A practical course of chemical physiology / by D. Noël Paton and Edward P. Cathcart.

PRACTICAL COURSE
OF
CHEMICAL PHYSIOLOGY

BY
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PREFACE

THE object of this Course is to give students a practical grounding in the study of Chemical Physiology.

It is divided into two sections. The Junior Section is intended for the ordinary medical student, and it has been planned to enable him to acquire the knowledge of the chemistry of the body which he will require in his future study of Physiology, and to train him in the methods of chemical investigation which he will afterwards use in his clinical work. It is really a course of Medical Chemistry. In our opinion such practical work should precede the systematic study of Physiology, and for this reason we have avoided, as far as possible, the blending of didactic with practical teaching.

The Senior Section is intended for advanced and science students who have completed the Junior Course. Its purpose is to give a training in the methods used in the prosecution of chemico-physiological and chemico-medical research, so that the student may find himself able to undertake such investigations with the aid of more elaborate and detailed books, such as those of Thierfelder, Hammarsten, etc. Since the time at the disposal of the student is limited, only a few typical methods have been selected, and no attempt has been made to cover the whole field of Chemical Physiology. To furnish a key to this domain is all that is attempted.

PRACTICAL CHEMICAL PHYSIOLOGY

JUNIOR SECTION

SYSTEM OF EXAMINATION OF PHYSIOLOGICAL SUBSTANCES

Use Urine, Milk, Salt, Fibrin.

I. Note Physical Characters.

A. Fluid.—1. Colour. 2. Transparency. 3. Deposit. 4. Odour. 5. Viscosity. 6. Specific Gravity. 7. Reaction.

B. Solid.—1. Colour. 2. Form—amorphous, crystalline, fibrous, etc. 3. Consistence—tough, elastic, hard, soft, etc. 4. Odour. 5. Solubility in various media—(1) Water (*a*) cold, (*b*) hot; (2) Sodium chloride, 1 per cent. solution; (3) Dilute KOH; (4) Dilute HCl; (5) Alcohol (*a*) cold, (*b*) hot; (6) Ether.

II. Investigate Chemical Characters.

Presence of Inorganic matter—Burn on platinum foil, and note ash. Nature of Inorganic matter—Ordinary Methods of Inorganic Chemistry.

Presence of Organic matter—Burn, noting odour and changes in the substance (exceptions).

Investigate the chemical nature of the Organic matter.

QUALITATIVE EXAMINATION OF THESE ORGANIC SUBSTANCES.

I. PROTEINS.

Proteins are the chief constituents of living matter. They are substances with very complex molecules. They yield on decomposition various derivatives, the chief of which are the monamino acids. They all contain Nitrogen and Sulphur ; many of them also contain Phosphorus.

Use Dried Egg Albumin and 5 per cent. Solution of White of Egg.

A. *Composition.*

Elements contained. Demonstration of the presence of—

Nitrogen.—Mix a pinch of soda-lime with a pinch of dried egg white, and heat in a dry test tube. NH_3 is given off. Test for this by means of moist litmus paper, or by HCl on a glass rod.

(For further tests, see Senior Section, pp. 36 and 43.)

Sulphur.—Heat another pinch of dried egg white in a dry test tube, and insert into the mouth of the test tube a piece of filter paper previously moistened with acetate of lead solution. Observe that it is blackened. (*Sulphide of lead.*)

Some proteins contain part of their sulphur in very loose combination. This may be tested for by boiling a solution of the protein with acetate of lead in alkaline solution. A black precipitate of PbS forms if loosely combined S be present. Try this test first with a small quantity of casein solution, then with egg albumin solution.

(For further tests, see Senior Section, p. 43.)

B. *General Tests for Proteins.*

i. Biuret reaction.

First add one drop of very dilute $CuSO_4$, then add KHO = violet or pink colour.

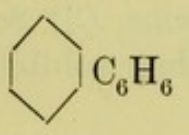
Depends on the presence of at least two $CO-NH_2$ groups, as in—

Oxamide $\begin{matrix} CO-NH_2 \\ | \\ CO-NH_2 \end{matrix}$ or Biuret $\begin{matrix} CO-NH_2 \\ | \\ NH \\ | \\ CO-NH_2 \end{matrix}$

ii. Xanthoproteic reaction.

$+HNO_3$ boil = yellow colour. Cool and add NH_3 to form a layer on the top = orange colour.

Depends on the presence of a benzene ring—



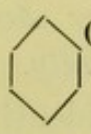
Probably a nitro-benzene compound is formed.

Repeat the test with benzene.

iii. Millon's reaction.

Add Millon's reagent ($Hg(NO_3)_2 + HNO_3$) and boil = rose-red precipitate.

Depends on the presence of a benzene ring with a single free hydroxyl group (OH) attached as in phenol—



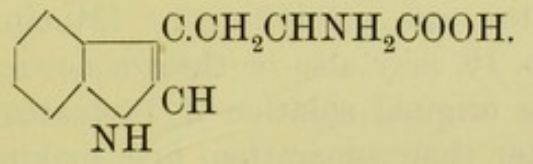
and in tyrosin.

Repeat the test on phenol—

iv. Adamkiewicz-Hopkins' reaction.

Add to the solution of protein a small quantity of glyoxylic acid, then pour concentrated H_2SO_4 carefully down the side of the test tube. A violet-red ring forms at the junction of the two liquids.

Depends on the presence of tryptophane—



v. Picric acid (or picric acid + citric acid = Esbach's reagent) gives a yellowish-white precipitate.

C. *Proteins are colloidal.* (*Demonstration.*)

Place some solution of egg white and common salt in a dialysing tube, and suspend in a beaker of water. After twenty-four hours test the water for proteins and chlorides. The protein has not dialysed.

Some proteins can be obtained in a crystalline condition (see Senior Section, p. 42).

D. *Identification of Various Proteins.*

The Proteins vary in character, and they are divided into three groups according to their composition—(1) Ordinary Proteins, (2) Conjugated Proteins, (3) Sclero-Proteins; and into sub-groups according to their solubilities.

A. Ordinary Proteins.

I. Those occurring in solution.

Use—

Solution of Egg Albumin, 1 *in* 20.	*Products of gastric digestion of Fibrin.*
Serum, diluted 1 *in* 10.	*Witte's Peptone*, 5 *per cent.*

To prepare Meta-Proteins, the student adds to half a test tube of Egg Albumin Solution either Acetic Acid or a few drops of Caustic Soda, and warms gently.

Table I. gives a method of identifying the various Proteins occurring in solution.

Before using this table always first test for the presence of Proteins.

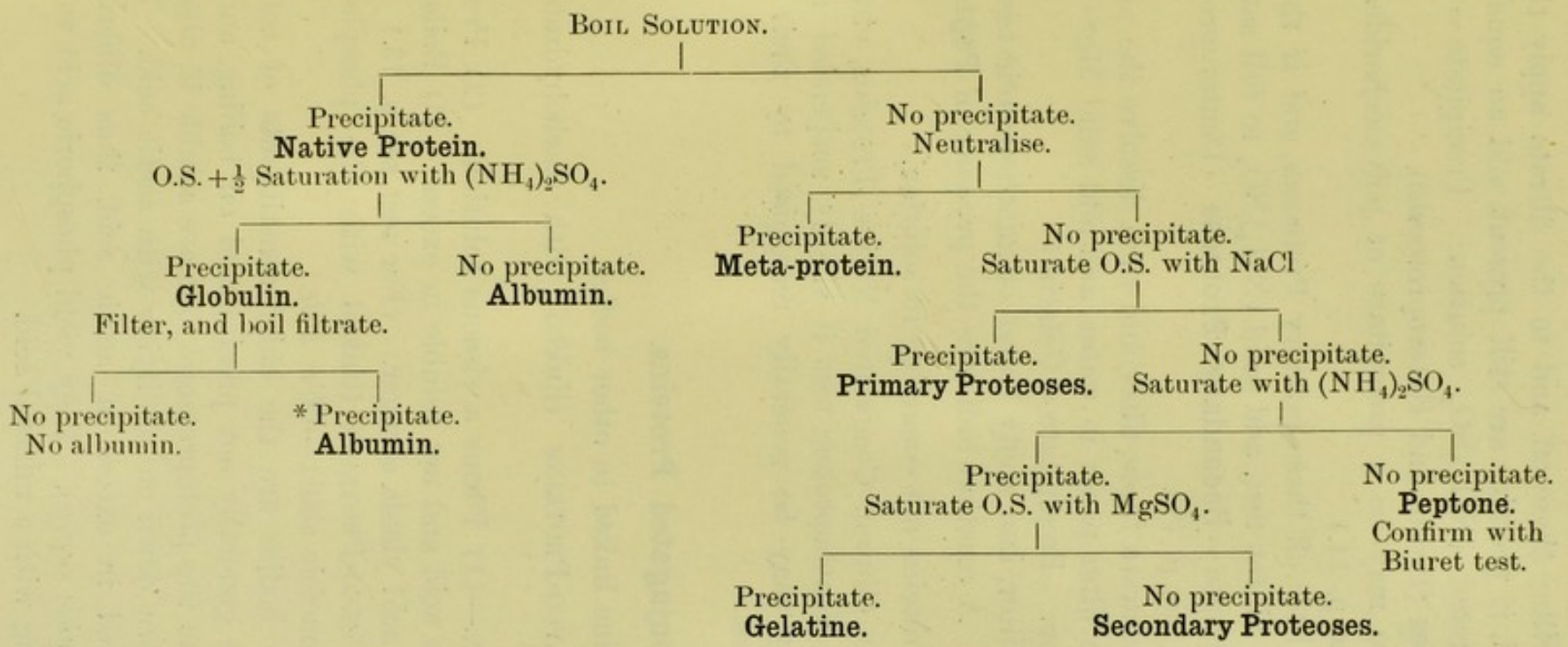
If more than one Protein be present.

(1) If the solution be markedly acid or alkaline separate any **Proteates** which may be present. If the solution be acid render it nearly neutral, or if alkaline make it faintly acid. Proteates are precipitated. (Mucin and Nucleo-Proteins, see p. 10, may also be thrown down.)

(2) Boil the original solution if Proteates be absent, or the filtrate after their separation, first making the solution faintly acid with acetic acid. If a coagulum forms **Native Proteins** are present.

TABLE I.

PROTEINS.



N.B.—Polysaccharides are also precipitated by neutral salts, and may have to be tested for.

* When both albumin and globulin are present in the same solution as, for example, in serum,

(3) Filter these off, and to the filtrate apply the biuret test, and, if proteins are still present, add an equal volume of saturated $(NH_4)_2SO_4$ solution. Precipitate = **Primary Proteoses** (Proto- and Heteroproteoses).

(There may be a precipitate of polysaccharides at this stage, see p. 14.)

(4) Filter off these primary Proteoses, and if the filtrate give the biuret test, add solid $(NH_4)_2SO_4$ to full saturation.

Precipitate = **Secondary Proteose** (Deuteroproteose, or **Gelatin**, see p. 11).

(To separate these, filter and then dissolve the precipitate from the filter paper in water, and add solid $MgSO_4$ to full saturation. Precipitate = Gelatin.)

(5) Filter, and apply biuret or xanthoproteic tests to the filtrate. A reaction indicates the presence of **Peptone.**

II. Proteins not occurring in solution.

Fibrin, Gluten, Coagulated Native Proteins, etc. These give the xanthoproteic test if simply suspended in water, and they may be generally recognised by their physical characters.

B. Conjugated Proteins.

Proteins linked to other bodies.

1. Glyco-Proteins (linked with carbohydrate).—(*Use Saliva.*)

Mucin.—(1) Forms a viscous solution. (2) Precipitated by acetic acid and not soluble in excess. (3) Boiled with a mineral acid yields a sugar. (For test see p. 13.)

2. Nucleo-Proteins (linked with the phosphorus-containing nucleic acid).—(*Use Bile.*)

These bodies are the chief constituents of cell nuclei. They are generally not precipitated on boiling, and behave much like the protoproteoses, but are soluble in alkalies and are thrown down on acidifying with acetic acid. They are redissolved in excess of acetic acid, thus differing from Mucin (*vide supra*). They yield phosphoric acid and purins on boiling with a mineral acid.

3. CHROMO-PROTEINS (linked with iron-containing molecule).—Hæmoglobin (see Blood, p. 18).

4. PHOSPHO-PROTEINS (linked with phosphorus-containing molecules, *not* nucleic acid).—(*Use Commercial Casein.*)

They are not precipitated by boiling, and generally behave like protoproteoses. They yield phosphoric acid on boiling with a mineral acid but no purins.

C. Sclero-Proteins.

These are the substances which build up many of the supporting, binding, and covering tissues of the body.

1. COLLAGEN.—(*Use White Fibrous Tissue.*) (*a*) Physical characters. (*b*) Chemical characters.—1. Insoluble in cold water. 2. Swells in acetic acid. 3. Soluble in hot KHO. 4. On boiling in water yields—

GELATIN.*—Hydrate of Collagen. — (*Use Commercial Gelatin and Gelatin Solution.*) 1. Swells in cold water, and dissolves on heating. 2. Forms a jelly on cooling. 3. Not precipitated by nitric acid. 4. Precipitated on full saturation with $MgSO_4$ (see p. 10). 5. Gives a violet colour with biuret test, and is thus distinguished from secondary proteoses which give a pink.

2. ELASTIN.—(*Use Elastic Fibrous Tissue.*) (*a*) Physical characters. (*b*) Chemical characters.—1. Insoluble in hot and cold water. 2. Soluble in strong KHO on heating. 3. Not readily precipitated from solutions, but tannic acid causes precipitation.

3. KERATIN. — (*Use Hair.*) (*a*) Physical characters. (*b*) Chemical characters.—1. Insoluble in water. 2. Soluble in alkalis. 3. Contains *sulphur* in loose state of combination.

Heat nails or hair with KHO solution in a test tube, and add a strong acid—H_2S is given off.

* If pure, does not give xanthoproteic test, but commercial preparations give it.

II. CARBOHYDRATES.

Carbohydrates are aldoses and ketoses. Those of chief physiological interest are derivatives of the Hexatomic Alcohol, $C_6H_{14}O_6$.

Use—

Glucose Solution, 2 *per cent.*
Lactose Solution, 2 *per cent.*
Maltose Solution, 2 *per cent.*
Cane Sugar Solution, 1 *per cent.*
Dextrin Solution, 1 *per cent.*
Glycogen Solution, 1 *per cent.*
Starch Solution, 1 *per cent.*

I. General Tests for Carbohydrates.

1. Boil for five minutes with dilute H_2SO_4. Make alkaline with NaHO. Apply Trommer's test (p. 13).

2. To half an inch of solution in a test tube add a drop or two of cold saturated alcoholic solution of α Naphthol and run down the side of the test tube about one inch of strong H_2SO_4. A violet ring forms at the junction of the fluids.

II. Classification.

1. Monosaccharides (Single Sugars), $C_6H_{12}O_6$. Aldoses—(1) Dextrose; (2) Galactose; Ketose—(3) Lævulose.

2. Disaccharides (Double Sugars), $2(C_6H_{12}O_6) - H_2O = C_{12}H_{22}O_{11}$, are formed by polymerisation of single sugars with accompanying dehydration:—(1) Lactose—Dextrose and Galactose; (2) Cane Sugar—Dextrose and Lævulose; (3) Maltose—Dextrose and Dextrose.

3. Polysaccharides, $x(C_6H_{12}O_6 - H_2O) = x(C_6H_{10}O_5)$, are formed by further polymerisation and dehydration, and form a series of advancing complexity from the simple dextrins to starch and cellulose:—

(1) Cellulose; (2) Starch; (3) Glycogen; (4) Dextrins or gums.

III. Distinctive Reactions of the Various Carbohydrates.

GROUP I. *Not Precipitated by Alcohol.*

THE SUGARS.

CLASS 1. **Monosaccharides.** *Reduce Barfoed's solution*—(Cupric acetate in acetic acid). Boil some of the reagent in a test tube, and, while boiling, add sugar solution drop by drop. The red suboxide appears in the solution.

They also, along with maltose and lactose, give the following reactions :—

A. They are oxidised when boiled with an alkali.

(1) *Moore's Test.* Sugar solution + KHO solution, and boil. A brown colour develops due to the formation of glucic and melassic acids. The oxygen is here taken from the air, and the test is not a delicate one.

(2) *Trommer's Test.* Add KOH solution to a small quantity of $CuSO_4$ solution till the precipitate of cupric hydrate is dissolved, and then boil the clear blue solution. Add to it about an equal quantity of the glucose solution. An orange-red or brown precipitate of cuprous oxide forms. The oxygen of the cupric hydrate is used to oxidise the sugar, which thus acts as a reducing agent.

(3) *Fehling's Test* is similar, but Rochelle salt is added. (See Quantitative Methods, p. 34.)

Many other tests depending on this same principle have been devised.

B. They ferment with yeast. Add a small piece of yeast, and keep in a warm place. Carbon dioxide gas is given off and alcohol formed.

C. They form acicular osazone crystals with phenylhydrazin.

To a dilute solution of the sugar add a drop or two of pure phenylhydrazin * and some glacial acetic acid, and keep near the boiling-point in a water-bath for about half an hour. On cooling, typical yellow crystals form, which must be examined microscopically. There must be sufficient phenylhydrazin added, as the formation of osazones depends

* Care must be taken not to let this touch the fingers.

on the presence of two molecules of phenylhydrazin to one of sugar.

D. They rotate the plane of polarised light—**Dextrose** and **Galactose** to the right, **Lævulose** to the left. (*Demonstration.*)

Lævulose may be distinguished from dextrose and galactose by Seliwanoff's test (general ketose reaction). To a solution of lævulose add an equal quantity of concentrated HCl and a small quantity of resorcin. A red colour develops *immediately* on boiling.

Class 2. **Disaccharides.** *Do not reduce Barfoed's solution.*

A. Those reducing Trommer's and other similar solutions.

a. **Maltose.** Ferments with yeast. Forms typical flat osazone crystals.

b. **Lactose.** Does not ferment with yeast. Forms typical osazone crystals—small spheres with spicules.

B. Not reducing Trommer's solution.

Cane Sugar. When boiled with a dilute mineral acid, it yields dextrose and lævulose, and reduces Trommer's solution. It forms no osazone crystals, and it chars with H_2SO_4. It gives Seliwanoff's reaction on account of its lævulose molecule.

Group II. *Precipitated by Alcohol.*

The Polysaccharides.*

1. Giving no colour with iodine. **Achroödextrin.** (Gives general carbohydrate tests, p. 12.)

2. Giving colour with iodine in cold, neutral or acid solutions, but not in hot or alkaline solutions. (*Always do a check experiment with a tube of water.*)

i. Solution not opalescent.

*The polysaccharides are precipitated by strong solutions of neutral salts, and may interfere with certain tests for proteins.

Erythrodextrin gives brown with iodine and is not precipitated by basic acetate of lead.

ii. Solution opalescent.

Glycogen gives brown with iodine and is precipitated by basic acetate of lead.

Starch gives a blue colour with iodine.

METHOD OF DISTINGUISHING THE VARIOUS CARBOHYDRATES.

(See Table II., p. 16.)

Pentose—$C_5H_{10}O_5$. (See Pentose, Senior Section, p. 45.)

Glycuronic acid ($C_6H_{10}O$) occurs in the urine under the administration of certain drugs (*e.g.* benzoates), combined with some substance generally of the aromatic group. The acid and its compounds give the reduction tests for sugars, and also give the orcin test for pentose (p. 45). They are thus distinguished from lactose, which does not give this test, and by the fact that even after boiling with a mineral acid and neutralising they do not ferment with yeast.

III. FATS, GLYCERIN, AND CHOLESTERIN.

Use Sheep or Ox Fat, and Cod-Liver Oil for Fats; use Gall Stones for Cholesterin.

Fats are *esters* formed by replacing the exchangeable atoms of H in the triatomic-alcohol glycerin with radicals of fatty acids, palmitic, stearic, and oleic. They occur in the cells of adipose tissue and as an emulsion in fluids like milk. A peculiar fat, **Lecithin**, occurs in many places, and abundantly in the yolk of egg. The colour of the yolk and of fats in other situations is due to pigments known as **Lipochromes**, which are soluble in the same media as the fats.

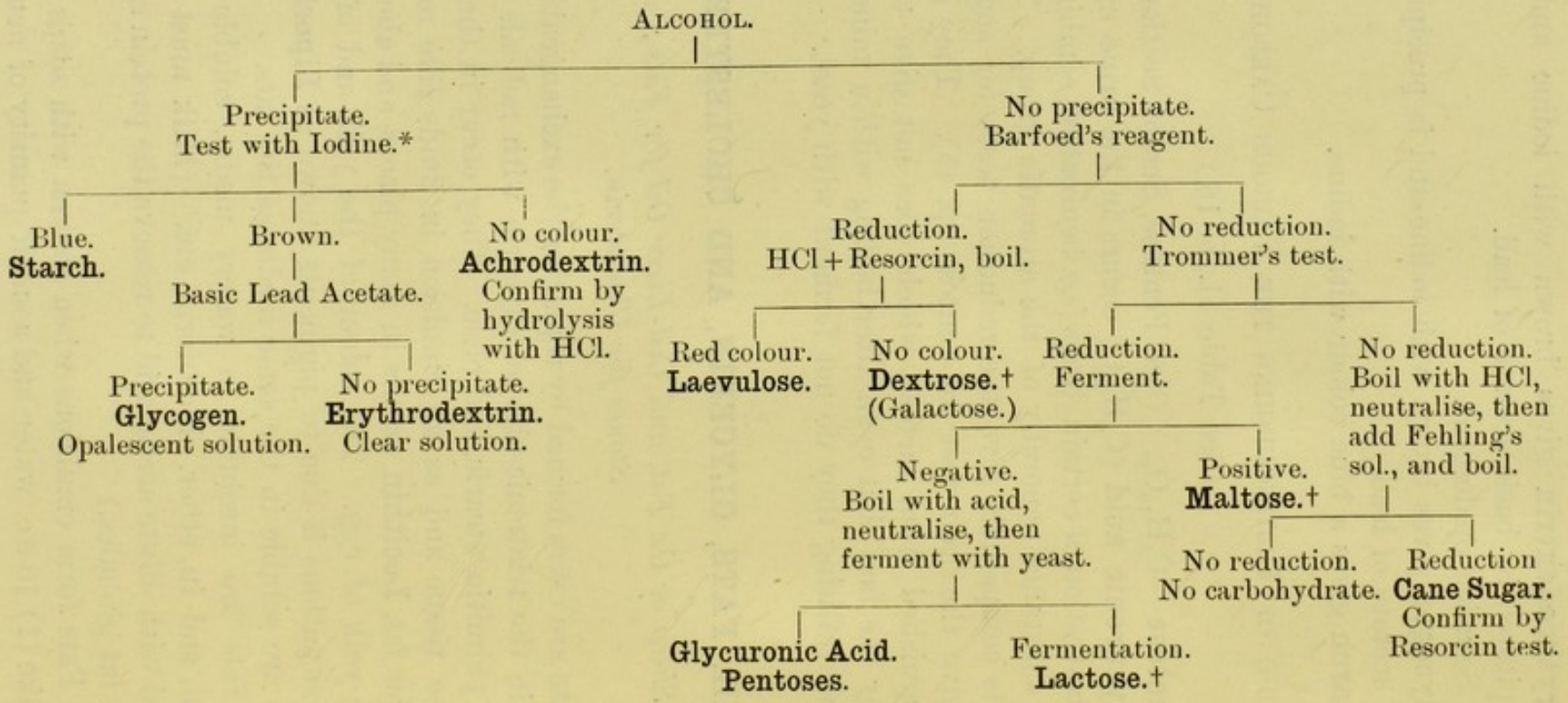
I. Fats are insoluble in watery media, soluble in hot alcohol, and in ether, chloroform, etc. (Milk must first be treated with dilute acetic acid to remove the protein envelope to the fat globules.)

II. Fats form emulsions when shaken with soaps.

Shake (1) 10 c.c. water and a small quantity of neutral oil.

TABLE II.

CARBOHYDRATES.



*Solution must be cool and not alkaline.

†Confirm by means of Phenylhydrazine.

(2) The same, with addition of a small quantity weak alkali (NaOH). (3) The same as in (2), but with the addition of a few drops of oleic acid in addition to the alkali. All emulsify, but (3) lasts longest, as a soap is formed.

III. Fats are saponified, *i.e.* converted into soaps and glycerin, on heating with a solution of caustic alkali (preferably an alcoholic solution). Heat a few drops of oleic acid or cod-liver oil with 10 c.c. 20 per cent. alcoholic NaOH ; a clear solution of soap is formed. If a small quantity of dilute H_2SO_4 is added to this clear solution, oleic acid will separate out.

To test for **Glycerin**—

Heat a few drops with a small quantity of acid potassium sulphate. Note smell of acrolein formed.

Detection of Fats.—Extract with ether. Evaporate. Note character of residue. Saponify as in previous section, or use Sudan iii as a stain (Histology).

Cholesterin is a monatomic alcohol. It occurs in bile and forms gall stones It also results from the breaking down of nervous tissue, and it is linked to fatty acids in the lanolin of sheep wool.

It behaves like Fats, but characteristic crystals form if it is dissolved in hot alcohol and this is allowed to cool on a microscope slide. These crystals on addition of strong H_2SO_4 (20 per cent.) assume a red colour at their edges.

FOOD STUFFS.

Practise the methods for the detection of proteins, fats, and carbohydrates on some of the common food stuffs, *e.g.* milk, flesh, liver, eggs, flour, etc. (For further study, see Senior Section.)

BLOOD.

Use Whipped Blood and a drop of Blood from the Finger.

I. Physical Characters.

1. *Colour.*—Disposition of colouring matter in erythrocytes (microscope). 2. *Opacity.*—Cause of. Take two test tubes

and put in each a drop of whipped blood, then add a small quantity of water to the one and to the other an equal volume of 0·75 per cent. NaCl solution. The pigment dissolves out of the corpuscles in the first, the mixture becoming transparent (*laked*), but not in the second. Confirm by the microscope. 3. *Smell.* 4. *Specific Gravity.*—Take solutions of sodium sulphate of different specific gravities about 1055, and find the one in which a drop of blood neither sinks nor floats. 5. *Reaction.*—Test with red glazed litmus paper.

II. Coagulation.—Formation of—*a.* Clot. *b.* Serum.

Use Blood from Finger.—Place a large drop of blood on a microscope slide, cover with a watch-glass, and observe clotting and separation of serum. (For structure of clot, see Histology.)

Use Oxalated Blood provided. Blood collected in $\frac{1}{10}$ *its Volume of* 0·2 *per cent. Solution of Oxalate of Soda.*

(1) 5 c.c. blood + 10 c.c. H_2O, no clot forms.
(2) 5 c.c. blood + 10 c.c. H_2O + 1 c.c. $CaCl_2$ 1 per cent. solution clots rapidly.

∴ Calcium is necessary for clotting.

III. Chemistry of Serum.

Proteins.

1. **Serum globulin.**—Precipitate from serum by half saturation with $(NH_4)_2SO_4$. Filter.
2. **Serum albumin.**—Boil the filtrate from the above = a precipitate.

IV. Chemistry of Erythrocytes.

1. **Hæmoglobin.**—Hb.

(1) *Physical characters.*—To a drop of defibrinated rat's blood on a microscope slide add water and stir till it becomes transparent. Then cover with a cover-glass and allow to stand for an hour and examine with a microscope. Draw the crystals.

(2) *Chemical characters.*—Similar to proteins, and gives protein reactions, but with the addition of an *iron* containing molecule. *Test for iron.* To the crystals under the cover-glass add a drop of dilute HCl and then a drop of ferrocyanide of potassium. The green reaction of iron appears.

(3) *Tests for hæmoglobin and its derivatives.*

Use Whipped blood 1 *in* 5 *of water, and dilute as required.*

(*a*) *Ozonic ether test.*—Add to dilute solution of blood a drop or two of tincture of guaiac, then a small quantity of ozonic ether. At the junction of the blood and ether a blue ring forms. This test is given by all pigmented derivatives of hæmoglobin and by many other substances.

(*b*) *Absorption spectra.*—Make a chart of the spectrum seen with a straight vision spectroscope. (See p. 49.)

A. Oxyhæmoglobin.

Put about quarter of an inch of the dilute blood in a test tube and then direct a steady small stream of water on to it. The solution will remain strong below and become more dilute above. Place the test tube close against the front of the slit of the spectroscope and examine in a good light. Draw the spectrum of the solution—weak, medium, and strong.

B. Reduced Hæmoglobin.

To a solution giving the two bands of oxyhæmoglobin very distinctly, add a drop or two of ammonium sulphide and warm gently. Note the change of colour and examine and draw the spectrum.

Then shake vigorously with air and get the return of the oxyhæmoglobin spectrum.

2. Methæmoglobin.

To a solution of HbO_2, which gives two very strong bands, add a few drops of a strong solution of ferricyanide of potassium and warm gently; the colour becomes brown and bubbles of oxygen are given off. Examine and draw the

spectrum. Then treat with ammonium sulphide and again study the colour and the spectrum.

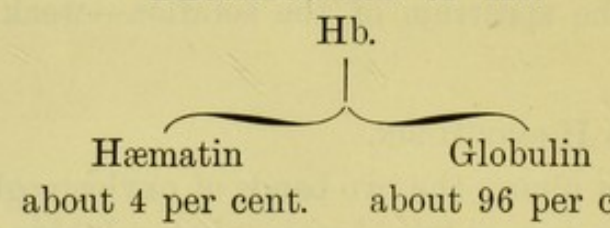
3. Carboxyhæmoglobin.

Pass coal gas through dilute blood giving two distinct bands; the colour and especially the froth becomes pink. A spectrum like HbO_2 is seen, but is not changed by the addition of ammonium sulphide.

Quantitative Examination of Hæmoglobin.

Haldane's modification of Gower's Method.—Set the tube containing the standard solution of 1 per cent. normal blood saturated with CO in the stand. Put a few c.mms. of water into the other tube. Prick the finger and draw up 20 c.mm. of blood in the pipette and blow it out into the water. Now pass a stream of coal gas into the tube, shake gently and dilute, taking care not to remove any fluid on the finger, and compare the colour with the standard. Note the point at which the colour is just too strong and the point at which it is just too pale, and take the mean of these. This gives the percentage of hæmoglobin expressed as per cent. of the normal—14 per cent.

Decomposition of Hb.



1. Hæmatin.—A pigment containing all the iron of Hb.

Acid Hæmatin.—Add acetic acid to diluted blood giving two very strong HbO_2 bands and warm. A brown solution with a spectrum closely resembling methæmoglobin, but not changed by ammonium sulphide, is produced.

Alkaline Hæmatin.—Add caustic potash to diluted blood, warm, and examine the spectrum (Oxidised Alkaline Hæmatin); then add ammonium sulphide and examine the spectrum (Reduced Hæmatin, Hæmochromogen). Repeat this, using some of a dried blood stain instead of the diluted blood.

2. Hæmatoporphyrin.—Add defibrinated blood drop by drop to strong H_2SO_4, stirring well. The acid removes the iron and leaves a purple solution of hæmatoporphyrin.

3. Hæmin.—Hydrochlorate of hæmatin. Place a fragment of dried blood on a microscope slide, add a drop or two of glacial acetic acid and a crystal of NaCl, put on a cover-glass and warm till it bubbles freely. On examination under the microscope after cooling small black crystals of characteristic shape will be seen.

Quantitative Estimation of Oxygen in the Blood.

Haldane Method.

Dupré's Apparatus.

(a) *Oxygen.*

1. Measure into the bottle 20 cm. of defibrinated blood.
2. Add to it 30 cm. of ammonia solution (1 in 500 of water). This "lakes" the blood.
3. Place about 4 cm. of saturated solution of potassium ferricyanide in the small tube and place it upright and without spilling in the bottle.
4. Connect with the burette and place the bottle in the basin of water, and, when the temperature becomes uniform, read the level of the fluid in the burette and note it. When several readings are the same—
5. Spill the ferricyanide into the blood and shake repeatedly, holding the bottle in a towel and replacing in water between times, and make readings of the level of the fluid in the burette till all the gas has come off.
6. Correct the volume of the gas evolved for temperature and pressure

$$v = \frac{v' \times 273 \times (b - b')}{(273 + t) \times 760}$$

b = pressure b' = tension of watery vapour at t = temperature and v' = volume read.

(b) *Carbon Dioxide.*

Now remove the cork, make the blood nearly neutral, then place some 20 per cent. tartaric acid solution in the tube. Put the tube in the bottle and repeat the process for the CO_2.

DIGESTION.

The student should start salivary, gastric, and pancreatic digestions (pp. 22, 23 and 24), and while they are in progress examine the characters of saliva and the methods of investigating the process of gastric digestion (pp. 22 and 23).

A. Salivary Digestion.

SALIVA.

I. PHYSICAL CHARACTERS. II. CHEMICAL CHARACTERS.—Reaction—neutral or alkaline. Composition. Organic solids.—1. Mucin (see p. 10). Add drop of acetic acid = Precipitate. 2. Proteins. Filter off mucin and test filtrate (p. 8). 3. Potassium sulphocyanide. *Test.*—Add ferric chloride with a drop of very dilute HCl to saliva on porcelain slab = red colour. (Discharged by mercuric chloride.)

III. PHYSIOLOGICAL ACTION.—Place some starch mucilage in a test tube. Add saliva, and keep at about 40° C. for five or ten minutes. Test with iodine, the blue colour gives place to the brown, indicating the presence of erythrodextrin. Test with Fehling's solution, get reduction indicating sugar (Maltose) (see p. 13).

Influence of various conditions upon the activity of salivary and other enzymes—

(1) boiling, (2) cold, (3) acids and alkalis.

Repeat the above experiment with saliva which has been boiled, with saliva and starch kept in tube surrounded with ice, with saliva to which a drop of mineral acid has been added, or with saliva to which a drop of strong alkali has been added. These experiments under varying conditions may be repeated with pepsin and trypsin.

B. Gastric Digestion.

Artificial Gastric Juice.—*Use an extract of the lining membrane of the stomach,* e.g. *a Pepsin powder in* 0·2 *per cent.* HCl *solution.*

I. Physiological Action.

1. *On Proteins.*

Digest a piece of fibrin in the artificial juice at about 40° C. for twenty minutes, and work out the proteins in solution (p. 8).

Soluble Native Protein.
|
Proteate (Meta-Protein).
|
Protoproteose.
|
Deuteroproteose.
|
Peptone.

2. *On Milk.*

Put the following into three test tubes, shake, and keep all at 40° C :—

(1) Milk + solution of rennet. Coagulation of caseinogen.

(2) Milk + 5 drops 0·2 per cent. pot. oxalate solution (to precipitate calcium) + rennin. No coagulation.

(3) Milk + 5 drops 0·2 per cent. pot. oxalate solution + 2 drops 1 per cent. $CaCl_2$ solution + rennin. Coagulation.

The enzyme rennin and calcium ions are both necessary.

II. Method of Investigating the Process of Gastric Digestion.

Œsophageal tube.—How to use it.

A. *Find if free* HCl *is present.*

At end of four hours after a meal, take a sample of the gastric contents and filter; test the filtrate.

1. *Phloroglucin Vanillin Test.*—Place a drop of this reagent in a small evaporating basin with a drop or two of the filtered gastric contents, and cautiously dry over a flame = a bright red colour develops if free HCl be present.

2. Uffelmann's Test.—Reagent. Add one drop of liq. ferri perchloridi to about 50 c.cms. of 2 per cent. solution of carbolic acid. A violet coloured solution is produced. This colour is discharged by mineral acids.

B. *To find if other Acids present.*

Lactic Acid.—(Normally present during first ¾ hour of gastric digestion, but not later.) Uffelmann's reagent gives a canary yellow colour.

Acetic Acid + Uffelmann's reagent gives a brownish colour.

Butyric Acid.—1. Smell. 2. Uffelmann's test gives a dirty grey colour.

C. *To find if Pepsin present.*

Filter the gastric contents, and, if the filtrate does not contain free HCl, add some 0·2 per cent. HCl, then add a small piece of fibrin stained with carmine and keep at 40°. If pepsin be present even in small amount the digestion of the fibrin is shown by the free carmine colouring the solution.

(For further study of gastric contents, see Senior Section, p. 47.)

C. Pancreatic Digestion.

For trypsin and diastase use a commercial extract of pancreas in 2 per cent. $NaCO_3$. *For lipase use fresh pancreas.*

Physiological Action on—

1. *Starch* (Diastase).—Digest starch paste in artificial juice, and examine for erythrodextrin and reducing sugars (p. 14).

2. *Proteins* (Trypsin).—Digest a piece of fibrin and work out the proteins in the solution (p. 8).

Allow the digestion to go on for twenty-four hours. Filter and evaporate the filtrate. Allow a drop to dry on a microscope slide, examine and draw the crystals. Do the biuret test on a digest which has been carried on for a prolonged period. Amino-acids, *e.g.* leucin and tyrosin, are ultimately split off by the breaking down of the protein molecule.

To another digest add bromine water; a purple colour will develop if tryptophane be present (see p. 7).

3. *Fats* (Lipase).

Mince fresh pancreas and mix with neutral cream to which a few drops of phenolphthalein have been added. Now add a single drop of very dilute sodium hydrate solution, just sufficient to cause the formation of a red colour. Keep the mixture at 40° C. The red colour is discharged owing to the liberation of fatty acids.

D. Bile.

I. Physical Characters.—1. Colour. 2. Transparency. 3. Viscosity. 4. Smell. 5. Specific gravity.

II. Chemical Characters.—1. Reaction. 2. Effect of heat. 3. Composition.

1. Mucin.—Precipitated by acetic acid (see p. 10).

2. Salts of the Bile Acids.—Glycocholic and taurocholic acids combined with *soda* as salts.

Tests for bile acids.—(1) Pettenkofer's test. Add syrup of cane sugar and shake thoroughly. Then add conc. H_2SO_4 slowly down the side of the test tube = rose red colour (other substances give this reaction). (2) Hay's test. (*a*) 50 c.c. dilute solution of bile; (*b*) 50 c.c. water alone. On sprinkling flowers of sulphur on the surface of (*a*) they sink, whereas they float in (*b*). Bile salts lower the surface tension.

3. Bile Pigments.—Bilirubin and biliverdin.

Tests for pigments.—Gmelin's test. To a small quantity of impure HNO_3 in a test tube add bile. A coloured ring forms at the junction of the two fluids. The colours are well seen on placing a drop of impure HNO_3 on a filter paper previously moistened with bile.

4. Cholesterin (see p. 16).

E. Detection of the Presence of Digestive Zymins.

Use various Commercial preparations.

Test their action—1. On fibrin for pepsin and trypsin (in acid and in alkaline solutions). 2. On starch for ptyalin and

other diastases. 3. On fat for lipase. 4. On milk for rennin.

(For examination of **Fæces**, see Senior Section, p. 41.)

URINE.

I. Physical Characters.—1. Colour. 2. Transparency. 3. Smell. 4. Specific gravity—urinometer—(i) Depends on per cent. of solids in solution. (ii) Method of calculating *solids* from sp. gr. Multiply last two figures of sp. gr. by 2·2 = solids in grms. per 1000 c.c.

Example.—Sp. gr., 1020 × 2·2 = 44 grms. per 1000 c.c.

5. Deposit.—(i) None when passed. (ii) Mucous cloud appears soon. (iii) As urea decomposes white precipitate of earthy phosphates. (Add a drop or two of ammonia.) (Triple phosphate.) (iv) If urine passed alkaline—Earthy phosphates (calcium and magnesium phosphate). (Add a drop or two of sodium hydrate.) (v) If urine very acid—Urates and uric acid.

Demonstration of these deposits.

II. Chemical Characters.

1. Reaction. Test with litmus paper. (*a*) When passed. —(i) Acid, not due to free mineral acids. (Apply phloroglucin vanillin test.) It is chiefly due to NaH_2PO_4. (ii) Sometimes neutral or alkaline after a meal. The alkalinity is fixed, and if litmus paper dipped in urine is dried the blue colour persists.

(*b*) After standing—Alkaline due to development of carbonate of ammonia from decomposition of urea. The alkalinity is volatile, and the blue colour of the litmus paper dipped in urine tends to disappear on drying. Earthy phosphates precipitated. Triple phosphate—$NH_4MgPO_46H_2O$.

(For estimation of acidity, see Senior Section, p. 41.)

2. Composition.—Percentage composition of little importance, but the amount of various constituents passed in twenty-four hours is of great importance. (*In examining urine, always take specimen from mixed urine of twenty-four hours.*)

Water.—Amount varies much, average about 1500 c.cm.

Solids.—More constant; 60–70 grms. Organic constituents form more than half.

A. Organic Solids.

I. **Urea**—$CO(NH_2)_2$.

(*a*) *Physical Characters.*

(*b*) *Chemical Characters.*

1. *Heat a small quantity of the crystals in a dry test tube.*

Note—

(i) Smell of ammonia. (ii) Continue heating till solidification commences. Biuret is formed. Do biuret test. (See p. 7.)

2. *Reaction with hypobromite of soda in alkaline solution* = bubbles of nitrogen given off—

$$CON_2H_4 + 3(NaBrO) = 3(NaBr) + CO_2 + 2H_2O + N_2.$$

3. *Reaction with nitrous acid* = bubbles of nitrogen if the solution of urea is strong.

$$CON_2H_4 + 2(HNO_2) = CO_2 + N_4 + 3H_2O.$$

4. *Reaction with nitric or oxalic acid.*—Pour a layer of the acid under a strong solution of urea in a test tube, and note the formation of crystals of nitrate or oxalate of urea.

II. **Purin Bodies.**

Uric acid is the most important member of this group present in human urine.

Physical Characters.—Crystalline form.—Very various, crystals usually pigmented. (Microscopic demonstration.)

Chemical Characters.—1. *Murexide test*; this depends upon the oxidation of uric acid. To a small quantity of uric acid in a porcelain basin add a drop or two of HNO_3 and heat cautiously to dryness. To the reddish-yellow residue add a drop of ammonia = purple colour (with KOH = bluish-violet colour).

This test is given by some other members of the purin group.

2. To urine add $(NH_4)_2SO_4$ (solid) to about saturation, then render alkaline with ammonia and allow to stand till

the following day = precipitate of ammonium urate. It is upon this reaction that Hopkins has based his quantitative method of estimating uric acid. (See Senior Section, p. 39.)

Urates and phosphates may occur as deposits in the urine.

Urates (usually brick coloured in acid urines).	*Phosphates* (white in alkaline urines).
1. Soluble on heating.	Not soluble on heating.
2. Soluble in KHO.	Not soluble in KHO.
3. Insoluble in acids.	Soluble in acids.

III. **Creatinine.**

Jaffe's test.—To urine add a small quantity of picric acid solution and then NaOH until it is alkaline. A deep brown colour develops. Do a check observation on a tube of water.

On this test Folin has based his colorimetric method of estimating creatinine quantitatively. (See Senior Section, p. 40.)

IV. **Hippuric Acid** = Benz-amino-acetic acid. (Of little importance.)

V. **Ether Sulphuric Acids.**

1. Indoxyl Sulphate of Potash. Indican. Formed by the linking of potassium sulphate to indoxyl, which is derived from indol produced in the intestine by putrefaction of proteins. 10 c.c. urine + 10 c.c. strong HCl + 2 or 3 c.c chloroform + drop by drop 1 in 20 calcium hypo-chlorite, shaking after each drop. Chloroform becomes bluish violet. (Does not always appear immediately.)

2. Skatoxyl Sulphate of Potash. Yields a reddish violet colour on oxidation.

The presence of these bodies frequently causes a coloured ring to form when HNO_3 is poured under a urine when testing for the presence of proteins.

3. Phenol is also found in the normal urine combined with sulphuric acid.

VI. **Neutral Sulphur Compounds.**—CYSTIN AND OTHER BODIES.

Cystin when present occurs in regular hexagonal plates. (Microscopic demonstration.)

VII. **Oxalic Acid.**—Sometimes occurs as crystals of oxalate of lime. (Microscopic demonstration.)

VIII. **Pigments** OF URINE.—Urochrome is the chief pigment present.

B. Inorganic Solids.

A. Volatile. **Ammonia.**—Add lime water and warm; the smell of ammonia may be detected. Red litmus paper moistened with water and held in the mouth of the test tube turns blue.

Carbonic Acid.—Add a mineral acid and effervescence may ensue, especially if citrates, etc., have been taken previously. [If HNO_3 be used it must be free from HNO_2, otherwise urea may be decomposed with the evolution of N.]

B. Fixed. **Acids.**—Hydrochloric, Phosphoric, Sulphuric **Bases.**—Sodium, Potassium, Calcium, Magnesium.

Chlorides.—Add a drop or two HNO_3 to prevent precipitation of phosphates, then sol. of nitrate of silver = white precipitate.

Phosphates.—Tribasic phosphoric acid, H_3PO_4, combined with alkalis and with earths.

A. Earthy phosphates.—Calcium and magnesium. (*a*) Render urine alkaline with KHO—these are precipitated. (*b*) Use NH_3, and observe crystals of triple phosphate, $NH_4MgPO_4.6H_2O$. (Feathery phosphates.) (*c*) Study the deposit in urine which has stood for some time. It contains triple phosphates. (Knife-rest crystals.) (Microscopic demonstration.)

B. Alkaline phosphates (of sodium and potassium). Precipitate the earthy phosphates and filter. Make the filtrate

acid with acetic acid, and then add uranium acetate and warm = precipitate of uranium phosphate $(UrO_2)HPO_4$.

Sulphates.

1. Preformed sulphates. Acidify the urine with HCl and then add barium chloride solution. A white precipitate forms.

2. Ethereal sulphates (see p. 28).

To precipitate the total sulphates the urine must be boiled with a mineral acid in order to decompose the ethereal sulphates (see p. 28) before the addition of the barium chloride.

C. Abnormal Constituents.

Proteins.—1. Add picric and citric acid (Esbach's solution)—all proteins are precipitated. 2. Add HNO_3 in a layer below the urine by pouring down the side of the test tube held obliquely. All proteins except peptones are precipitated. 3. Acidify and boil—serum albumin and serum globulin are coagulated. Filter and test filtrate for proteoses and peptones. (See Proteins, p. 8.)

Blood Pigments.—1. Guaiac and ozonic ether test. (*Fallacies.*) 2. Spectroscopic examination.

Bile.—1. Pigments. Gmelin's test (see p. 25). 2. Salts. Pettenkofer's test. Hay's test with flowers of sulphur (see p. 25).

Sugar (Glucose). (See p. 13.)

Aceto-acetic Acid.—Add a dilute solution of perchloride of iron. This gives a purple colour.

Acetone.—Note the fruity smell. (1) Add a small quantity of freshly prepared nitroprusside of sodium solution, then NaOH = red colour. On adding acetic acid till the reaction is acid the colour changes to violet. (2) Iodoform test. Add NaOH and warm, and then solution of iodine in potassium iodide and again warm—smell of iodoform. On standing iodoform separates out.

METHOD OF EXAMINING A FLUID OF UNKNOWN COMPOSITION.

I. Physical Characters. If opaque, examine microscopically. If a deposit is present, examine it microscopically and chemically as a solid (see below).

II. Chemical Examination.

Test for **proteins,** and work out by table Table I. (p. 9). (If native proteins very abundant, separate them by boiling and filtering before testing for carbohydrates.)

Test for **carbohydrates**, and work out by Table II. (p. 16).

Test for **fats**—if the fluid is milky or shows other sign of their presence.

Test for **urea** (p. 27), **purins** (**uric acid***) (p. 27), **bile salts** (p. 25), **diacetic acid** (p. 30), and **acetone** (p. 30).

(If the fluid is coloured, examine with the spectroscope for **blood pigments** (p. 18), and test for **bile pigments** (p. 25).)

METHOD OF EXAMINING A SOLID OF UNKNOWN COMPOSITION.

I. Physical Characters. Use microscope if finely divided.

II. Chemical Examination.

(More than one substance may be present.)

1. Proteins. (Apply Xanthoproteic test direct.)

(1) Freely soluble in dilute NaCl. Work out by Table I. (p. 9).

(2) Insoluble in dilute NaCl.

(*a*) Readily soluble in dilute acids or alkalies. Proteates (Meta-proteins).

(*b*) Insoluble in these—

Soluble in hot water—Gelatin (p. 11).

Insoluble in hot water—Collagen, Elastin, Keratin (p. 11), Coagulated Proteins, Gluten of wheat, etc.

* Uric acid is very insoluble, but it may occur as a salt.

1. Carbohydrates. (General tests, p. 12.) (Apply Iodine direct.) All freely soluble in warm water, except raw starch. Work out by Table II. (p. 16).

3. Fats and Cholesterin. Insoluble in watery media. Soluble in hot alcohol. Work out by p. 15.

4. Bile Salts. Freely soluble in water. Pettenkofer's and Hay's tests (p. 25).

5. Urea. Freely soluble in water (p. 27). Effervesces with hypobromite of soda. Crystals with HNO_3 in strong solution.

6. Uric Acid (p. 27). Insoluble in cold water and in alcohol, soluble in alkaline solutions, especially when hot. Murexide test.

7. Creatinine (p. 28).

8. Blood Pigments (p. 18). Soluble in dilute NaCl solution. Spectra.

9. Bile Pigments (p. 25). Soluble in water. Gmelin's test.

10. Lipochrome Pigments (p. 15). Insoluble in watery media. Soluble in alcohol and ether.

QUANTITATIVE METHODS.

(For exact methods for the estimation of total nitrogen, nitrogen in urea, ammonia, and uric acid and of creatinine, see Senior Section, p. 36).

Urea.

Hypobromite Method.

This depends on the reaction (see p. 27).

$$CON_2H_4 + 3NaBrO = N_2 + CO_2 + 2H_2O + 3NaBr.$$

It gives only a rough indication of the amount of area.

1 grm. urea = 372 c.c. N at 0° C. and 760 mm. pressure. But only 354 c.c. are given off. Therefore 1 grm. urea = 354 c.c. N.

At 60° *F.*, 1 *grm. urea* = 370 *c.c. N.*

1. *Apparatus.* (Dupré.)

2. *Chemicals.* Hypobromite solution.

(1) Caustic potash, 40 per cent. solution. (2) Bromine. When required mix—5 c.c. of (2) with 50 c.c. of (1).

3. *Method.*—(1) Place about 15 c.c. of hypobromite solution in the bottle and 5 c.cm. of urine in the tube, and cork. Allow to stand for quarter hour. (2) Read off level of fluid in burette (A). (3) Mix urine and hypobromite. Allow to stand for quarter hour. (4) Read off level of fluid in burette (B).

The difference between A and B is the amount of nitrogen given off from the urea in the urine. The volume may be corrected for temperature and pressure (p. 21).

In making readings, see that the level of the fluid is the same inside and outside the burette.

Purins.

Purinometer (Demonstration).

Volumetric Methods.

A. A Normal Solution is one which contains the equivalent weight of a given substance in grammes dissolved in one litre of water.—As an example, take hydrochloric acid. The equivalent weight of HCl = H : 1 : Cl : 35·5. Therefore a normal solution would contain 36·5 grms. in each 1000 c.c., and thus a decinormal solution = 3·65 grms. per litre.

Normal solution is written $\frac{N}{1}$, tenth normal $\frac{N}{10}$, and so on.

The same holds good for, say, NaOH. Of these solutions 1 c.c. alkali will exactly neutralise 1 c.c. acid.

Where the acid is dibasic, as in the case of H_2SO_4, only half of the molecular weight is dissolved in one litre, and if it be tribasic, then only one-third of the molecular weight would be taken, as in the case with such a substance as phosphoric acid.

B. A Standard Solution (S.S.) is prepared containing per unit of volume enough of some substance to combine with a definite weight of the substance to be estimated.

Chlorides.

Mohr's Method.—Take 10 c.c. of *acid* urine in a beaker, add a drop or two of cold sat. sol. yellow chromate of potash as *indicator*. Then run in from a burette the standard solution, nitrate of silver, which is prepared so that 1 c.c. = 0·01 grm. NaCl.

End reaction.—Red colour.

Volhard's method is more accurate, but it is not so readily carried out. The principle is the same. (See Senior Section p. 41.)

Phosphates.

1. S.S uranium nitrate solution (1 c.c. = 0·005 grm. P_2O_5). —2. Sodium acetate and acetic acid solution. 3. Indicator —Potassium ferrocyanide.

Use 50 c.c. urine + 5 c.c. of 2. Boil and add S.S. from burette, adding a drop of the mixture from a glass rod from time to time to drops of the indicator placed on a porcelain slab. End reaction.—Brown.

Glucose.

A. With Fehling's solution.

1. *Solutions.*—Fehling's solution. (*a*) Cupric sulphate. 34·64 grms. in 500 c.c. water. (*b*) Rochelle salt, 173 grms.; Caustic soda, 50 per cent., 100 c.c., made up to 500 c.c. Keep in separate bottles, and mix equal parts when required. 10 c.c. = 0·05 grm. glucose.

2. *Method.*—If urine has sp. gr *over* 1030, make up 10 c.c. to 100 c.c. with water, and if *under* 1030, 10 c.c. to 50, and fill a burette. Place 10 c.c. Fehling in a porcelain basin, dilute very freely till the colour is pale blue, and boil. While boiling run in urine till the blue colour *entirely* disappears and the suboxide falls to the bottom. The amount of urine and water used contains 0·05 grms. glucose. To find the amount of glucose in the urine, correct for dilution.

B. With Pavy-Fehling solution.

(1) *Solution*.—120 c.c. of Fehling's solution + 300 c.c. ammonia, and made up to litre with water. 10 c.c. = ·005 grm. glucose.

(2) *Method*.—Place 20 c.c. of the solution in the flask provided and dilute with 80 c.c. of water, connect with the burette, and place the exit tube in another flask. Then boil and run in the urine until the blue colour goes. The whole estimation must be completed within three minutes.

C. With Polarimeter. (See Senior Section, p. 41.)

Proteins.

Fill an Esbach's tube to the mark U with urine, and then to the mark R with Esbach's solution; mix, and allow to stand for twenty-four hours. Read off the level of the precipitate. This gives a rough indication of the amount of proteins in parts per thousand.

SENIOR SECTION.

I. QUANTITATIVE EXAMINATION OF VARIOUS CONSTITUENTS OF THE URINE.

I. Total Nitrogen by Kjeldahl Method.

Pipette off 5 c.c. of the urine into a 300 c.c. combustion flask, add 20 c.c. N free H_2SO_4 and a small piece of copper. Heat this mixture in a fume chamber, carrying on the combustion until the solution is colourless, then allow to cool. Now transfer the solution into the distilling flask, washing the combustion flask thoroughly out with distilled water. Almost neutralise the contents of the distilling flask, to which have been added a drop or two of alizarin red solution, with strong NaOH solution, then cool. When cold the contents are rendered distinctly alkaline by a further addition of NaOH, and the flask is *immediately* connected up with the distillation apparatus, the other end of the condenser being attached to a tube which dips under the surface of a measured amount of $\frac{N}{10}$ H_2SO_4 contained in a flask. The burner beneath the distilling flask is now lighted and the distillation continued until all the NH_3 is driven off. When the distillation is ended the distilling flask is removed, and the condenser tube is well washed down with distilled water into the flask containing the $\frac{N}{10}$ acid. When cool this acid is titrated with $\frac{N}{10}$ NaOH, using alizarin red as indicator, and the difference

between the amount of $\frac{N}{10}$ alkali in c.c. required to completely neutralise the $\frac{N}{10}H_2SO_4$ and the amount of $\frac{N}{10}H_2SO_4$ originally taken gives the amount of $NH_3 = N$ distilled over.

Ex. If 60 c.c. $\frac{N}{10}H_2SO_4$ be taken in the first place, and during the titration 25 c.c. $\frac{N}{10}$ NaOH are required, then

60 c.c. − 25 c.c. = 35 c.c. $\frac{N}{10}H_2SO_4$ neutralised by NH_3 evolved

$$35 \text{ c.c. } \frac{N}{10}H_2SO_4 = 35 \text{ c.c. } \frac{N}{10}NH_3 = 35 \text{ c.c. } \frac{N}{10}N;$$

$$\text{and as 1 c.c. } \frac{N}{10}N = \cdot0014 \text{ grms. N,}$$

then in 5 c.c. urine there is $35 \times \cdot0014 = \cdot049$ grms. N.

II. Estimation of Urea.

1. *By Folin's Method.*

Weigh 20 grms. crystallised magnesium chloride into an Erlenmeyer flask (250-300 c.c. capacity), then add with a pipette 5 c.c. of urine, 6 c.c. concentrated HCl, a piece of hard paraffin about the size of a hazel nut, and finally a drop or two of an aqueous solution (1 per cent.) of alizarin red. A special safety tube is then inserted, and the mixture boiled until distinct "bumping" commences. When this appears the heat is lowered so that bumping still goes on, but not quite so energetically. Heating is continued for at least two hours. The contents of the flask must never become alkaline. If the indicator shows any appearance of red a few drops of the acid distillate which collects in the safety tube must be added by tilting the tube forward. [When the safety tube is running properly there ought to be a slow but steady return constantly going on.] At the end of the necessary period the contents of the Erlenmeyer flask are transferred to a distilling flask, as in the Kjeldahl method, and the same

process carried through as described above. Here it is better to boil the $\frac{N}{10}$ H_2SO_4 with the distillate before titrating.

2. *Mörner-Sjöqvist Method.*

Solutions necessary. 1. A saturated solution of barium chloride containing 5 per cent. $Ba(OH)_2$. 2. A mixture of 1 vol. ether + 2 vols. absolute alcohol.

5. c.c. of the urine + 5 c.c. of the barium mixture and 100 c.c. of the alcohol-ether mixture are mixed together in a small stoppered flask and allowed to stand over night. The contents are then filtered, the precipitate being well washed out with alcohol-ether mixture. The filtrate is next evaporated at 55° C. after the addition of a small quantity of water and a pinch of magnesium oxide to get rid of the ammonia. When there is about 10 c.c. of the filtrate left it is transferred to an Erlenmeyer flask (250 c.c. capacity) and treated as in Folin's method, or it can be transferred to a combustion flask and treated as in the Kjeldahl method. The Mörner-Folin is more accurate than the Mörner-Kjeldahl method.

III. Estimation of NH_3.

1. *Folin Method.*

25 c.c. of urine is pipetted off into a tall cylinder, about 1 grm. dry sodium carbonate and some petroleum or toluol (to prevent frothing) added. This cylinder is connected on one side with the air and on the other with a special flask containing $\frac{N}{10}$ H_3SO_4 to absorb the NH_3 liberated. Air is then made to pass rapidly through the urine either by means of a blast pump or else by a good suction pump. Operation lasts two and a half to three hours.

2. *Formol Method.*

20 c.c. of urine are diluted by adding 50 c.c. water then made neutral using phenolphthalein as the indicator, after the addition of 15 grams of powdered neutral potassium oxalate. Then 20 c.c. of 20 per cent. commercial formalin, previously made neutral, are added, and the solution titrated

with $\frac{N}{10}$ NaOH to neutralisation. This method is only suitable for normal urines from which amino acids are absent.

$$1 \text{ c.c. } \frac{N}{10} \text{ NaOH} = 0{\cdot}0017 \text{ grm. } NH_3.$$

IV. Estimation of Uric Acid.

1. *Hopkins-Folin Method.*

Reagents necessary. (1) Uranium solution; 500 grms. ammonium sulphate, 5 grms. uranium acetate, and 60 c.c. 10 per cent. acetic acid in 650 c.c. water. (2) $\frac{N}{20}$ potassium permanganate solution.

150 c.c. urine is measured into a tall, narrow cylinder, and 37·5 c.c. of the uranium solution is added. The mixture is allowed to stand without stirring for about half an hour. The uranium precipitate has then settled, and the clear supernatant fluid is removed by decantation. 125 c.c. of this fluid is measured into a beaker, 5 c.c. of strong ammonia added, and the mixture allowed to stand over night. The precipitate is then filtered off, washed with 10 per cent. $(NH_4)_2SO_4$ solution until chloride free. The filter is removed from the funnel, unfolded, and the precipitate washed back into the beaker with water. Enough water to make about 100 c.c. is added, and finally the precipitate is dissolved by the addition of 15 c.c. concentrated H_2SO_4, and at once titrated with the $\frac{N}{20}$ potassium permanganate solution, the end reaction being the first appearance of a diffuse pink coloration.

$$1 \text{ c.c. } \frac{N}{20} \text{ pot. permanganate} = 3{\cdot}75 \text{ mgrms. uric acid.}$$

A correction of + 3 mgrms., due to the solubility of ammonium urate, is added to the result.

2. *Wörner's Method.*

Warm 100 c.c. urine in a beaker to 40°–45° C., and then add 20 grms. $(NH_4)_2SO_4$ and stir till dissolved. When cool, add 5 c.c. ammonia, and allow to stand for several hours.

Filter off the precipitate of ammonium urate and wash chlorine free with 10 per cent. $(NH_4)_2SO_4$ solution. Next dissolve the urate from the filter by means of hot 1 per cent. NaOH, followed by thorough washing with hot water. Put the filtrate in a porcelain basin, and heat in a water-bath until ammonia ceases to come off. Then transfer to a combustion flask and proceed as in the Kjeldahl method.

$$1 \text{ c.c. } \frac{N}{10} H_2SO_4 = 4{\cdot}2 \text{ mgrms. uric acid.}$$

V. Estimation of Creatinine.

The following method was devised by Folin, and is based on Jaffe's test (p. 28).

10 c.c. urine measured into a 500 c.c. volumetric flask, 15 c.c. picric acid (sat. sol.), and 5 c.c. NaOH (10 per cent.) solution added, mixed together and allowed to stand five to six minutes. At the end of this time the flask is filled up to the mark with water. A portion of this solution is compared in a Duboscq colorimeter with a standard solution of potassium bichromate $\frac{N}{2}$. Under the above conditions a column of 8 mm. of the half-normal bichromate solution gives the same depth of colour as 8·1 mm. of a solution of pure creatinine (10 mgrms. creatinine in 500 c.c. of the solutions used).

Ex. $10 \times \frac{8{\cdot}1}{7} = 11{\cdot}5$ mgrms. creatinine in 10 c.c. urine (supposing 7 to be the reading obtained with the colorimeter).

Estimation of Creatine.

10 c.c. urine measured into a small flask and 6 c.c. dilute HCl (one part concentrated HCl in ten parts of water) added. The flask, with a small funnel as a condenser, is then put on to the steam bath (or water bath) and heated for 3–4 hours at 100° C. The acid urine mixture is then cooled, neutralised with NaOH (10 per cent.), and the total creatinine estimated as in creatinine method. Preformed creatinine subtracted from total creatinine = creatine expressed as creatinine.

VI. Estimation of the Acidity of Urine.

To 25 c.c. of urine are added 15 to 20 grms. powdered potassium oxalate (Folin) and one or two drops of a 1 per cent. phenolphthalein solution. The mixture is rapidly rotated, for two minutes, and titrated at once with $\frac{N}{10}$ NaOH. The appearance of a faint but permanent red tint gives the end point.

VII. Estimation of Chlorides by Volhard's Method.

Pipette 10 c.c. urine into a 100 c.c. volumetric flask, add 6 c.c. pure HNO_3, 15 c.c. S.S. $AgNO_3$ (1 c.c. = ·01 grm. NaCl), and 10 to 15 c.c. of water. Shake thoroughly and then fill up to the 100 c.c. mark. Allow to stand for a few minutes before filtering. Take 50 c.c. of the filtrate and titrate with S.S. potassium sulphocyanide (8·3 grm. per 1000 c.c.), using iron alum as the indicator.

2 c.c. sulphocyanide sol. = 1 c.c. $AgNO_3$ sol.

VIII. Estimation of Sugar by Polarimeter (*Saccharimeter*).

Decolorise the urine by means of charcoal and filter. Fill the polarimeter tube and read off rotation (7). Then, if the tube be 1 decimeter long—

$$52{\cdot}8 : 7 :: 100 : x$$

(x gives percentage of glucose).

II. QUANTITATIVE ANALYSIS OF FÆCES.

Before beginning analysis, see that the fæces are thoroughly mixed so that a fair sample may be taken.

1. Estimation of the Water Content and Ash.

(*a*) Put about 2 grms. of moist fæces on a small watch glass, weigh exactly, shake the fæces carefully into a small weighed platinum crucible, and again weigh the watch glass. The difference between the two weighings gives the amount of fæces used in the determination. Dry the fæces in the

crucible at 110° in a hot air oven to a constant weight in the usual method. The difference gives the water content.

(*b*) To find the amount of ash. Heat the crucible at first gently, and then complete the ashing with a strong flame. Cool and weigh.

II. Estimation of N. by Kjeldahl Method.

Take 2–3 grms. of fæces weighed carefully as in the estimation of water content, and place in a combustion flask with H_2SO_4. A globule of mercury may have to be added instead of copper, as fæces are somewhat difficult to combust fully. If mercury is used, before distilling some sodium sulphide or hyposulphite must be added in order to break up certain Hg–N compounds which are formed during combustion.

III. Estimation of Fat by Soxhlet Method.

Take a weighed quantity of the fæces (about 5 grms.) dried as in I. (*a*), and extract for three to four hours in a Soxhlet apparatus with ether, as free as possible from water. Distil off the ether in the previously weighed small Erlenmeyer flask. When the distillation is complete heat the flask for a short period at about 100° C. Cool and again weigh.

III. EXAMINATION OF PROTEINS.

I. Crystallised Egg Albumin (*Hopkins' Method*).

Beat up egg white from perfectly fresh eggs to a froth with exactly its own bulk of saturated ammonium sulphate solution. Let the mixture stand over night and then filter. Measure the filtrate and then add to it drop by drop from a burette 10 per cent. acetic acid until a well-marked permanent precipitate forms,—the solution ought to be distinctly milky. Now add 1 c.c. additional acetic acid for each 100 c.c. of filtered mixture. A bulky precipitate forms, at first amorphous, but crystallisation commences in an hour or two. Shaking the mixture occasionally aids the process of crystal-

elements be absent, add ammonia to a large quantity of the acid solution. It should remain clear.]

III. Decomposition Products of the Proteins.

Proteins on prolonged boiling with mineral acids (acid hydrolysis), or on prolonged digestion with proteolytic ferments, break down into products which no longer give the biuret reaction.

20 grms. casein are boiled with 100 c.c. 25 per cent. sulphuric acid for 10–12 hours. To 20 c.c. of this biuret free product, add 10 per cent. phosphotungstic acid in 5 per cent. H_2SO_4 solution so long as a precipitate forms. When precipitation is complete, filter, best with the aid of a water pump, wash the precipitate with a small quantity of dilute phosphotungstic acid and then dry. The nitrogen in the precipitate and in the filtrate may be estimated by Kjeldahl's method.

By the above method the monamino are separated from the diamino acids.

The monamino acids, the most important being glycocoll, alanin, leucin, tyrosin, are to be found in the filtrate. In the precipitate are found the diamino acids, arginin and lysin and the base histidin.

[With ferment digestion, but not after acid hydrolysis, polypeptides are found. These polypeptides are condensation products arising by the union of two or more amino acids. The simplest is glycyl-glycin from 2 mols. of glycocoll. The majority of polypeptides are precipitated by phosphotungstic acid.]

Tryptophane also is found among the decomposition products. It is obtained from the digest by precipitation with mercuric salts.

IV. EXAMINATION OF NUCLEO-PROTEIN.

Boil 100–200 grms. finely minced pancreas with about a litre of water for ten to fifteen minutes and then filter. To the filtrate, whilst still warm, add acetic acid drop by drop

until a finely divided precipitate begins to settle out. Filter this precipitate off, wash with water, then very thoroughly with absolute alcohol, and finally with ether. It is best to allow the precipitate to stand for some hours in ether, then dry. The dry substance is nucleo-protein.

I. Detection of Pentose in the Nucleo-Protein.

To a small quantity of the substance in a test tube add several c.c. HCl and a little orcin, then boil = Reddish-blue colour (a blue pigment forms). Extract with amyl alcohol, the colour goes from reddish to greenish. Examine with a spectroscope. (Bands between C and D.)

Bial's modification, *i.e.* the addition of a trace of Fe_2Cl_6 to the HCl, is said to render the test more delicate.

II. P in the Nucleo-Protein may be detected by the Fusion Method (see p. 43).

III. Purin Bodies. For the method of isolating the total Purins, see Muscle, *infra*.

V. EXAMINATION OF MUSCLE.

I. Creatine and Purin Bodies.

Digest about a pound of lean meat with about 600 c.c. water at 55° C. for half an hour, and at the end of this time strain through a linen cloth. To remove the soluble proteins from the filtrate, add a few drops of acetic acid and then raise fluid to the boiling-point, stirring vigorously all the time. Filter off the coagulum which has formed, and to the filtrate add basic lead acetate so long as a precipitate forms. Filter off this precipitate, and free the filtrate from excess of lead by means of H_2S. Filter off the precipitate of lead sulphide, and concentrate the filtrate to a syrup on the water-bath. Creatine crystallises out on standing. The purin bodies are contained in the mother liquor.

To prove the presence of the creatine, convert the crystals into creatinine by heating a small quantity of the crystals with dil. HCl for several hours at about 90° C. Estimate by the colorimetric method (p. 40).

THE PURIN BODIES may be obtained as silver salts. Stir up the mother liquor with a small quantity of water, render distinctly alkaline by means of ammonia, and then add silver nitrate solution. A precipitate of the purins as silver compounds results.

II. THE PROTEINS.

Rub 100 grms. of muscle (best from white fish) with 50 grms. of sodium chloride thoroughly together in a mortar, then to the pulp add 500 c.c. of water, so as to make a 10 per cent. solution of NaCl. Stir the mixture well and strain through calico. Salt extract (*A*). Rub the residue up with 0·2 per cent. NaOH solution in the mortar, and again strain. Soda extract (*B*). Residue (*C*) is left on the calico.

(*A*) Salt extract. Boil a portion of this solution. A coagulum forms = NATIVE PROTEINS. Filter off the coagulum. The filtrate is protein free. To another portion of the original solution add an equal volume of saturated $(NH_4)_2SO_4$ solution. A precipitate of GLOBULINS falls. Filter off the precipitate. The filtrate is protein free,—no albumins are present. With another portion of the extract find the heat coagulation points of the contained globulins.

(*B*) Soda extract. On careful addition of acetic acid to this extract a precipitate forms which is redissolved in a large excess of the acid. It is the nucleo-protein of muscle.

(*C*) Residue. This consists of collagen (p. 11).

VI. EXAMINATION OF FLOUR.

I. PROTEINS.

Knead some flour with water to form a dough, place this in a small muslin bag, and continue kneading in a beaker of water. Starch grains pass through (prove by iodine test and microscope), leaving eventually a yellowish, sticky mass.

This is GLUTEN, the chief protein of wheat. Prove its protein nature by the ordinary tests. Prove that it has the characters of a globulin.

This gluten can be separated into an alcohol soluble protein—GLIADIN—and an alcohol insoluble protein—GLUTEN CASEIN.

Boil a small amount of the GLUTEN with alcohol and filter. Evaporate the alcoholic filtrate—GLIADIN is left.

Test both the gliadin and gluten casein by the ordinary protein reactions. Further, test their solubilities in water, acid, and alkaline solutions.

II. CARBOHYDRATES.

The amount of carbohydrate present can be estimated by converting all forms into glucose and estimating it by Fehling's solution.

Take about 10 grms. of dried bread, and place it in an Erlenmeyer flask (300 c.c. capacity) with 100 c.c. 2 per cent. HCl, connect with a reflux condenser and boil for one and a half to two hours. Almost neutralise and then filter into a litre flask and make up to 1 litre. Use this solution for the estimation of the sugar by the ordinary Fehling method. For small amounts the titration method is not delicate enough. The amount of copper oxide formed must then be weighed.

VII. EXAMINATION OF GASTRIC JUICE.

I. QUANTITATIVE ESTIMATION OF HCl.

1. HCl may be estimated quantitatively by means of barium.

25 c.c. of 0·2 per cent. HCl are mixed with 25 c.c. of 1 per cent. lactic acid + several drops of a solution of Witte's peptone to make artificial gastric contents.

To 10 c.c. of this acid mixture in a small porcelain basin a small quantity of pure barium carbonate is added, and it is evaporated to dryness on the water-bath. The basin is now heated on the free flame in order to ash the protein material. After cooling the contents of the basin are

repeatedly extracted with hot water and then filtered. The filtrate which contains the barium chloride should not exceed 50 c.c. in amount. The barium chloride, and hence also the barium content, represents the total amount of HCl present in the 10 c.c. contents. The amount of barium present is now estimated as the sulphate. The united filtrate, acidified with several drops of concentrated HCl, is heated in an Erlenmeyer flask (200–250 c.c. capacity) to boiling-point, then 5 c.c. dilute H_2SO_4 are slowly added. The flask is now allowed to stand for from two to three hours, *i.e.* until it is at room temperature, and then the barium sulphate is filtered off on asbestos in a weighed Gooch crucible, thoroughly washed with cold water, dried, ignited, and weighed. 1 mol. $BaSO_4 = 2$ mols. HCl, *i.e.* 233 parts $BaSO_4 = 73$ parts HCl.

2. Tœpfer's method for estimation of the acidity of the stomach contents.

In each test use 5 c.c. filtered stomach contents.

(1) Estimate the total acidity by means of $\frac{N}{10}$ NaOH, using 2 drops of a 1 per cent. solution of phenolphthalein as the indicator. Note the number of c.c. of alkali required.

(2) Titrate another sample as above, but using 4 drops of a 0·5 per cent. alcoholic solution of dimethylamidoazobenzol as indicator. End point is reached when the red colour changes to yellow.

(3) Titrate another sample as in (1), but using 3 drops of a 1 per cent. watery solution of alizarin red as indicator. End point is the change of the yellow colour to red violet.

Results.

(1) Gives the total acidity of the juice.

(2) Gives the amount of free hydrochloric acid.

(3) Gives the amount of combined hydrochloric acid.

1 − 2 = combined HCl, organic acids and acid salts.

(1 − 2) − 3 = organic acids and acid salts (chiefly acid phosphates).

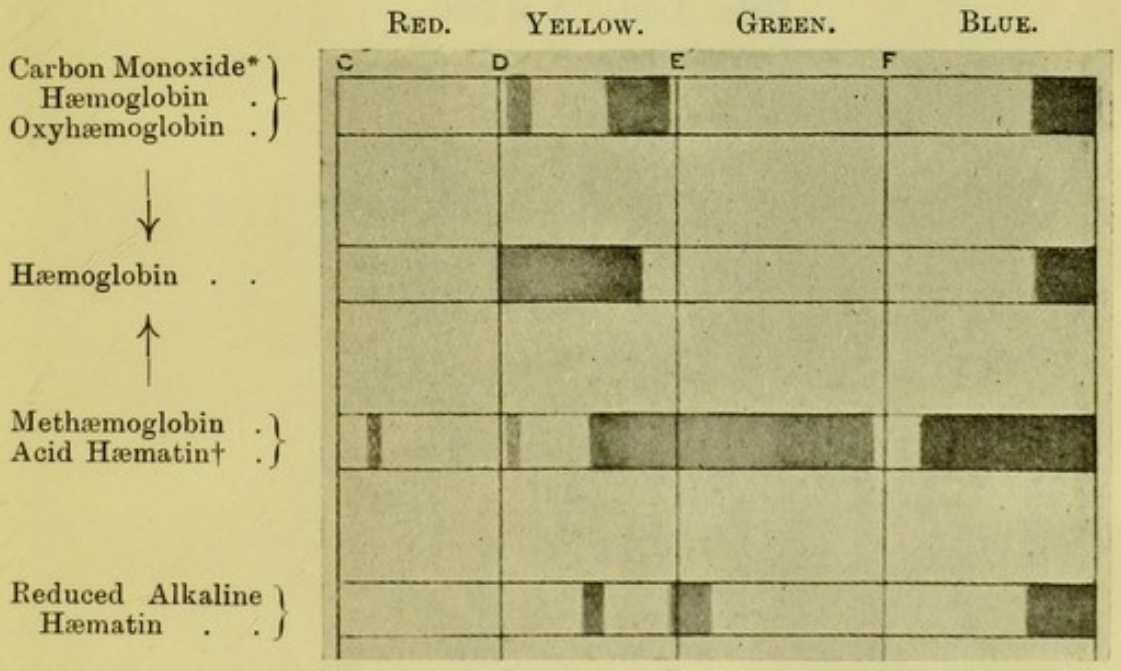
The number of c.c. $\frac{N}{10}$ NaOH required for each 5 c.c. of gastric juice for neutralisation, multiplied by 0·73, gives the acidity for 100 c.c. gastric juice in grms. HCl.

II. Quantitative Estimation of Pepsin.

This estimation is carried out by means of Mett's tubes. These tubes are small tubes of glass of some 1–2 mm. diameter, filled with coagulated egg white. To find the relative activities of two solutions of pepsin, pieces of prepared tube 1–2 c.m. long are placed in the solutions and kept at 37° in the incubator for several hours (eight to ten). The length of albumin digested is then measured, and the square of the length gives the degree of activity. Thus if one column was digested 3 mm., and the other 4 mm., the activities of the two solutions are as 9 : 16.

Estimate the strength of two enzyme solutions given.

SPECTRA OF MORE IMPORTANT BLOOD PIGMENTS.



*These are not absolutely identical.

†The spectrum of Acid Hæmatin is not absolutely identical with that of Methæmoglobin.

APPENDIX.

From the amount of nitrogen found to get the amount of substance multiply by the following factors :—

Urea = N × 2·145.
Ammonia = N × 1·214.
Uric Acid = N × 3·00.
Creatinine = N × 2·695.

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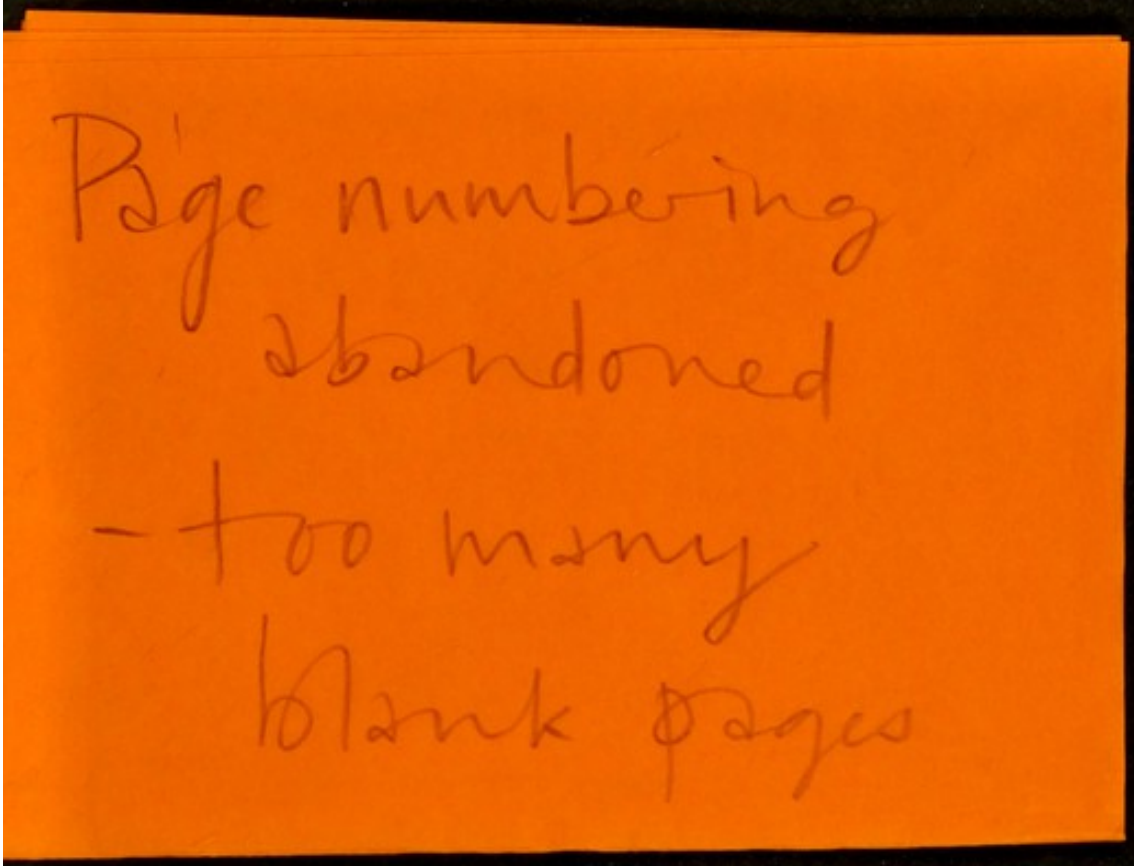
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