chloroform till the blood drop remains suspended in the mixture; if blood drop rises to surface, add benzene.

Stir carefully with glass rod, after adding chloroform or benzene.

After the blood has been brought to a suspended position in the mixture, determine specific gravity of mixture.

What is the specific gravity of your blood?

4. *Hæmolysis, or Laking of Blood.*—Note the opacity of ordinary defibrinated blood.

(*a*) Place two c.c. of defibrinated blood in a test tube, fill with 0.9 per cent. sodium chloride solution, and note that hæmolysis takes place. Set this test tube aside to use as a standard.

(*b*) To two c.c. of defibrinated blood add tap or distilled water. Note that hæmolysis takes place.

(*c*) Grind a little blood in a mortar with some clean sand. Add saline solution, filter, and notice if any hæmolysis has occurred.

(*d*) Shake two c.c. of blood with a little ether, and dilute with normal saline. Note hæmolysis.

(*e*) Add a 2 per cent. solution of urea in water to defibrinated blood. Note hæmolysis.

(*f*) Repeat (*e*), using a 2 per cent. solution of urea in normal saline. Does hæmolysis occur in this case?

(*g*) To a solution of two c.c. of blood in a test tube of normal saline, add two or three drops of saponin solution.

(*h*) Rapidly pith and decapitate a frog. Collect the blood on a watch-glass, and allow it to stand until the serum separates. Transfer the drop of serum to a clean watch-glass, and allow a drop of your own blood to fall upon it. Note results.

CHEMISTRY OF HÆMOGLOBIN

Blood Spectra

Direct the spectroscope to the window, and carefully focus Fraunhofer's lines. Note carefully D in the yellow, and E, the next well-marked line, in the

green. Direct the spectroscope to a Bunsen flame. Notice the bright yellow line in the position of the D line, on sharply tapping the burner.

The absorption spectra of blood with which we are concerned are all found in the middle portions of the spectrum between the Fraunhofer lines C and F, a region easily observed.

Oxyhæmoglobin

Take a series of test tubes of about equal size. Fill the first with diluted defibrinated blood (1–32); then fill the second with the same mixture diluted with an equal bulk of water (1–64): half-fill the third with this, and then fill up with an equal bulk of water, (1–128), and so on; the sixth tube will contain 1 part of blood in 1024 of water, and will be nearly colourless.

Hæmoglobin

1. Into another series of six tubes add a few drops of Stokes' fluid, then pour in some of the contents of each of the first series.

2. Examine the tubes with the spectroscope, and map out in note-book, typical absorption bands of *Oxyhæmoglobin* in first series, and *Hæmoglobin* in second. Notice that in the more dilute specimens of hæmoglobin the bands are no longer seen, whereas those of oxy-hæmoglobin in specimens similarly diluted are still visible.

3. Take a tube which shows the single band of

reduced hæmoglobin, shake it with air; the bright red colour returns, and it shows spectroscopically the two bands of oxyhæmoglobin for a short time. Continue watching bands, and note that they fade, and are replaced by single band as reduction again occurs.

Carbon Monoxide Hæmoglobin

The preparation of this pigment may be accomplished by passing coal-gas through defibrinated ox-blood. Notice brighter tint (carmine) assumed by blood thus treated. Dilute some CO-hæmoglobin blood with water, and examine spectroscopically. What do you observe? How does spectrum of CO-hæmoglobin differ from that of oxyhæmoglobin? Add some Stokes' fluid, and again examine with spectroscope. Has any change taken place? How does this reaction compare with action of Stokes' fluid upon oxyhæmoglobin?

Neutral Methæmoglobin

Dilute a little defibrinated ox blood (1–10), and add a few drops of freshly-prepared saturated potas. ferricyanide. Shake mixture, and observe bright red colour of blood is displaced by brownish red. Now dilute a little of this solution and examine spectroscopically. Note single very dark absorption band lying to left of D, and if dilution is sufficiently great also observe two rather faint bands lying between D and E in somewhat similar positions to those

occupied by absorption bands of oxyhæmoglobin. Add a few drops Stokes' fluid to methæmoglobin solution while it is in position before spectroscope, and note immediate appearance of oxyhæmoglobin spectrum, which is quickly followed by that of hæmoglobin.

Hæmin Test

The formation of hæmin crystals is one of the best methods for testing minute quantities of blood, blood-stains, etc. Thus:

Place a drop of blood on a glass slide, and allow to dry, or heat gently over a flame, so as to drive off water. Scrape residue into a little heap, and add a drop of glacial acetic acid from end of a glass rod. Rub up into a paste, and put a little on a clean slide; add a drop of glacial acetic acid, cover with coverslip, and heat till solution begins to boil; remove for a few seconds, and heat again; repeat two or three times. Allow slide to cool, and examine under microscope (high power). Observe small black or brownish-black crystals of hæmin—Teichmann's crystals.

N.B.—Allow the slide to cool slowly, and when complete the preparation should be moist with acetic acid. If stain examined is an old one add a minute crystal of sodium chloride.

Acid Hæmatin

To a little diluted blood (1:5) add ¼ of its volume of 33 per cent. acetic acid; mix thoroughly, and warm

in water bath at 40–50° C. for 5–10 minutes. Dilute a small portion, and examine with spectroscope. An absorption band in the red between C and D will be seen. This is acid hæmatin.

Make the solution faintly alkaline by gradually adding dilute caustic soda, and filter.

Hæmochromogen

To some of the aqueous solution of alkaline hæmatin add a few drops of Stokes' reagent or ammonium sulphide. The solution becomes red in colour, and on spectroscopic examination shows two absorption bands in the green, the one nearer D being very prominent and sharply defined, the other being much fainter.

In very dilute solutions, only the one band is seen, but the absorption of light here is very great. A solution of oxyhæmoglobin where the absorption bands can scarcely be seen on conversion into hæmochromogen will show this single band. The conversion of hæmoglobin into hæmochromogen is a very delicate test for blood stains: the stain is heated with 1 per cent. caustic soda, the solution is cooled and filtered, and then reduced with Stokes' reagent. Examination with spectroscope shows one or both absorption bands.

Acid Hæmatoporphyrin

To 2 c.c. of concentrated sulphuric acid in a t.t. add 4–5 drops defibrinated blood, and mix thoroughly

after addition of each drop. A purple solution will be obtained which, when examined with spectroscope, will show two well-marked absorption bands. The solution should be diluted with glacial acetic acid if necessary. One of the absorption bands lies in the orange between C and D, the other, which is broader and darker, lies in the yellow-green between D and E.

Alkaline Hæmatoporphyrin

Pour the acid solution of hæmatoporphyrin into water; cool; a pigmented precipitate separates out which contains most of the hæmatoporphyrin. Sodium acetate makes precipitation more complete. Filter off ppte., wash with water, and dissolve in a little warm dilute caustic soda. The solution of alkaline hæmatoporphyrin so obtained shows, when examined with spectroscope, four absorption bands—a narrow one in red, a broader and darker in green, a third in green extending to violet side of E, and a fourth at junction of blue and green. Hæmatoporphyrin, when occurring in urine, is always alkaline.

CHEMISTRY OF FIBRINOGEN AND THE CLOTTING OF BLOOD

Two plasma have been prepared in the following way: (A) one part blood collected in one part saturated sodium sulphate; (B) nine parts blood collected in one part potassium oxalate solution, 1 per cent. As a source of thrombin, use a little blood serum. Use small amounts of plasma.

Plasma A

(i.) Dilute a small quantity with five times its volume of water, and keep at 40° C. Clotting occurs.

(ii.) Dilute a small quantity with five times its volume of water, add a drop or two of serum, and keep at 40°; it clots more quickly than (i.).

Conclusions.—The plasma contains fibrinogen and fibrin ferment, which are prevented from reacting by the presence of neutral salts.

Plasma B

(i.) Dilute a small quantity with nine times its volume of distilled water; keep at 40°. There is no clot.

(ii.) Dilute as (i.), add a few drops of $CaCl_2$ solution, keep at 40°; clotting occurs.

(iii.) Dilute as (i.), add serum to which a little oxalate has been added. It clots. Prove by filtering and testing the filtrate with $CaCl_2$ that excess of oxalate was present. This proves that calcium free fibrinogen *plus* calcium free ferment form a clot.

Conclusion.—The oxalate has prevented coagulation by removing calcium salts, hence on their addition the blood coagulates. The calcium salts are concerned in the formation of thrombin.

Chemistry of Serum Albumin and Globulin

(*a*) Dilute 10 c.c. blood serum with 100 c.c. water. Is there any ppte.? Boil and acidulate at boiling point

with dilute acetic acid. Filter; ppte. is serum albumin and globulin. Test ppte. for proteins by Biuret, Xanthroproteic.

(*b*) Concentrate and test filtrate from (*a*) for sugar by osazone.

(*c*) Half saturate 10 c.c. blood serum with ammon. sulphate. What is ppte.? Filter and completely saturate with solid ammon. sulphate. What is precipitate? Filter and test filtrate with proteins.

CHEMISTRY OF BLOOD CORPUSCLES

(*a*) *Lecithin.*—Evaporate 30 c.c. of defibrinated blood on water bath until dry. Cool and extract with a mixture of 10 c.c. alcohol and 10 c.c. ether for one hour, with occasional shaking. Filter. Evaporate filtrate to thick oil on water bath, and test for lecithin.

(*b*) Incinerate completely a portion of residue from lecithin extraction. Dissolve residue in hot dilute HCl., filter, and test filtrate for phosphates, sulphates and iron (KCNS); Ca., Na. and K., by flame test.

ENZYME REACTIONS OF BLOOD

(A) Catalase

Fill a fermentation tube with hydrogen peroxide solution and introduce one or two drops of fresh blood (obtained by pricking). Note bubbles of oxygen collecting on standing. Repeat, using boiled blood.

(B) Peroxidase

The peroxidase reactions given by blood are used in the detection of blood, and are very delicate.

1. *Guaiacum Reaction.*—Add a few drops of tincture of Guaiacum to a dilute solution of blood. Add a few c.c. of ozonised ether. Note formation of blue colouration. Repeat, using boiled blood.

2. *Benzidine Reaction.*—Reagent is a solution of Benzidine in acetic acid. Acidify a dilute solution of blood with acetic acid, add 2 or 3 c.c. of hydrogen, peroxide solution, and the benzidine reagent. A green or blue colour develops on standing. Repeat, using boiled blood.

Test solutions A, B, and C, for blood.

Demonstration.—Abell's vivi-diffusion experiments.

MUSCLE

Experiments on "Living" Muscle

Muscle Plasma.—Prepared in the following manner: A rabbit is killed, and its muscles washed free from blood by a stream of salt solution injected through the aorta. The muscles are then quickly removed, minced and extracted with 5 per cent. solution of magnesium sulphate. "Muscle plasma" solution prepared as above will be given out to the class.

1. Test reaction of muscle plasma solution.

2. Dilute a portion of the plasma with three or four times its volume of water, and place on water bath at

40° C. for about an hour. A typical *myosin clot* should form. Test the reaction of the muscle serum surrounding the clot. Has the reaction changed? Test for lactic acid as follows: Take 5 c.c. of Uffelmann's solution (1 grm. phenol in 75 c.c. water, to which are added 5 drops of a strong solution of ferric chloride) and add the muscle serum. Compare with solution of lactic acid.

3. Remove the clot of myosin; observe it is soluble in 10 per cent. sodium chloride and in 0.2 per cent. HCl, forming acid meta-protein.

4. Place a little muscle plasma in a t.t., put in cold water on warm bath; raise temperature of water very carefully, and note temperature at which coagulation occurs (Paramyosinogen). Filter off, and then heat clear filtrate as before, being careful to note the exact temperature at which the next coagulation occurs. A little cloudiness usually occurs before the complete coagulation, therefore do not mistake real coagulation point and filter at wrong time.

What are the coagulation temperatures of these two proteins? Which protein was present in greater amount?

Experiments on "Dead" Muscle

Some lean meat has been extracted with sodium chloride solution for several hours; the solution has been strained through cheese cloth to remove the large

pieces of meat. The solution thus obtained will be given out to the class.

1. Test reaction.

2. Filter, test for lactic acid.

3. Take about 20 c.c. of meat solution, saturate with sodium chloride, filter off ppte. of myosin; myosin collects as a film on sides of filter paper, and may be removed and tested before the entire volume of fluid has been filtered.

Apply the protein colour reactions.

4. *Extractives of Muscle.*—Gently boil a few pieces of lean meat with a little water for about 20 minutes; faintly acid with acetic acid, filter. Concentrate filtrate to about one quarter its bulk, and test for (*a*) Phosphates, (*b*) Creatinine (Bio. Chem., Synop. XXIII, B).

5. *Preparation of Glycogen.*—Grind a few scallops in a mortar with sand. Transfer to an evaporating dish, add water and boil for 20 minutes. Faintly acidify with acetic acid to precipitate proteins. Filter, and divide filtrate into two parts. Note the opalescence of the solution. Test one portion of the filtrate as follows:

(*a*) *Iodine Test.*—To 5 c.c. of the solution in a t.t. add 2–3 drops of iodine solution and 2–3 drops of ten per cent. sodium chloride. Warm this slightly, and then allow to cool. What do you observe? Is this similar to the iodine test upon any other body with which we have had to deal?

(*b*) Does the solution reduce Fehling's solution?

(*c*) Hydrolyse about 10 c.c. of solution with concentrated HCl. Cool and neutralise with solid KOH. Test with Fehling's solution. What do you conclude?

(*d*) Place 5 c.c. of solution in a t.t., add 5 drops of saliva, and place on the water bath at 40° C. for ten minutes. Does this now reduce Fehling's solution?

6. To the second part of the glycogen filtrate add 3–4 volumes of 50 per cent. alcohol. Allow the glycogen precipitate to settle, decant the supernatant fluid, filter the remainder, and upon the glycogen make the following test (place all filtrates and residues in residue bottle on the centre table) :—

Iodine Test.—Place a small amount of glycogen in an evaporating dish, add a drop of dilute iodine solution and a trace of sodium chloride solution. The same red colour is observed as in the iodine test upon the glycogen solution.

PHYSIOLOGICAL URINE

Collect the urine passed by yourself for 24 hours, mix, measure the volume and use about 300 c.c. for the following experiments.

Note the colour and reaction to litmus of normal urine. What is the average normal output per diem?

Specific Gravity.—Take the specific gravity of normal urine with the urinometer. Test the urinometer by taking the sp. gr. of distilled water, which should be 1000 at 15° C. Correction for temperature : Every

three degrees above 15° requires the addition of one unit of the last order; every three degrees below 15° requires the subtraction of one unit. Thus a sp. gr. of 1018 at a temperature of 21° would be 1020 at 15°. Urinometers are usually graduated to read at 15° C.

Calculation of Total Solids in Urine.—Multiply the last two units of the specific gravity at 15° by 2.6 (Long's coefficient). This gives the number of grammes of solid matter in 1000 c.c. of urine. Calculate the output per diem.

Determination of Urea. Ambard-Hallion Method.—(Two students working together perform two determinations.)

The apparatus consists of a graduated tube with a measuring cylinder, attached and a rubber bulb which can be attached to the lower end.

Two or three glass beads are placed in the rubber bulb, which is then attached to the graduated apparatus. The cock is opened, the bulb emptied of air, by compression, and the cock closed again. Ten c.c. of urine diluted 1 : 9, is then placed in the measuring cylinder, and allowed to run into the ureometer. Next 10 c.c. of 18 per cent. sodium hydrate are run into the ureometer, and the apparatus filled with distilled water with the exception of the rubber bulb, which must remain compressed to allow the entry of the sodium hypobromite. Five c.c. of sodium hypobromite solution are then allowed to enter the ureometer *slowly* and the tap is closed. After the lapse of about five

minutes, when the evolution of gas has slackened, a second 5 c.c. of hypobromite may be added. To ensure the complete evolution of the whole of the nitrogen, the liquid is gently agitated by means of the glass beads. The apparatus is allowed to remain undisturbed for fifteen minutes. Then place the ureometer in a cylinder of water, remove the rubber bulb, and depress the apparatus in the cylinder until the level of the liquids inside and outside is the same. Read the volume of gas evolved, observe the temperature and pressure, and from the chart read off the weight of nitrogen. Calculate the weight of urea; and from this the daily excretion.

Determination of Acidity and Ammonia.—1. Pipette out 25 c.c. urine, add 25 c.c. water and 15 grammes solid potassium oxalate. Shake thoroughly for one or two minutes, and whilst the solution is still cold from the effect of the oxalate titrate with decinormal NaOH until a permanent pink colour results. Note number of c.c. added. (Keep this solution.) Calculate the acidity of 1500 c.c. urine in terms of HCl, or multiply the number of c.c. of alkali used by four, and call it "per cent. of acidity."

2. Neutralise 20 c.c. of formalin solution with decinormal NaOH, using phenol phthalein as indicator, and add this neutralised solution to the neutral solution of urine obtained in the preceding "determination of acidity (1)." The two neutral solutions become acid. Why is this? Now titrate again with

decinormal NaOH, and note the number of c.c. used. This last titration gives the number of c.c. of NaOH equivalent to the ammonia. Explain fully the reason? Calculate the number of grammes of NH_3 excreted per diem.

Decomposition of Urine Urease.—Take 100 c.c. of the urine which you have just used for the urea and ammonia determinations, infect it with a little stale urine and set it aside in the draught cupboard until next period. The urine will undergo decomposition, a sediment will be formed which on examination under the microscope will be found to consist partly of "triple phosphate" crystals. What is the composition of "triple phosphate"?

At the beginning of the second period determine the urea and ammonia in the decomposed urine. The urea must be determined as before. If the urine is alkaline to phenol phthalein, it must be acidified with two or three drops of very dilute hydrochloric acid before proceeding with the determination of ammonia, and the acidity results neglected.

Note how the urea content has fallen and the ammonia risen owing to the decomposition by the urease. Give full equations for this decomposition. Calculate the percentage of urea converted into ammonia.

Uric Acid.—Saturate 100 c.c. of urine with solid ammonium chloride (27 grammes necessary), and add one or two drops of strong ammonia. After fifteen to

twenty minutes filter off the gelatinous precipitate of ammonium urate. Test for the presence of uric acid in this precipitate by placing a portion of it in an evaporating basin and applying the murexide test. (Cp. Bio. Chem., Synop. XXIII, A.)

Creatinine.—Picramic acid reaction.—To 5 c.c. of urine add 0.5 c.c. picric acid solution and 1 c.c. of KOH. Note the bichromate red colour of the solution.

Repeat the reaction with urine diluted with an equal volume of water, and compare the depth of colour in the two experiments.

What do you know about the output of creatinine?

Potassium Indoxyl Sulphate (*Urine Indican*).—Decolourise about 10 c.c. urine by precipitation with 1 c.c. lead acetate and filtration. To clear filtrate add 2 c.c. chloroform and a volume of Obermeyer's reagent (solution of $FeCl_3$ in strong HCl.) equal to that of the urine. Shake vigorously, and on standing note the blue colour of the chloroform.

Carry out a second experiment with a specimen of urine diluted with an equal volume of water. What is the source of the potassium indoxyl sulphate, and what equations represent its conversion into indigo?

Excretion of Hippuric Acid.—A capsule of two grammes of ammonium benzoate will be given to each student. The student is expected to take the ammonium benzoate before retiring at night, to collect the urine voided the following morning, and bring the

specimen to the laboratory for examination for hippuric acid, as follows :

Add to the urine twice its volume of saturated ammonium sulphate solution, and 2 c.c. of concentrated sulphuric acid. Allow the mixture to stand several hours, and filter off the crystals of hippuric acid, recognising them by their crystalline form.

Chlorides.—Acidify 5 c.c. with nitric acid, add a few drops of silver nitrate, precipitated chloride soluble in ammonia. Which are present in greater quantity, chlorides or sulphates ?

Sulphates.—Acidify urine with HCl, add $BaCl_2$. Note precipitate of $BaSO_4$. Filter this precipitate until a *clear* solution is obtained. Now boil with a drop of concentrated HCl and note further precipitation of $BaSO_4$. This is due to the decomposition of *ethereal* or *organic sulphates*.

Filter off the second precipitate of $BaSO_4$; to filtrate add a small piece of zinc and, if necessary, HCl to give gentle evolution of gas. Test issuing gas for H_2S by lead acetate paper.

This shows the presence of *neutral sulphur*. What are the sources of these forms of sulphur in the urine, and what are the explanations of the reactions ?

Phosphates.—(*a*) Try reaction of the three sodium phosphates with litmus paper ; solutions provided.

(*b*) Make urine alkaline with ammonia, add a drop of magnesium sulphate, shake up, warm, allow to stand until end of period. Examine a drop of sedi-

ment under low power for triple phosphate; sketch crystals. See action of Urease.

(*c*) Earthy phosphates. Make urine alkaline with ammonium or potassium hydrate; warm, and note precipitate of calcium and magnesium phosphates.

(*d*) Alkaline phosphates. Filter off earthy phosphates from (*c*); divide filtrate; add magnesia mixture to one, acidify the other with nitric acid, and boil with ammonium molybdate.

What are the normal sources of phosphoric acid in urine?

PART III

CLINICAL CHEMISTRY

SYNOPSES OF THE COURSE OF TWELVE LABORATORY PERIODS IN CLINICAL CHEMISTRY GIVEN AT THE END OF THE WINTER TERM OF THE THIRD YEAR

THIS short course is intended to include the essentials of routine chemical examination of urine and stomach contents.

It is a systematic course in the technique of tests and analyses which the student will be required to use in the study of his cases in the hospital, and which the practitioner must be familiar with and regularly employ in his daily work.

The laboratory practice is supplemented by lectures, in which the clinical bearing of the various processes is fully explained.

R. F. R.

FIRST LABORATORY PERIOD

URINE

1. *Colour.*—Observe and record colour of A, B, C, D, E, and F (central table). D, E, and F abnormal. Colour of A pale, B normal, C high coloured or febrile, D dark.

Test reaction, samples A, B, and E, using rod to bring drop in contact with litmus paper. A is alkaline from ammon. carb., B amphoteric, E is alkaline from fixed alkali. Note red colour of litmus moistened by A is restored on drying slowly over Bunsen flame or on hot porcelain plate, while that moistened by E remains blue after drying.

(See Part II regarding the reaction of urine.)

Blood-red colour of E from alkaline chrysophanate, resembles blood, but gives no absorption spectrum; loses red colour on acidification. Sample F, green colour from methylene blue; turns red when boiled with HCl; green colour restored when carefully neutralised; shaken with chloroform, pale blue pigment is dissolved out.

Sample G, Iodides. Add drop or two of chlorine water or bromine water, shake with chloroform, violet colour; or a drop of starch solution and a drop of chlorine water—green to blue colour.

2. *Solids in Urine.*—Take specific gravity of G and H with urinometer. Test your urinometer, using distilled water and the solution having a sp. gr. of 1020 at 15° C., make correction if necessary. Calculate approximate solids in each excreted in 24 hours, taking the quantity per 24 hours to be 1½ litres (or 50 fluid oz.). What is the average normal amount? Multiply last two figures of sp. gr. by 2.6 (Long's coefficient); product is grammes per litre, or parts per 1000.

3. *Transparency.*—Describe samples 1, 2 and 3.

Note normal mucus cloud in 1, silk opalescence-bacteria in 2, and sediments in 3, 4, 5, and 6.

4. *Urea.*—Determine urea in sample H, using Doremus' apparatus; graduation is in grammes per 1 c.c. urine. Fill to mark with sodium hydrate (40 per cent.), add at draught cupboard, 1 c.c. bromine, mix, just fill by adding water. Clean pipette. Fill with urine, and add exactly 1 c.c. slowly to the alkaline hypobromite in the tube as demonstrated; allow to stand a few minutes; read the percentage (normal about 2), and calculate quantity per 24 hours (normal about 30 grammes).

Repeat, using sample G.

SECOND LABORATORY PERIOD

Sediments and Albuminuria

1. Repeat urea determination, sample A; calculate to daily excretion of 1800 c.c.

Urinary Sediments

For the proper examination of sediments a hand centrifuge is desirable. The sediment obtained should be examined microscopically, and chemically if necessary.

The sediments obtained are either organised or unorganised. Organised sediments consist of casts of the renal tubules, epithelial cells from different parts of

the urinary tract, pus, blood cells, spermatozoa, parasites, etc. (see Clinical Microscopy).

Unorganised sediments vary with the reaction of the urine. The more common varieties are given below.

Examine a drop of suspended sediment under the microscope. (See microscopes with prepared slides—central table.) Test as described below a few drops in a small test tube.

IN ACID URINE

URIC ACID :

Light yellow to dark reddish-brown in colour. Crystalline form very varied: rhombic prisms, wedges, rosettes, dumbbells, whetstones, butcher's trays, &c., insoluble in HCl. Soluble in sodium hydroxide and reprecipitated by hydrochloric acid.

URATES :

Pinkish, soluble on warming, and in alkalies, sometimes amorphous, sometimes crystalline, as "thorn-apples," fan-shaped clusters of prismatic needles. These are always coloured.

CALCIUM OXALATE :

Small octahedra, with an envelope-like appearance (squares crossed by two diagonals); also in dumbbells. Insoluble in acetic acid, easily soluble in hydrochloric acid.

CALCIUM HYDROGEN PHOSPHATES (stellar phosphates):

In rosettes of prisms and in dumbbells. Rather rare.

CYSTINE:

Colourless hexagonal plates, soluble in ammonia, insoluble in acetic acid. Very rare.

IN ALKALINE URINE

AMMONIUM MAGNESIUM PHOSPHATE (triple phosphate):

Colourless prisms ("coffin-lids" and "knife-rests") or feathery stars. Easily soluble in acetic acid. Not dissolved on warming.

ALKALINE EARTHY PHOSPHATES of Ca and Mg:

Colourless amorphous. Insoluble on warming and in alkalies, soluble in acetic acid.

CALCIUM HYDROGEN PHOSPHATE: see above.

CALCIUM CARBONATE:

Dumbbells or spheres with radiating structure. Soluble in acetic acid, with evolution of gas.

AMMONIUM URATE:

Yellow, or brownish amorphous masses, or showing "thorn-apple" crystals. Soluble on warming.

ALBUMINURIA

For clinical purposes minute quantities of serum albumin and serum globulin in urine are best detected by coagulation through effect of heat and chemical reagents.

Preparation of Sample.—Urine should be acid and quite bright; if not, it should be acidified with a drop or two of acetic acid, and filtered. If filtration alone does not clarify, shake up with a pinch or two of Kieselguhr (infusional earth), or add about 1 c.c. of magnesia mixture, filter again, and acidify.

The two most generally used tests are the Heat and Cold Nitric Acid reactions.

(1) *Cold Nitric Acid contact method, Heller's test.*—(*a*) Pipette method. Make a pipette, using moderately wide glass tubing. Take one inch clear acid urine in test tube, float up slowly with strong nitric acid by filling the pipette about half full of strong nitric acid, closing and introducing to bottom of test tube, allow acid to slowly escape; close again and carefully withdraw. Work near or over a sink so as to avoid dropping acid on the bench.

Apply test to A, B, C, and D. A is normal urine; observe oxidised pigments at line of contact, no opalescence; B contains 0.05 per cent. of albumin; C is alkaline, containing albumin; apply before and after acidification and clarification; D contains alkaloids and no albumin.

(*b*) *Albumoscope method.*—This method is a much more convenient and cleaner way of applying any contact test than by using a pipette. Pour clear acid urine in larger tube until it reaches the bottom of the cup at top of capillary tube forming the other arm. Pour about 1 c.c. of strong nitric acid in the cup, and

observe reaction at line of contact. Use samples A and B. Dilute B with normal urine, sample A, by measure until reaction is no longer obtained on standing five minutes. Use either pipette method or albumoscope. Calculate the percentage of albumin you are able to detect. Keep in small flask about 50 c.c. of that which gave the faintest reaction until next period. To prevent fermentation, add a few drops of toluol, and shake up.

THIRD LABORATORY PERIOD

ALBUMINURIA—(*continued*)

1. *Boiling and Acidification.*—Fill a long test tube ¾ full of sample B, properly prepared; hold it by the bottom, and apply small flame half way up the tube until the urine boils. Compare upper with lower part of test tube contents. Add a few drops of acetic or nitric acid. Does it affect the coagulum?

Repeat, using sample E, containing an excess of earthy phosphates. Note effect of adding acid to the precipitate. Apply this heat test to B after some dilution and to the portion retained from last period, which just reacted to Heller's test. Which is the more delicate test?

2. *Picric Acid Reaction.*—Make a saturated solution by boiling about half a gramme of the acid in a test tube of water. Cool under tap, and either pour off the clear liquid, or filter. Apply test thus: Test tube ¾ full of clear urine (B), hold obliquely, fill upper

¼ with reagent; observe reaction where the mixture occurs. Repeat, using sample D, containing alkaloids, and note results. Apply this test to a portion of the urine retained since last period. Is there a reaction visible?

3. *Ferrocyanide Method.*—Make urine acid with acetic acid, and add a few drops of solution of ferrocyanide of potassium.

4. *Other Tests.*—Test A, B, and D (after diluting B 1–50) with each of the following reagents, applied by the contact methods, using pipette or albumoscope: (*a*) Trichloracetic acid, (*b*) potassio-mercuric iodide.

Make careful notes of the limitations and sources of error in these reactions. Read text book and lecture notes on reactions for albumin.

FOURTH LABORATORY PERIOD

ALBUMINURIA—ALBUMOSURIA—HÆMOGLOBINURIA AND ICTERIC URINE

1. Test samples A and B for traces of Albumin by nitric acid contact method. Report condition of urine before testing, and state how you prepared it.

2. Determine the *Quantity of Albumin* in sample C, using Esbach's Albuminometer. Fill to line U with clear acid urine, then to line R with Esbach's reagent (10 grms. picric acid, 20 grms. citric in 1 litre). Invert tube slowly five or six times to thoroughly mix the fluids; do not shake up. After 24 hours the

figure showing the depth of the precipitated albumin represents grammes per litre of albumin.

3. *Albumosuria.*—By preliminary test show absence of albumin. Use sample D. Apply biuret test (1) by floating up urine with Fehling's solution, allowing to stand ten minutes; reaction seen but obscured by precipitated phosphates; (2) fill test tube ¾ full of urine made strongly alkaline with potas. hydrate, pour about 1 c.c. of very dilute copper sulphate solution carefully on top. Allow to stand, and observe reaction.

For further reactions and distinctions of proteoses and albumoses, see Laboratory Notes on Biological and Physiological Chemistry.

4. *Hæmoglobinuria.*—Note colour and spectra of samples provided. Draw hæmoglobin and methæmoglobin spectra.

Guaiacum Test: A few drops of freshly made alcoholic solution of guaiacum (1 grm. in 60 c.c. of 95 per cent. alcohol) to urine in test tube: shake up with 2 or 3 c.c. ether containing hydrogen peroxide ("ozonised ether"), prepared by shaking two volumes of hydrogen peroxide solution with one volume of ether, and decanting the ether. Observe blue colour—Oxidase reaction.

Benzidine Test: Reagent, about half c.c. of a fresh saturated solution of Benzidine in glacial acetic acid and 2 or 3 c.c. hydrogen peroxide solution; acidify the urine with acetic acid, and add an equal volume to the reagent. A green or blue colour results.

5. *Bile Pigments.—Icteric Urine*—Note colour and appearance of the froth on shaking the bottle of icteric urine. Filter about 20 c.c. through a dry filter, draw up the filter paper in the funnel when empty, allow to stand a few minutes, then touch filter paper where pigment is marked with a drop of diluted fuming nitric acid. Observe colours green and red.

FIFTH LABORATORY PERIOD

Carbohydrates in Urine

Examine urines A and B for trace of albumin by nitric acid contact method.

1. *Glycosuria.*—Test sample A for glucose by each of the following: [1] Fehling's, [2] Nylander's, [3] Phenylhydrazine and [4] Fermentation tube. Carefully review notes on Carbohydrates in Biological Chemistry for details of methods. [5] Safranine test. This is a convenient reaction. To about 2 c.c. of a 0.1 per cent. solution of safranine add an equal volume of the urine and about 2 c.c. of potas. hydroxide. Boil the mixture, but do not shake up. The deep red colour fades in presence of sugar to a yellow. The safranine is reduced to a "Leuco-base."

When using Fehling's solution for qualitative test in urine, proceed always as follows: Take about 2 c.c. Fehling's, 3 volumes water, boil, and add not more than 2 c.c. of the urine; a reduction indicates glucose.

Repeat the five tests on sample D containing reducing substances not glucose.

2. *Lactosuria.*—Examine urine B for lactose: Use Fehling's, Safranine test, and Fermentation tube. How does B differ in reaction from A?

Apply mucic acid reaction, using 100 c.c. of urine. Groups of four students working at draught cupboard. To the urine add 20 or 25 c.c. of strong nitric acid, evaporate on water bath in cupboard until the volume is reduced to 20 c.c. Urine should then be clear and contain crystals of mucic acid while warm or on partly cooling. (See notes on Biological Chemistry.)

3. *Pentosuria.*—Test C for pentose, using Bial's solution [1 grm. orcin, 500 c.c. HCl, 25 drops ferric chloride solution]; boil about 5 c.c. of reagent in test tube; while still boiling add two or three drops urine. Note green colour in a few minutes without further heating.

SIXTH LABORATORY PERIOD

Quantitative Determination of Glucose

1. What sugars are present in samples A and B?

2. *Benedict's Method.*—Determine the quantity of glucose in D, using Benedict's method. (See Bio. Chem., Synopsis IX, 5.) Repeat the determination until concordant results are obtained.

3. *Polariscope.*—Read the percentage of glucose in the solution in long tube, using Schmidt & Haensch's clinical saccharimeter. Decolourise and clarify a test

tube full of sample D by (1) shaking up with about 20 c.c. of Kieselguhr (infusional earth), or (2) adding 1/10th volume exactly (using flask provided) of lead acetate solution, and shaking up. Filter in either case, using a dry filter, into shorter observation tube until just too full; slide on cover and cap it firmly. Read, and multiply result by 2 to get the percentage. When using lead acetate to clarify, the reading should be multiplied by 11/10ths to allow for the lead solution.

Students will use the polariscope in the dark room in groups of four.

SEVENTH LABORATORY PERIOD

Quantitative Glucose; Acetone and Diacetic Acid

1. Determine the quantity of sugar in A and B, using Benedict's method.

2. *Roberts' Fermentation Method.*—Take two bottles, one of about 150 c.c. (a 4 oz. vial) and the other 8 oz., or 400 c.c., with a large neck (small pickle bottle suitable). Fill small one and cork it; use sample A. To wide-mouth bottle add 6 oz. urine rubbed up with a lump of yeast size of filbert. This bottle should be loosely stoppered with cotton wool. Place both bottles in the fermentation cupboard for 18 to 24 hours. Then take sp. gr. of both, each degree of sp. gr. lost is nearly equivalent to 1 grain of sugar per fluid oz. or 0.21 p.c.

3. *Acetone.*—Test sample C for acetone; make

urine alkaline with potas. hydrate; filter. To the clear filtrate add drop by drop strong iodine solution (Lugol's solution) until permanently slightly coloured. An opalescence or precipitate of iodoform indicates acetone.

Confirm as follows: Filter off this precipitate, wash twice with water, when filter is quite empty remove any water in stem of funnel, add to the precipitated iodoform a few drops of warm alcohol and collect on watch-glass. Allow to evaporate at room temperature and examine under microscope for crystals of iodoform.

Dilute the sample 1 in 100 with water; repeat test. Distil over 2 or 3 c.c., and apply test to distillate. The distillation may be conveniently carried out without a condenser by tying a test tube to the side tube of a 200 c.c. distilling flask; acidify about 100 c.c. of dilute urine with a drop or two of sulphuric acid, and slowly distil over about 2 or 3 c.c. The flask can be held in the hand with a cloth about the upper part of the neck. Without testing the distillate the absence of acetone is not proven.

4. *Diacetic Acid.*—To filtered urine D add neutral ferric chloride. Filter the precipitated ferric phosphates, add a drop or two more to filtrate, and observe claret-red colour of clear urine.

Boil the urine; filter; repeat test; negative or very faint colour if due to diacetic acid, as it is converted into acetone on boiling.

Make another portion of D faintly acid with drop or two of sulphuric acid; shake up with ether; draw off ether; add equal volume of water and drop of ferric chloride; shake up; aqueous layer is coloured violet red.

The following is an excellent and ready test for the mixture of acetone and diacetic acid which is met with in cases of acidosis (Jackson Taylor).

To the urine in a test tube add an equal volume of freshly prepared sodium nitro-prusside solution. Carefully float on the surface of the mixture some strong ammonia water. Allow to stand for five minutes. The presence of acetone and diacetic acid is shown by a permanganate colour at line of junction spreading up into the ammonia. This test is not so sensitive when applied to the distillate. If the urine or the distillate is saturated with ammonium sulphate before the elements of the test are applied, it is much more sensitive, as the acetone is thrown out of solution.

Saturating the urine with ammonium sulphate before applying the above test causes the reaction with acetone to be much more distinct (Rothera).

EIGHTH LABORATORY PERIOD

Estimation of Chlorides—Ehrlich's Test

Estimation of Chlorides in Urine.—Volhard's method. Solutions are: (1) a standard silver nitrate solution 1 c.c.=0.01 sod. chloride (29.059 grms. per

litre); (2) a standard solution of potas. sulphocyanide, 1 c.c.=1 c.c. silver nitrate solution; (3) a saturated solution of ferric sulphate.

Measure exactly 10 c.c. urine into 250 c.c. flask, dilute with about 50 c.c. water, add about 4 c.c. strong nitric acid; add slowly exactly 15 c.c. silver nitrate from one of the large burettes; shake thoroughly, pour into 100 c.c. measure; rinse flask with a little water; add this, and make up exactly to 100 c.c. with water.

Filter through dry filter into dry graduate until exactly 75 c.c. have passed through; pour into beaker, add 5 c.c. strong ferric sulphate solution, then titrate with sulphocyanide solution from burette until first faint pink colour appears. Repeat, using same sample; add 1 c.c. at a time till about 1 c.c. short of end reaction, then add 1/10th c.c. to finish.

Example. Suppose 6 c.c. sulphocyanide are required to combine with excess of silver nitrate in the 75 c.c., therefore 8 c.c. are required for total 100 c.c. filtrate representing 10 c.c. urine. Since 1 c.c. sulphocyanide solution=1 c.c. silver nitrate, 15–8=7 c.c. silver nitrate were required for 10 c.c. urine, 1 c.c. silver nitrate=0.01 sod. chloride, therefore 0.07 grm. sod. chloride in 10 c.c. urine, *i.e.* 7 per cent. or 0.07 × 150, × 10.5 grms. in 1500 c.c.

Ehrlich's Test.—The Diazo reaction. Two solutions used: (1) a 5 p.c. solution HCl, with 1 p.c. sulphanilic acid; (2) an 0.5 p.c. solution sod. nitrite. These two

are mixed immediately before use, 40 of No. 1 solution to 1 of No. 2 solution. Two or three c.c. of urine are mixed with an equal volume of the reagent, and well shaken. Ammon. hydrate is allowed to flow down t.t. and overlie mixture; at line of junction red characteristic colour develops. Any urine may give a yellow colour.

NINTH LABORATORY PERIOD

Clinical Chemical Analysis of Urine—Study of Indicators—Lactic Acid

1. Fill out the following Report [see opposite page] on the urine marked A, stating presence or absence of the various pathological constituents and the quantity of chlorides, urea, sugar and albumen (if the last is present in more than traces).

2. *Special Indicators.*—Observe and record colour reactions of indicators, Phenolphthalein, Tropaolin oo and Töpfer's reagent, by adding two or three drops of indicator to test tube of water and one drop of potas. hydrate, then make each just acid with a drop or two of dilute HCl. Compare effect of one drop of (1) dilute lactic, (2) acetic, and (3) hydrochloric acids on a test tube of water coloured by these indicators.

3. *Lactic Acid* —Test solution B by (1) Thomas' reaction. To about 6 c.c. of solution B, containing 0.2 per cent. lactic acid add three or four drops of the 30 p.c. solution of chromic acid. Observe clear yellow

solution which turns reddish-brown on heating in water bath for 10 minutes.

(2) Uffelmann's test: The reagent is prepared by adding ferric chloride to a 1 p.c. solution of phenol until a purple colour is developed; half a test tube of this reagent is turned yellow by a few drops of dilute lactic acid. Use solution B. Try effect of 0.2 p.c. solution HCl, and compare results.

Report No..... *Date*.....

Name.....

ANALYSIS OF URINE

Colour..... Transparency.....

Reaction .....

Specific gravity.....

Amount voided in 24 hours = 1750 c.c.

	Per Cent. by Weight.	*Amount in 24 Hours.*
Urea.....	.....	.....
Chlorides.....	.....	.....
Glucose	.....	.....
Albumin	.....	.....

Acetone ..... Indican.....

Diacetic acid..... Diazo reaction.....

Bile .....

Blood .....

Character of sediment.....

TENTH LABORATORY PERIOD

Acidity of Stomach Contents—Occult Blood

Stomach Contents.—Filter a little of sample A, HCl, B, lactic, and C, both acids. Test each for free HCl by Tropaolin and Töpfer's reagent—one drop of reagent to half a test tube filtered solution. Compare value of Tropaolin oo and Töpfer's reagent to distinguish lactic from HCl.

To A, B, and C apply Gunzberg's test for hydrochloric acid as follows: Place two drops of reagent in a clean evaporating dish, and two drops of filtered stomach contents; evaporate to dryness on a water bath, and note colour of residue—red to purple red with HCl. Test B and C for lactic acid, using both Thomas' and Uffelmann's reactions.

Clinical methods in general use for acidity: Measure exactly 10 c.c. sample C, use the 10 c.c. graduate, pour into small beaker and add 2 drops Töpfer's reagent. Add decinormal sod. hydrate from burette until red changes to orange yellow. Note and calculate percentage of HCl. Add a few drops of phenolphthalein, and continue adding the standard alkali until pink colour of phenolphthalein just appears=total acidity.

The number of c.c. of alkali required for 100 c.c. of stomach contents is called degrees of acidity; normal total acidity 50–75 degrees=0.2 to 0.3 p.c.; free HCl 25–50 degrees=0.1 to 2 p.c.

Repeat determination, using 10 c.c. and phenolphthalein alone; result = total acidity. Take another 10 c.c., and use Töpfer's reagent or Tropaolin 00, and reaction gives the free hydrochloric acid. Compare results.

Occult Blood.—Phenolphthalin Test—To 2 c.c. stomach contents, D, add about 4 or 5 drops alkaline solution of phenolphthalin and 4 or 5 drops of hydrogen peroxide (alkaline phenolphthalin prepared—2 grms. phenolphthalein, 5 grms. potas. hydrate, 10 grms. zinc dust, 100 water boiled for a few minutes after it is decolourised and filtered). Note red colour of alkaline phenolphthalein produced by action of oxidase. Repeat, using dry material marked E; shake up quantity size of half pea with 2 to 3 c.c. water in cold for some time. Apply test as above.

Benzidine Test (see Lab. period IV).—Apply this test to both stomach contents and dry material E.

ELEVENTH LABORATORY PERIOD

Acidity of Stomach Contents—Milk Preservatives

Determine total acidity of stomach contents A, using 10 c.c. as before. Take a second 10 c.c., determine acidity due to free HCl twice, using Tropaolin 00 for one and Töpfer's reagent for the other. Use indicator alone with water as standard for end reaction in a second beaker. Repeat until constant results are obtained.

Stomach contents have frequently a sour rancid odour due to lactic acid (see above), and also to presence of butyric acid. To recognise the latter shake up with ether, evaporate the extract to dryness, add a drop or two of sulphuric acid, and a drop or two of ethyl alcohol. Warm on water bath, and note odour of ethyl butyrate (pineapples).

MILK PRESERVATIVES

1. *Borax or Boric Acid.*—Take a few grains borax, add in test tube or watch-glass 2 drops sulphuric acid, stir well ; add a little alcohol, stir again, and test colour of flame with drop on rod.

Evaporate about 50 c.c. milk marked B to dryness, then char residue, rub up thoroughly with 2 or 3 drops sulphuric acid to a paste to set free the boric acid, add about 1 c.c. alcohol ; mix thoroughly with rod, and hold wet rod in Bunsen flame ; green colour indicates boric acid or borax. In practice use 150 to 200 c.c. milk.

2. *Salicylic Acid.*—Add a drop of ferric chloride to a very dilute solution of sodium salicylate, note colour of ferric salicylate.

Evaporate about 50 c.c. of milk (C) to dryness, do not char ; rub up with 4 or 5 c.c. water, filter and test filtrate with ferric chloride. In practice use 150 to 200 c.c. milk.

3. *Formic Aldehyde* (*Formalin*).—Reagent : 5 grms.

ferric chloride in 100 c.c. water, 100 c.c. sulphuric acid and 40 of water, mix slowly and cool. Mix 35 c.c. of ferric chloride solution with 100 of sulphuric acid to make the reagent. Reagent already prepared.

Add half volume of reagent to milks A and D, shake up, allow to stand 10 to 15 minutes; coagulated proteins and fat rises to top; solution below is pink if traces of formic aldehyde are present.

Compare reaction of the two milks.

PART IV

ADVANCED CLINICAL AND BIOLOGICAL CHEMISTRY

SYNOPSES OF AN OPTIONAL COURSE OF TWELVE OR FOURTEEN LABORATORY PERIODS IN ADVANCED CLINICAL AND BIOLOGICAL CHEMISTRY GIVEN AT THE END OF THE THIRD YEAR

THIS optional course is offered to those students who have had more than the average training in chemistry required for students in Medicine, and the class is limited.

The exact quantitative methods studied in this course are essential for the investigation of problems in metabolism, whether physiological or pathological.

It is designed especially for those who intend taking hospital positions after graduating, or who desire to prepare themselves for study abroad and for research in Medicine.

The course is required from all candidates for the Gold Medal in Medical Chemistry (Sutherland Medal).

R. F. R.

FIRST LABORATORY PERIOD

URINE—NEGATIVE IONS

I. *Acidity of Urine* (Naegeli-Folin): Measure 25 c.c. urine in 200 c.c. Erlenmeyer flask, add 3 or 4 drops of phenolphthalein and 15–20 grms. powdered

potas. oxalate. Shake up, and at once titrate to rose colour with decinormal sodium hydrate with constant shaking. Calculate to 1500 c.c. urine in terms of HCl, or multiply the number of c.c. of alkali used by 4 and call it the "per cent. of acidity."

II. *Phosphates in Urine.* (Sutton's *Vol. Analysis.*) Solutions required: (*a*) Standard uranium acetate; (*b*) standard solution of a tribasic phosphate (microcosmic salt); and (*c*) solution of cochineal. The uranium solution contains 35 grms. uranium acetate with 50 c.c. pure acetic acid per litre. The phosphate solution contains 5.886 grms. microcosmic salt, powdered and dry, per litre, and hence 50 c.c. contains 0.1 grm. P_2O_5. (Work out.)

To standardise the uranium solution, add a few [illegible] of cochineal solution to 50 c.c. of the phosphate [illegible] 1 in a small flask, heat to boiling point, and while boiling run in uranium solution from a burette slowly until mixture turns a dirty green colour. Calculate from this how much P_2O_5 1 c.c. of the uranium acetate solution is equivalent to, if exactly correct, 20 c.c. uranium acetate are required for 50 c.c. of microcosmic salt, 1 c.c. = 0.005 P_2O_5.

Take 50 c.c. clear urine—sample A—and estimate total phosphates. Titrate while boiling with the uranium solution as before, using the cochineal indicator, and the first appearance of the green colour is the end point. Repeat until the results are con-

cordant. Prove that the number of c.c. divided by 100 gives per cent. P_2O_5.

Earthy and Alkaline Phosphates.—Boil about 60 c.c. of sample A after adding a few drops of conc. sod. hydrate (thus precipitating the earthy phosphates); filter; take 50 c.c. of the filtrate, neutralise with acetic acid, and determine the phosphates as above with the uranium solution. This value is the alkaline phosphates. The difference between this and the previous determination gives the earthy phosphates.

Save all of the uranium residues. Put the mixture from the titration into the vessel on the centre table.

III. *Sulphur compounds in Urine.*—(A.) *Total Sulphates* (Folin).—Place 25 c.c. of urine in a 250 c.c. Erlenmeyer flask, add 20 c.c. of hydrochloric acid (1 volume of concentrated HCl to 4 volumes of water), and boil gently for twenty minutes, covering the mouth of the flask with a small watch-glass. Cool the flask under the tap, and dilute to about 150 c.c. with water. Add 10 c.c. of 5 per cent. barium chloride solution, slowly, drop by drop, to the cold solution, which must not be stirred or shaken during the addition, nor for at least one hour after. Then shake well.

In the meantime heat a crucible, cool it in desiccator, and weigh.

Filter contents of flask through 9 cm. ashless filter after greasing lip of flask and decanting with use of rod; do not fill the filter paper more than three-fourths full each time. Decant the clear supernatant liquid

without disturbing the precipitate any more than possible; then add a little hot water; stir up with rod. Gradually wash out all the precipitate with hot water. Filtrate must be quite bright. Wash carefully the filter and precipitate with hot water until a drop from funnel gives no reaction with silver nitrate. Dry filter in funnel in water oven.

Transfer to the crucible as much as possible of the precipitate, using platinum wire. Fold the dry filter tightly lengthwise with remaining precipitate inside, perforate top, and attach to platinum wire as instructed. Burn it over crucible as instructed; crucible should rest on glazed paper to collect any particles which might escape.

After ashing filter cover crucible, add 1 drop of dilute sulphuric acid, and heat to red heat for half an hour or until ash is quite white. Cool in desiccator, and weigh.

Calculate weight of sulphate ions; Barium sulphate multiplied by 0.4206 = H_2SO_4 by 0.412 = SO_4 ions.

SECOND LABORATORY PERIOD

URINE—NEGATIVE IONS (*continued*)

B. *Inorganic Sulphates* (Folin).—Place 25 c.c. of urine and 100 c.c. of water in a 250 c.c. Erlenmeyer flask. Acidify with 10 c.c. of hydrochloric acid (1 volume of concentrated HCl to 4 volumes of water). Add 10 c.c. of 5 per cent. barium chloride, drop by drop, as in the previous exercise, and proceed as there directed.

C. *Ethereal Sulphates* are found by taking the difference between the total sulphates and the inorganic sulphates.

D. *Total Sulphur in Urine* (Benedict).—Reagent: Crystallised copper nitrate 200 grms., potas. chlorate 50, water to 1 litre.

10 c.c. urine and 5 c.c. reagent in evaporating dish. Evaporate to dryness on water bath; place on triangle, gradually heat with small flame until black and dry, holding burner in hand. Then heat to redness for 10 minutes after it has become dry. When cool add 20 c.c. HCl (reagent bottle), warm until completely dissolved; transfer with rod to Erlenmeyer flask, dilute 100 to 150 c.c. with washing of dish; add 10 c.c. barium chloride solution slowly; allow to stand one hour, filter barium sulphate, and weigh as before.

Difference between A and D is "Neutral Sulphur"; calculate both to SO_4 ions.

Tabulate the results obtained from analysis of the sulphur compounds in the sample of urine.

IV. *Chlorides* (Volhard).—For method see Clinical Chemistry, Period VIII.

THIRD LABORATORY PERIOD

URINE

NITROGENOUS EXCRETION

I. *Total Nitrogen in Urine* (*Kjeldahl*). (Hawk, p. 401).

Principle: The nitrogen of most organic substances

and mixtures becomes converted into ammonium sulphate on heating with strong sulphuric acid. The ammonia is set free by strong alkali, distilled into excess of decinormal acid and the acid not neutralised determined by titration.

1. *Digestion.*—Measure exactly 10 c.c. of urine in Kjeldahl flask, about 10 c.c. sulphuric acid and 5 grms. dry potas, sulphate, heated until decolourised in draught cupboard, and heat continued for half an hour. Cover with paper cap until next period.

2. *Distillation.*—Add rapidly 300 c.c. distilled water to colourless liquid; add a drop or two of indicator and some pieces of porous earthenware to prevent bumping; add slowly 40 c.c. strong sodium hydrate to make strongly alkaline. Connect with distilling apparatus at once. This apparatus with receiver containing 100 c.c. of decinormal sulphuric acid should be ready. Distil for about 45 minutes. Disconnect receiver and test a drop of distillate with red litmus or turmeric paper; connect again quickly if distillate is still alkaline.

Titrate distillate with decinormal sodium hydrate, using alizarin as indicator, and calculate nitrogen in 1500 c.c. of urine; 1 c.c. decinormal sulphuric acid = 0.0014 nitrogen.

Determine the total nitrogen in sample A in duplicate as part of the partition of nitrogen.

II. *Ammonia.*—1. *Formaldehyde method.*—See Biological Synopses, XVII. 5, and Physiological Urine, Part II.

2. *Folin's microchemical method.*—Use the apparatus provided.

Into the test tube measure 1 to 5 c.c. of urine, so that 0.75 to 1.5 mgms. of ammonia-nitrogen are dealt with. For normal urine, 2 c.c. are usually about right. With diabetic urine, even 1 c.c. may be too much, and the urine must be previously diluted.

Add water, if necessary, to bring the volume to about 5 c.c. Add 3 c.c. of a saturated solution of potassium hydroxide, also a few drops of kerosene or heavy, crude machine oil (to prevent foaming).

Measure 2 c.c. of decinormal hydrochloric acid into the 100 c.c. graduated flask, add about 20 c.c. of distilled water, connect up the apparatus, and pass a strong current of air through for 30 minutes. Nesslerise as described below, and compare with 1 mgm. of nitrogen obtained from the solution of standard ammonium sulphate, similarly and simultaneously Nesslerised.

Calculation.—The number of mgms. of ammonia-nitrogen in the volume of urine taken are readily calculated, and so the number of grammes per 100 c.c. The amount of ammonia is obtained from this by multiplying by $\frac{17}{14} = 1.214$.

Preparation of the Nesslerised Solutions.—In another 100 c.c. measuring flask place 5 c.c. of standard ammonium sulphate solution, containing 1 mgm. of nitrogen, and dilute it to 60 c.c. To each flask add 3 c.c. of a cold saturated solution of Rochelle salt (to prevent the formation of a cloud on adding Nessler's

solution). Nesslerise both solutions as nearly as possible at the same time with 5 c.c. of Nessler's reagent diluted immediately beforehand with 25 c.c. of ammonia free water. Fill both flasks to the mark with water, and mix.

Determination of the Depth of Colour.—This may be done by means of a Duboscq colorimeter or by means of Nessler's tubes; the former method is the more rapid and convenient. In one of the chambers place some of the unknown solution, in the other some of the standard ammonia solution. Place the tube of the standard at a certain depth (20 mm. is usually the best), and adjust the other tube until the colours match. Several readings should be taken, moving the unknown from below and from above.

Calculation of results.—Example:

$$\frac{\text{Height of standard}}{\text{Height of unknown}} = \frac{20 \text{ mm.}}{21.3 \text{ mm.}}$$

So the 1 c.c. of fluid taken contains $\frac{20}{21.3} = 0.94$ mgm. nitrogen.

Urine was diluted to 1 in 10.

So 100 c.c. of urine contains 0.94 grm. nitrogen.

For methods of preparing the standard solution of ammonium sulphate and Nessler's solution, see Cole's *Practical Physiological Chemistry*, p. 178. For diagram and description of the colorimeter, see Cole, p. 191. Read this carefully before using the instrument.

FOURTH LABORATORY PERIOD

URINE

NITROGENOUS EXCRETION (*continued*)

III. *Uric Acid* (*Folin's microchemical method*).

Principle.—Urine is evaporated to dryness, and extracted with ether and alcohol to remove polyphenols. The residue is dissolved in dilute alkali, and treated with Folin's uric acid reagent. The fluid becomes coloured blue, and is compared colorimetrically with a standard solution of uric acid similarly treated.

Method.—2 to 5 c.c. of urine (depending on the specific gravity) are measured into an evaporating basin, a single drop of a saturated solution of oxalic acid is added and the whole evaporated to complete dryness on the water bath. Allow to cool, and add 10 c.c. of a mixture of two parts of dry ether (distilled over sodium) and one part of pure methyl alcohol. Allow to stand for five minutes without stirring. Carefully pour off the fluid, and extract similarly once more. To the residue add 10 c.c. of water and a drop of saturated sodium carbonate solution, and stir until solution is complete. Add 2 c.c. of Folin's reagent as provided, and then 20 c.c. of a saturated solution of sodium carbonate. Transfer the blue solution to 100 c.c. graduated flask, wash the evaporating basin out with water into the flask, and make the volume up to 100 c.c. By means of

Duboscq's colorimeter, compare the colour of this solution with that of a standard solution of uric acid prepared as described below.

Preparation of the Standard Solution.—Weigh out 250 mgms. of Kahlbaum's uric acid. Transfer it to a 250 c.c. measuring flask by means of 25 to 50 c.c. of water. Add 25 c.c. of a 0.4 per cent. solution of lithium carbonate, and shake at intervals for an hour before making the volume up to 250 c.c. The solution does not keep for longer than five or six days. (Solution provided.)

1 c.c. of this solution is carefully measured by means of a reliable pipette into a 100 c.c. measuring flask, 10 c.c. of water, 2 c.c. of Folin's reagent, and 20 c.c. of saturated sodium carbonate solution are added and the volume made up to the mark with water. The reagent and sodium carbonate should be added as nearly simultaneously as practicable both to the unknown and to the standard solution. Five minutes is the maximum allowable interval.

Calculation of Results.—Set the standard at a depth of x mm.

Let the depth of the unknown be y mm., when an equality of tint is obtained.

Suppose 3 c.c. of urine were taken.

Then 3 c.c. of urine contain $\frac{x}{y}$ mgm. uric acid.

So 100 c.c. contain $\frac{100\,x}{3\,y}$ mgm.

FIFTH LABORATORY PERIOD

URINE

NITROGENOUS EXCRETION (*continued*)

IV. *Creatinine.*—Determine the creatinine in sample A, using Folin's colorimetric method with Duboscq's colorimeter. (See Hawk, p. 415.) Calculate the percentage of creatinine in sample, also nitrogen as creatinine.

V. *Urea.*—Determine the urea, using Folin and Pettibone's Method No. 2. (Hawk, 4th edn. p. 307.)

State the results of the above nitrogen determinations in terms of Total Nitrogen—nitrogen as ammonia, nitrogen as urea, nitrogen as uric acid, nitrogen as creatinine, and undetermined nitrogen.

SIXTH LABORATORY PERIOD

1. URINE

ACETONE AND DIACETIC ACID

2. FÆCES

1. *Quantitative Estimation of Acetone and Diacetic Acid.*—Determine the combined acetone and diacetic acid in terms of acetone, by the Messinger-Huppert method (see Hawk, p. 422), and subsequently determine the acetone (the same day) by Folin's method (see Hawk, p. 423). Subtract the value obtained by the second method from that obtained by first method, to secure data regarding the diacetic acid content of

the urine in terms of acetone. Calculate this to diacetic acid.

(Sets of apparatus for these determinations are to be set up in private laboratory.)

2. *Fæces.*—The moisture is determined by drying at low temperature under reduced pressure in specially constructed vessels.

Determine the total nitrogen in the sample of dried fæces. Weight about one to two grammes from a weighing bottle by difference. Calculate the nitrogen to proteins by the factor 6.25.

SEVENTH LABORATORY PERIOD

Stomach Contents

1. Determine the *total acidity* of 10 c.c. filtered stomach contents—titration with phenolphthalein and decinormal NaOH. (See Clinical Chemistry, X.)

2. Determine *free HCl* with another 10 c.c. of same solution, using tropaolin 00; 2nd, using Töpfer's reagent.

3. Take 10 c.c. same solution, titrate to end point for HCl, using Gunzberg's reagent as outside indicator, as follows: Place cupped porcelain plate on top of water bath, boil water until plate is hot, evaporate to dryness, one drop of the reagent in each cup. Keeping plate hot, add 1 drop stomach contents to dry reagent in cup; observe red salt left after evaporation; add standard alkali, test after each c.c. until reaction ceases.

Note reading that gives last reaction; repeat titration, adding standard alkali, at once to this point, then one drop at a time until reaction is no longer visible. Compare with results obtained with tropaolin and dimethyl amidoazobenzol (Töpfer's reagent).

4. Titrate another 10 c.c., using alizarin as indicator.

By use of three indicators, viz.: 1 (2 or 3) and 4, the total acidity, free HCl, half combined HCl, total HCl, acid salts, and organic acids can be calculated.

A. Phenolphthalein gives free HCl *plus* acid salts, *plus* organic acids, *plus* half-combined HCl.

B. Alizarin gives free HCl, acid salts, organic salts.

C. Tropaolin, Töpfer's, and Gunzberg's reagent give free HCl.

Example A=6.4 c.c. B=4.8 c.c. C=3 c.c.

Total acidity—A=64 or 0.23% calculated to HCl.

Free HCl— C=30 or .11%. „ „

Half-combined HCl— A—B=16 or 0.06% calculated to HCl.

Total HCl— C+(A—B)=46 or 0.17% calculated to HCl.

Organic acids and acid salts—B—C=18 or 0.07% calculated to HCl. (See Clinical Chemistry, X.)

Test for lactic acid by Uffelmann's or Thomas' method. If absent, the last calculation represents the acid salts.

(Carbonic acid is always included in total acidity.)

EIGHTH LABORATORY PERIOD

URINE

CRYOSCOPY

Cryoscopy.—Note the construction of the two forms of apparatus for determining freezing points, viz., the Beckmann Heiden-hain and its modification for the cryoscopy of urine.

Each consists of the following parts:

1. A heavy glass jar to hold the freezing mixture, with ring-shaped stirrer.

2. An air jacket (sometimes alcohol or glycerine is used instead of air) shaped like a large test tube and held in position by the metal cover of the outer jar.

3. An inner tube with a short side arm to carry the liquid to be tested. This also carries through its cork a stirrer and a thermometer.

4. The thermometer fitting the cork of the innermost tube, which is graduated in hundredths of a degree centigrade.

For very exact determination of the lowering of the freezing point of water by the constituents in solution, the mercury in the stem of the Beckmann differential thermometer should be adjusted so that 0° C. corresponds to a point about half way up the stem. (Demonstrated.)

A freezing mixture—salt, fine ice, and water—is made, giving about $-3°$ C.

About 25 c.c. of urine to be tested is cooled to 0° C. in a mixture of ice and water. Pour enough urine into tube to cover the bulb of the thermometer; replace the cork, carrying the thermometer and stirrer. Manipulate the two stirrers, and watch the thermometer very carefully. A gradual fall of the mercury occurs, followed in a few minutes by a sudden rise. After a few oscillations it comes to rest. The temperature which the thermometer shows when the mercury is at rest after the sudden rise is the freezing point.

Before freezing, liquids are usually super-cooled. The thermometer then registers a temperature below the true freezing point. By constant stirring the over-cooling is lessened, but occasionally excessive super-cooling occurs. Then a minute fragment of ice introduced by the side arm causes instant freezing.

Results are stated as the number of degrees or fraction of a degree below 0° C. at which the urine freezes. D (the depression) varies from 1.30° C. to 2.20° C.

See Hawk, pp. 279.

NINTH LABORATORY PERIOD

Foods

MILK

1. Take the *specific gravity* of the sample of milk using a picnometer and the chemical balance. Regulate the temperature of the milk carefully, and avoid bubbles in the picnometer.

2. *Total Solids.*—Dry in the oven at 100° the extraction cup filled loosely with asbestos fibre, provided. Cool it, and weigh. Measure 10 c.c. of milk with pipette, run in slowly so it may be completely absorbed. Weigh again. Place in electric oven over night at 85–90° C. Cool, and weigh next morning. The loss is the water. Keep cup in a desiccator until ready for extraction.

3. *Butter Fat.*—Place the dry cup in a Soxhlet, and extract five or six times, using light petroleum spirit. Dry in oven, cool, and weigh again; the loss in weight is the butter fat, the residue is the solids other than fats, viz. salts, proteins, and lactose.

4. *Proteins.*—Determine the total nitrogen by Kjeldahl process; use a little copper sulphate. Weigh in the milk by difference from a weighing bottle. Pour directly into the digestion flask; do not use a funnel. Wash down any drop of milk high up on the stem of the flask with a wash-bottle before adding the acid, etc. Boil very slowly at first. Do not heat strongly until the caking is finished. The fat in the milk causes the digestion to be very slow.

Use the factor 6.37 (and not 6.25) in converting the nitrogen into milk proteins.

5. *Lactose.*—To about 350 c.c. of water in a beaker add 20 grammes (carefully weighed) of milk, mix thoroughly, acidify the fluid with about 2 c.c. of 10 per cent. acetic acid, and stir the acidified mixture continuously until a flocculent precipitate forms. At

this point the reaction should be distinctly acid to litmus. Heat the solution to boiling for one half-hour, filter, rinse the beaker thoroughly, and wash the precipitated proteins and the adherent fat with hot water. Combine the filtrate and wash water, and concentrate the mixture to about 150 c.c. on a water bath. Cool the solution, and dilute it to 200 c.c. in a volumetric flask. Titrate this sugar solution according to directions given under Benedict's method. (See Biological Chemistry Synopses, IX, III.)

Calculate the food value of half a litre of the milk.

1 gramme of carbohydrates or proteins = 4.1 Cal.

1 gramme of fat = 9.3 ,,

TENTH LABORATORY PERIOD

Foods (*continued*)

BREAD

1. *Water* (Leathes).—Take about 10 to 15 grammes of fresh bread, rub it up lightly to crumbs and weigh on torsion balance, 10 to 12 grammes to second decimal place. Spread it on a flat evaporating dish exposed to air to dry, then reduce to powder as fine as possible and dry in oven at 105° C. to constant weight after transferring the powder quantitatively to a weighed watch-glass.

Calculate the percentage of water.

2. *Proteins.*—Determine the total nitrogen, using about half a gramme of the dried bread, by the Kjeldahl

process. Calculate the percentage of protein, using the factor 6.25.

3. *Carbohydrates.*—Weigh carefully about 1 grm. of the dried bread. Place in a 200 c.c. flask; add about 60 c.c. of dilute sulphuric acid (2 per cent.). Fit the flask with a reversed condenser, and boil gently for two hours. Filter the contents into a 100 c.c. graduated flask, rinse the original flask twice with a few c.c. of water, pass these through the filter, and wash the filter until the solution and washings reach the containing mark. Mix and determine the glucose present by Benedict's method. The starch in the bread corresponds to nine-tenths of the glucose found.

Calculate the food value of a kilo. of this fresh bread allowing 1.2 per cent. of fats.

Approximate percentage composition of bread and milk:—

	Water.	Carbohydrates.	Proteins.	Fats.	Salts.
Milk	87	5.0	3.3	3.0–4.0	0.7
Bread	35	53.0	9.5	1.2	1.2

ELEVENTH LABORATORY PERIOD

Blood

1. *Quantitative Estimation of Urea.*—Use MM. Ambard and Hallion's apparatus. (See Part II, Urine.) Great care must be taken to exclude error when determining he urea in blood, as the volume of nitrogen is small. 10 c.c. of blood serum, or defibrin-

ated blood, are treated with an equal volume of 20 per cent. solution of trichloracetic acid—the acid added drop by drop, with stirring to precipitate the protein. Filter into the ureometer directly to the 10 c.c. mark. This volume contains 5 c.c. of the original blood. Proceed as described in the determination of urea in the diluted urine. Calculate to grammes of urea per litre of blood.

2. The *total nitrogen* in blood may be determined on 1 c.c. by the microchemical method of Folin. (See Cole, p. 175.)

3. *Ammonia.*—This is determined by the Folin method described under Urine; 5 c.c. of blood is a convenient quantity.

4. *Chlorides.*—Use Volhard's method, taking 10 c.c. of blood. (See Chlorides in Urine.)

Printed by Ballantyne, Hanson & Co
at Paul's Work, Edinburgh