PRACTICAL PHYSIOLOGICAL CHEMISTRY

BY

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WITH COLOURED PLATE AND OTHER ILLUSTRATIONS IN THE TEXT

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PRACTICAL PHYSIOLOGICAL CHEMISTRY

PREFACE

THIS book was originally compiled as a handbook for practical work in Physiological Chemistry at University College, London, since no single text or class book covered the complete course, or treated Physiological Chemistry as part of the subject of Organic Chemistry, or even as an independent subject.

The present book must still be regarded mainly as a compilation. It represents an attempt to give to the worker a nearly complete statement of the whole subject. Each section has a short explanatory summary of the essential points, so as to connect the various sections together. The essential points are illustrated by the practical experiments, which are printed in different type.

The illustrations are also compiled from various sources. These are mentioned underneath each figure. The illustrations of apparatus not so mentioned have been drawn from my own sketches. For those of the osazone crystals, haemin, and tyrosine, I am indebted to Miss V. G. Sheffield, who has also kindly helped in reading the proof sheets.

In most physiological chemistry laboratories the strengths of the reagents employed are very various, e.g. dilute acetic acid may be 1 per cent., or 2 per cent., or 5 per cent., or even 10 per cent. In order that all workers may employ a reagent of standard concentration, a list of reagents has been carefully drawn up and is appended.

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DEFINITION.

THE substances composing the organic material upon which the phenomena of life depend, and the great majority of the products of vital activity, are mixtures of compounds of the element Carbon.

From these substances the chemist has isolated numerous pure carbon compounds and prepared others; he has also synthesized carbon compounds from their elements.

Some 100,000 carbon compounds are now known. The possibility of their existence is due to the unique property which the element carbon possesses of being able to combine with itself; compounds are known which contain in their molecules from one to sixty atoms of carbon directly joined together.

Of these 100,000 carbon compounds only a small number are directly concerned in vital processes.

The chemistry of all the carbon compounds is termed organic chemistry.

The chemistry of those carbon compounds which are the constituents of living matter and are concerned in vital processes is physiological or biological chemistry. The changes which they undergo and the functions which they fulfil in the living plant or animal form the subject of chemical physiology.

RECOGNITION OF AN ORGANIC COMPOUND.

Organic compounds are distinguished from inorganic compounds by being combustible, that is, when heated they will char, sometimes take fire, and will, on prolonged heating, completely burn away leaving no ash. Inorganic compounds when heated do not char and they leave a residue.

There are a few exceptions to this general rule, e.g. oxalic acid and its salts amongst the organic compounds, and the ammonium salts amongst the inorganic compounds. An oxalate will leave a residue of the oxide of the metal with which it is combined.

The following experiments exemplify these statements:—

1. Heat a small piece of paraffin wax upon platinum foil; it will melt, take fire, and will completely burn away leaving no residue.

2. Heat a crystal of cane sugar in the same way; it will melt, char, and on further heating will disappear completely.

3. Heat a few crystals of common salt on platinum foil; they will melt and unless heated very strongly, e.g. with a blowpipe flame, they will remain as a solid white mass when allowed to cool.

4. Heat a small piece of soap: as before, it will char, the vapours evolved may take fire, and when the charred particles have all vanished a white or nearly white residue will remain.

In this way substances composed of organic and inorganic matter can be recognized. The composition of the inorganic residue can only be found out by the usual methods of inorganic analysis when the organic matter has been destroyed by heating.

5. In the same way heat a little oxalic acid or an oxalate, e.g. calcium oxalate, and observe the results.

6. Also heat some ammonium chloride and note that it leaves no residue.

CONSTITUENTS OF A CELL.

A living cell consists of a mixture of organic and inorganic substances dissolved or suspended in water; water makes up about three quarters of the total mass of the cell, the other constituents about one quarter.

The twelve elements: carbon, hydrogen, nitrogen, oxygen, sulphur, phosphorus, chlorine (iodine), sodium, potassium, calcium, iron, and magnesium enter into the composition of the various compounds which compose the material of all living organisms; of these, only oxygen and nitrogen exist in the free state dissolved in the liquid.

The inorganic compounds are water and salts, chiefly the chlorides, sulphates, and phosphates of sodium, potassium, calcium, magnesium.

The organic compounds are very numerous in their variety. Proteins, carbohydrates, and fats are the essential substances, also the most complex. Alcohol, lactic acid, urea represent stages in the processes of their building up and breaking down, i.e. in their synthesis and decomposition or anabolism and catabolism.

DETECTION OF THE ELEMENTS IN A CELL.

1. Carbon, Hydrogen, Nitrogen.

Heat gently in a crucible about a gramme of dried yeast, previously powdered finely in a mortar. The mass chars, and volatile and combustible substances are evolved—presence of carbon and hydrogen. Continue the heating until these substances cease to be given off, and the mass no longer changes on further heating.

Notice the smell of burning flesh and hair—presence of nitrogen.

2. Sodium, Potassium, Chlorine, Phosphorus, Sulphur, etc.

Allow the mass to cool, extract it with warm water, filter and wash. Examine the filtrate A and the residue B.

Filtrate A.

The filtrate A contains those substances which are soluble in water and which are volatilized on heating to a higher temperature, e.g. sodium chloride.

Ascertain the reaction. It is generally alkaline owing to the presence of alkali carbonates and phosphates. Test separate portions for sulphuric acid, phosphoric acid, carbonic acid, hydrochloric acid, potassium, sodium; calcium sulphate is occasionally found in this extract, but rarely.

Residue B.

Heat the residue B still further until all the carbon has been oxidized away and a nearly white mass remains; this mass contains the substances insoluble in water, e.g. calcium phosphate.

Dissolve it in hydrochloric acid and note the presence or absence of carbonates by effervescence of carbon dioxide. Filter from any unburnt carbon and test the filtrate for phosphoric acid, calcium, magnesium, iron, as follows:—

Add ammonium chloride and ammonia: filter.

(*a*) The precipitate is white or yellowish-red in colour, and can consist of the phosphates of calcium, magnesium, iron. Dissolve a small portion in nitric acid and test for phosphoric acid with ammonium molybdate.

Treat the remainder with acetic acid. Boil for some time; yellowish-red flakes consisting of ferric phosphate remain undissolved; filter them off, dissolve in dilute hydrochloric acid and test for iron with potassium ferrocyanide or potassium thiocyanate.

Test the filtrate for calcium with ammonium oxalate, completely remove it by gently warming and filtering, and treat the filtrate with ammonia. If magnesium be present a crystalline precipitate will form either at once or after a short time.

(*b*) The filtrate may contain calcium and magnesium. Treat it with ammonium oxalate to remove calcium if present, and test for magnesium in the filtrate with sodium phosphate.

LIFE-CYCLE OF THE ELEMENTS.

During the life processes of animals and plants an interchange of inorganic into organic and of organic into inorganic compounds occurs. The change of inorganic into organic is effected by the plant from the carbon dioxide in the atmosphere, ammonia and salts in the soil. The change of organic into inorganic is effected by the animal. The organic compounds synthesized by the plant enter the animal as food and are converted into carbon dioxide, which leaves the body by the lungs, salts, urea and other simple organic compounds, which leave the body in the urine. The fæces contains matter which has not been utilized by the animal. The urea and the other organic compounds in the excreta are finally broken down into carbon dioxide, ammonia, etc., by bacteria. There is thus a complete life-cycle of the elements.

DETECTION OF THE ELEMENTARY END PRODUCTS IN THE LIFE-CYCLE IN ANIMALS.

1. Carbon.

(*a*) On breathing into lime or baryta water, there is formed a large deposit of calcium or barium carbonate. Nearly all of the carbon is eliminated as carbon dioxide by the lungs; a small quantity only by the kidney.

(*b*) Evaporate down about 5 c.c. of urine in a porcelain basin to dryness over a small flame. Continue the heating when the residue is dry. It chars —presence of carbon.

2. Nitrogen.

Boil about 5 c.c. of urine in a small flask with caustic soda. Ammonia, due to the decomposition of the urea, is evolved, which may be recognized by its smell and the blueing of moistened red-litmus paper.

3. Chlorine.

To some urine add silver nitrate. A white precipitate of silver chloride and silver phosphate is formed. Acidify with nitric acid. This dissolves the silver phosphate, leaving the silver chloride, which is soluble in ammonia after filtering off. The silver phosphate is again precipitated if the filtrate be made alkaline with ammonia.

4. Phosphorus.

Phosphorus is present in urine as acid phosphates of the alkalies, and of calcium and magnesium.

(*a*) Render some urine alkaline with caustic soda, and heat gently. Calcium phosphate and magnesium phosphate are precipitated. Filter. To the filtrate add magnesia mixture. The soluble alkaline phosphates are precipitated as ammonio-magnesium phosphate (triple phosphate).

(*b*) Acidify some urine with acetic acid containing sodium acetate, and then add uranium acetate solution so long as there is a precipitate. Heat to 80° C. to complete the precipitation.

(*c*) Acidify with concentrated nitric acid, add excess of ammonium molybdate solution and heat to 50° C. A yellow coloration and then a precipitate of ammonium phosphomolybdate appears.

5. Sulphur.

Add baryta water to some urine, so long as a precipitate forms. Barium phosphate and barium sulphate are precipitated. Acidify with hydrochloric acid. The barium phosphate is dissolved, leaving the barium sulphate.

6. Sodium.

Evaporate 20 c.c. urine to dryness, and introduce some of the residue into a Bunsen flame by means of a platinum wire. Yellow colour. Spectroscope shows yellow sodium line.

7. Potassium.

Evaporate 100 c.c. urine to $\frac{1}{8}$ of its volume. Cool and filter. To filtrate add some concentrated tartaric acid solution, or sodium hydrogen tartrate. Mix well and allow to stand in cool place. Potassium hydrogen tartrate separates out, mixed with some ammonium hydrogen tartrate.

8. Calcium and Magnesium.

Render 200 c.c. urine alkaline with ammonia. After a few minutes filter off the precipitate of calcium and magnesium phosphates. Dissolve this precipitate in acetic acid, and filter if necessary.

To the clear filtrate add ammonium oxalate and heat gently. Calcium oxalate is precipitated. Filter.

Make the filtrate alkaline with ammonia. Ammonium magnesium phosphate forms on standing.

ISOLATION AND PREPARATION OF PURE ORGANIC COMPOUNDS.

CRITERIA OF THEIR PURITY.

The organic substances which compose all animal and vegetable cells consist of a mixture of numerous compounds. In order to investigate their evolution and their degradation in nature it is necessary to separate these compounds from one another and to prepare each of them in a state of purity. The chemical and physical properties of the pure substance can then be ascertained. Knowledge of the pure compounds shows their chemical relationship to one another and an idea of their rôle in nature is obtained. This idea is proved or disproved by an investigation of the changes which the organism, as a whole or individual portions of it, can effect in these substances.

In the study of the chemical properties of the compounds, other compounds are formed by their interaction. These compounds also require isolation and purification. The principal operations in organic and physiological chemistry will thus consist in the isolation and preparation of pure compounds.

Solid organic compounds are more numerous than liquid; gases are comparatively rare.

I. Solids.

(*a*) When two or more solids are present, only one may be soluble in a given solvent, e.g. a mixture of fat and protein may be separated by extracting with ether, which dissolves the fat leaving the protein. See under "Fats," p. 55.

(*b*) If the constituents of a mixture are all soluble in the same solvent they can be separated by fractional crystallization. On dissolving the mixture in a given solvent and evaporating the more insoluble compound separates out first when crystallization occurs, and is filtered off; the filtrate contains the more soluble compound, and this is obtained by evaporating until crystallization again occurs. The several crops of crystals require recrystallization from a suitable solvent, e.g. water, alcohol, benzene, to obtain a pure preparation.

(*c*) A mixture of solids in a solution may be separated by acidifying or making the solution alkaline, when precipitation may occur. In this case the substance precipitated will be either an acid or a base: thus benzoic acid and hippuric acid (pp. 132, 106) are precipitated when hydrochloric acid is added to a solution of their salts. Caseinogen (p. 177) is precipitated when milk is acidified with acetic acid. Aniline, quinine, strychnine are precipitated when caustic soda is added to their solution in acids.

These reactions depend upon the fact that the acid or base precipitated is insoluble or soluble only with difficulty in the acid or alkaline solution.

Acid substances may again be separated by precipitation as salts with heavy metals. Basic substances may be separated as salts with acids, e.g. urea nitrate, or as double salts with gold or platinum chloride, e.g. choline platinochloride.

(*d*) Two solids in a solution may be separated by extraction with a new solvent, which is not miscible with the first. The liquids are then separated, and the substances are obtained on evaporation of the two solvents, e.g. a mixture of succinic acid and urea in water is separated by extracting the solution with ether which dissolves the succinic acid leaving the urea in the water.

Lactic acid is usually separated in this way from an aqueous extract of muscle.

1. Separate Benzoic Acid from Solution and Purify it by Recrystallization.

To the given solution of sodium benzoate add a slight excess of dilute hydrochloric acid. Benzoic acid is precipitated. Filter off the precipitate and wash it free from hydrochloric acid with water.

The filtration of crystalline organic compounds is best effected by means of a perforated porcelain plate placed in a funnel or a complete funnel of porcelain of this pattern (Buchner), and suction as in the accompanying figure.

The perforations are covered over with a filter paper of the right size; this is wetted with the liquid and sucked down. The substance is then placed on the paper when the liquid is sucked off; it is then washed with water and pressed down with a spatula so that the wash water is drained off as completely as possible.

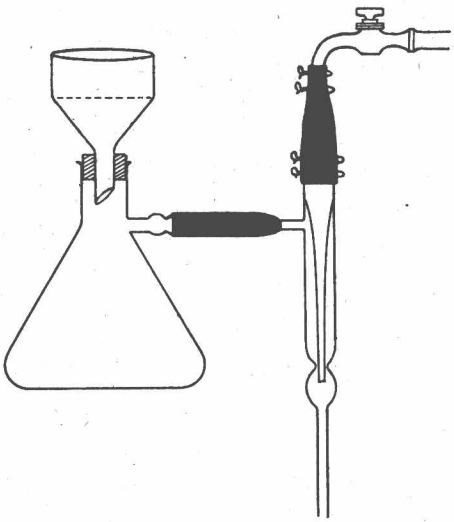

FIG. 1.

Then place the substance in a small flask or beaker and dissolve it in boiling water, sufficient only to just dissolve it being used. Whilst hot, filter the solution through a folded or pleated filter paper into a clean beaker, cover the beaker with a clock glass or a piece of clean paper and allow to cool. Pure benzoic acid will crystallize out. Filter off the crystals, wash them with a little fresh solvent and dry the crystals thoroughly between sheets of filter paper. Determine its melting-point (p. 11).