Fig. 1—Diagram of Biological Processes
BIOLOGICAL CHEMISTRY

BY

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THE aim of this book is to give a readable account of the chemical processes that take place in living organisms. Although the discussions of various problems represent my own views I have endeavoured to give references to original sources for actual statements of recorded facts and for deductions from the same. These references are of two kinds, those giving the first statement of the point mentioned, and those giving recent details or general discussions from which the reader can trace the development of the subject.

I have much pleasure in thanking the following for use of illustrations as recorded in the text: The Editor of the "Annals of Botany," Mr. J. Barcroft, F.R.S., Prof. W. M. Bayliss, F.R.S., Prof. F.F. Blackman, F.R.S., Prof. H. Cushing, Dr. R. Hutchison, Messrs. Longmans, Green & Co., The Medical Research Council, The Editor of the "Journal of Physiology," The Editor of "The Practitioner, The Council of the Royal Society," Sir E. S. Schafer, F.R.S., Prof. Sutherland Simpson and Prof. A. D. Waller, F.R.S.

H. E. ROAF

LONDON HOSPITAL MEDICAL COLLEGE
LONDON, E.I.
INTRODUCTION

BIOLOGICAL Chemistry or the Chemical Reactions occurring in living cells presents a perplexing multiplicity and complexity of reactions. Not only does it involve a knowledge of Organic Chemistry, but it also requires an acquaintanceship with the laws of chemical reactions in solution: that is, an appreciation of the statics and dynamics of Chemistry.

On surveying the whole field involved in these reactions we see that the subject can be divided into two portions. The first of these is associated with an accumulation of available energy and is thus concerned with the synthesis of organic material by green plants, under the influence of sunlight. The second is concerned with the liberation of available energy in performing the life processes of plants and animals. The former is the constructive or anabolic phase; the latter is the destructive or catabolic phase.

The above two phases constitute a cycle (see Frontispiece). Starting with the simple inorganic substances, water and salts from the soil, and carbon dioxide from the air, the plant cells form carbohydrates, fats and proteins. During the activities of plants and animals carbohydrates, fats and proteins are decomposed, with the liberation of their available energy, so that ultimately the simple inorganic substances are set free once more.

Examined more closely, the one main cycle can be subdivided into cycles involving different elements, such as one for nitrogen, one for sulphur, etc., but each of these is dovetailed into the general description given above.

The arrangement of the subject matter of this book follows the lines indicated above.

The first section consists of a few elementary chapters giving a brief outline of the main groups of organic substances found in cells, and a summary of the laws of chemical reactions
in solutions with their application to some biological processes. These must necessarily be brief and further observations on the chemical reactions and physico-chemical processes will be given in the other portions of the book.

The second section deals with the accumulation of available energy by the absorption of the radiant energy from the sun by the green plant. It is true that the immediate products of photosynthesis may be used for the formation of more concentrated energy stores, but this concentration ultimately depends on the energy supply from the sun.

The third section traces the use, by plants and animals, of the products of photosynthesis. This forms the largest part of the subject, as it comprises so many different processes each of which can form a speciality by itself.

In tracing the cycle of changes it is obvious that many branches of chemical science are involved. Depending on whether one is interested mainly in plant or animal life, one or other portion assumes preponderating importance, but the aim of this book is to show the relation of the parts to the whole and to illustrate the various processes by the more illuminating examples.
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BIOLOGICAL CHEMISTRY
ERRATA

Page 2, line 3. —C— should be —C—

Page 10, fourth line from bottom. For "Methyl glucose" read "Methyl glucoside."

Page 14, line 10. For "Palmityl" read "Stearyl."
do. footnote, 3rd line from bottom. For "gram" read "grams."

Page 15. Kekulé's formula should be

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C
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Page 16, line 4. For "benzine" read "benzene."

Page 17, lines 18 and 19. For "but if . . . oxygen atom," read "but if nitrogen and oxygen are united to the same carbon atom."

Page 19. For "Aminazol" read "Iminazol."

Page 66, Table XI. For "α-Glucose" read "α-Glucase"; for "β-Glucose" read "β-Glucase"; and for "1-Xyloside" read "d-Xyloside."

Page 92, first line. For "on hydrogen" read "in hydrogen ion."

Page 106, line 5. For "all" read "most."

Page 111, footnote. For "W. H. Dudley" read "H. W. Dudley."

Page 125, footnote formula. For "C_{26}" read "C_{20}".

Page 155, line 3. For "finally" read "mainly."

Page 183, line 23. For "pyramidine" read "pyrimidine."

Page 192, third and second lines from bottom. For "a crystal-line iodine . . . compound" read "a crystalline compound containing iodine."

Fig. 44. Transpose A and B.

The heat of combustion obtained in the ordinary way. Further, any subsidiary reaction that results may furnish energy to the reacting system.

Four elements are sufficient for the construction of the groups of substances dealt with in this chapter. Each of these elements possesses certain valencies or combining
THE energy required for living organisms is obtained from compounds containing carbon. The branch of science that deals with compounds containing carbon is called organic chemistry.

The heat energy that can be obtained from an organic substance is measured by burning it in the presence of oxygen: the heat so produced is absorbed and measured in calories. This heat of combustion is not the same as the available or usable energy, but until the available energy has been measured we must confine our attention to the heat value obtained on combustion.

A calorie (c) is the amount of heat required to raise one gramme of water from 15° to 16° C. For many purposes this unit is too small, so a large calorie (C) is used which is equal to 1,000 calories.

In dealing with biochemical oxidations, we must remember that the reaction takes place in solution, therefore we must add the heat of solution of the products of reaction to and subtract the heat of solution of the original substance from the heat of combustion obtained in the ordinary way. Further, any subsidiary reaction that results may furnish energy to the reacting system.

Four elements are sufficient for the construction of the groups of substances dealt with in this chapter. Each of these elements possesses certain valencies or combining...
equivalents and they unite with each other so that the valencies are mutually satisfied.

The four elements are carbon with four valencies (\(\text{C}\)), hydrogen with one (\(\text{H}\)), oxygen with two (\(\text{O}\)) and nitrogen with three or five (\(\text{N} \equiv \text{N} \equiv \text{N} \equiv \text{N} \equiv \)). Thus carbon can unite with four hydrogen or two oxygen atoms, but owing to the odd number of valencies associated with nitrogen, carbon and nitrogen cannot form by themselves such simple and even unions.

Models of these atoms in three planes are extremely useful in understanding the organic compounds, but for purposes of description we must adhere to the plane of the paper and represent the elements as shown above with dashes to represent the valencies. The symbol \(\text{R}\) is used to represent a molecular group which is of minor importance, and to it are attached the special groups which are of importance in the reaction which is being studied.

For the present we can confine our attention to three large groups of compounds as these furnish the basis on which we can build up the greater part of Biological Chemistry. As the discussion of the subject requires it we shall introduce additional groups of organic compounds.

The three classes are carbohydrates, fats and proteins.

One carbon atom can unite with two oxygen atoms, or with four hydrogen atoms. In the former case we have formed the important gas carbon dioxide (\(\text{CO}_2\)), and in the latter marsh gas or methane (\(\text{H}_2\text{C}_2\text{H}_4\)). On removing one of these hydrogen atoms the methyl group (\(-\text{CH}_3\)) is left. Two methyl groups unite to form ethane (\(\text{H}_2\text{C}_2\text{H}_6\)), and on removing one hydrogen atom from ethane the ethyl group (\(\text{C}_2\text{H}_5\)) is left. By uniting carbon and hydrogen in this way a series of compounds, each containing one \(\text{CH}_2\) group more than the preceding member, forms what is called the marsh gas or paraffin series.

Our first consideration must be the substances formed by oxidation, i.e. the introduction of oxygen into these compounds.
By introducing one atom of oxygen so that its two valencies are united one with carbon and the other with hydrogen, the hydroxyl group (—O—H or —OH) is formed. This converts the paraffins into alcohols, of which there are three varieties depending on the number of hydrogens attached to the carbon atom, to which the hydroxyl group is attached.

A primary alcohol retains two hydrogen atoms, a secondary alcohol one, and a tertiary alcohol has no hydrogen united directly to the carbon atom to which the hydroxyl group is attached. The introduction of a second atom of oxygen should lead to the presence of two hydroxyls but water (H$_2$O) is split off and one oxygen atom is left united to the carbon by its two bonds. By oxidation a primary alcohol is converted into an aldehyde, and a secondary alcohol into a ketone: a tertiary alcohol is decomposed on oxidation, because a valency must be set free in order to furnish the two valencies required by oxygen. Further oxidation of the aldehyde produces an acid, whilst a ketone like a tertiary alcohol cannot be oxidised further without decomposition.

\[
\begin{align*}
R_2 & \quad H \quad R_2 \\
R_1 & \quad C-\text{OH} & R_1 & \quad C-\text{OH} & R_1 & \quad C-\text{OH} \\
H & \quad \text{or} \quad R & \quad \text{or} \quad R & \quad \text{or} \quad R & \quad \text{or} \quad R \\
& \quad \text{or} \quad \text{CHOH} & \quad \text{or} \quad \text{CH}_2\text{OH} & \quad \text{or} \quad \text{COH} & \\
& \quad \text{Secondary Alcohol.} & \quad \text{Primary Alcohol.} & \quad \text{Tertiary Alcohol.} & \\
& \quad \downarrow & \quad \downarrow & \quad \downarrow & \\
& \quad R_2 & \quad R_2 & \quad R_3 & \\
& \quad R_1 & \quad C=\text{O} & \quad R_1 & \quad C=\text{O} & \quad R_1 & \quad C=\text{O} \\
& \quad \text{or} \quad R & \quad \text{or} \quad R & \quad \text{or} \quad R & \quad \text{or} \quad R & \quad \text{or} \quad R & \quad \text{or} \quad R \\
& \quad \text{Ketone} & \quad \text{Aldehyde} & \quad \text{Acid} & \\
& \quad \text{or} \quad \text{CHO} & \quad \text{or} \quad \text{RCOOH} & \\
& \quad \text{oxygen—water} & \quad \text{oxygen} & \quad \text{water} & \\
& \quad \text{or} \quad \text{H} & \quad \text{or} \quad \text{O}
\end{align*}
\]

The groups $R_1$, $R_2$ and $R_3$ may also contain other alcohol, aldehyde, ketone or acid groups.

**Carbohydrates**

A substance in which several alcohol groups occur is known as a polyhydroxy alcohol. One of these, containing three alcohol hydroxyl groups, is the substance glycerine in which two primary and one secondary alcohol groups occur. By oxidising one of the primary alcohol groups to an aldehyde, or the secondary alcohol group to a ketone, simple sugars are formed, and these belong to our first group: the carbohydrates.
Glycerine, like other alcohols, can unite with acids, giving rise to organic salts or esters, to which we must return under the heading of "Fats."

The most frequently occurring carbohydrates, unlike glycerine, do not contain three carbon atoms (triose), but six (hexose), or some multiple of six carbon atoms. The six carbon atom sugars are called monosaccharides, and multiples of six are called di-, tri- and poly-saccharides.

When the hexoses unite to form disaccharides water is removed and the process is known as dehydration or condensation. The reverse process of breaking down the polysaccharides into hexoses is accompanied by the addition of water and is known as hydrolysis.

Some sugars are known which contain less than six carbon atoms, and those with five carbon atoms (pentoses) are found in most cells.

The reactions of the various sugars can be foretold from a knowledge of the groups contained in them and the relative position of the groups to each other.

We shall now examine the chief reactions for carbohydrates.

*Trommer's Test.*—This well-known reaction can be described as taking place in two stages. On adding copper sulphate to a solution of sugar and then rendering alkaline by sodium or potassium hydroxide, a blue precipitate occurs, which dissolves in excess of alkali, to form a clear blue solution. The sugar owes its power of holding copper hydrate in solution to the hydroxyls of the alcohol groups. Many other substances besides sugar form a blue solution with copper hydrate, e.g. glycerine, tartrates, citrates, ammonia, etc.

On heating the clear blue solution the cupric hydroxide is reduced to cuprous hydroxide, and the latter loses water to form cuprous oxide: hence a yellow precipitate is formed which soon turns red. The sugar is oxidised. This portion of the test is due to the presence of the free aldehyde or ketone group. Although ketones do not reduce copper salts the presence of alcohol hydroxyl groups on the neighbouring

carbon atoms renders the ketone sugars capable of reducing metallic hydroxides.

Fehling’s Test.*—Instead of using copper sulphate and alkali, a mixture is made containing copper sulphate, a tartrate and alkali. This, in spite of the absence of sugar, gives a deep blue solution. A little of this is added to the sugar solution and the mixture is boiled. As in Trommer’s test the sugar is oxidised and the cupric hydroxide is reduced and dehydrated to the cuprous oxide so that a red precipitate occurs.

Pavy’s Test.† consists of an ammoniacal copper solution. Ammonia not only holds the cupric hydroxide in solution but it also dissolves the cuprous hydroxide to a colourless solution; therefore on heating with sugar the solution is decolourised and no red precipitate occurs.

Other metals are also reduced by sugar solutions. An ammoniacal silver solution can be reduced to form a silver mirror. Nylander’s reagent consists of bismuth hydroxide held in solution by tartrate and on heating with sugar a black precipitate of metallic bismuth occurs.‡

The alkaline copper solutions are frequently used for the quantitative estimation of reducing sugars; for the details the reader must be referred to books on practical chemistry.§

These tests are given by all reducing sugars, that is those which possess a free aldehyde or a ketone associated with an hydroxyl group. This includes all monosaccharides and some disaccharides. Those carbohydrates which do not reduce metallic hydroxides can be converted into reducing substances by hydrolysis with acid. Alkali is not used for hydrolysis, as the sugar is oxidised in the presence of alkali to form dark brown coloured products. The formation of these coloured products in the presence of alkali is the basis of Moore’s Test.||

Barfoed’s Test.—The monosaccharides differ from the reducing disaccharides in that they are more powerful reducing substances, and under standard conditions will reduce copper

acetate in acetic acid solution whilst the disaccharides will not.*

**Phenyl Hydrazine Test.**—The aldehyde or ketone oxygen atom can be replaced by a molecule of phenyl hydrazine forming a hydrazone. On heating with an excess of phenyl hydrazine the neighbouring alcohol group is oxidised either to an aldehyde or to a ketone group depending on whether the neighbouring group was a primary or secondary alcohol.

![Fig. 2. Crystals of Phenyllosazones.](image)

A second molecule of phenyl hydrazine can now be added and the resulting product is called an osazone. The osazones are therefore compounds formed by two molecules of phenyl hydrazine united to one molecule of sugar.

Fischer found these compounds extremely useful in isolating the various sugars, because they are relatively insoluble and crystallise well.* The crystals are yellow in colour, and they can be identified by their shape and melting point. Substituted phenyl hydrazines are used for special purposes.†

**Seliwanoff Reaction.**‡—On heating ketone sugars in a solution containing 18 per cent. of hydrochloric acid (equal parts of concentrated hydrochloric acid and sugar solution) a red colour is given, but the addition of resorcinol gives a deeper red colour and later on a red precipitate which dissolves in alcohol to form a red solution. This test is used for the identification of fructose and sucrose (cane sugar).

**Pentose Tests.**§—On heating a sugar containing an odd number of carbon atoms with 18 per cent. hydrochloric acid and phloroglucinol or orcinol, a cherry red colour is produced with the former and a violet followed by blue green or blue with the latter, both of which become precipitated on standing. These coloured condensation products can be dissolved in amyl alcohol, and their solutions examined spectroscopically. The phloroglucinol product shows an absorption band between D and E, and the orcinol product one between C and D, but near to D.

**Mucic Acid Test.**—On oxidising galactose (and lactose) with nitric acid a crystalline product (m.p. 213° C.) is easily separated. This is mucic acid, and serves to identify galactose in its compounds.

Molisch Reaction.—By adding a few drops of an alcoholic solution of \( \alpha \)-naphthol to a solution of carbohydrate, and by floating the mixture on strong sulphuric acid a purple red ring is produced at the junction of the two liquids.* This test, unlike some of the previous tests, which depend upon some special molecular arrangement, is given by all carbohydrates.

**Polysaccharides**

The union of two or more hexose groups usually leads to the masking of the aldehyde and ketone groups. Under these circumstances many of the carbohydrate tests fail until the compound has been hydrolysed by acid. The substances of high molecular weight, containing a number of monosaccharide groups can be distinguished by certain colours given by them with iodine.

Starch, the most frequently occurring carbohydrate in plants, gives a blue colour; glycogen, a similar substance from animals, gives a mahogany brown colour; and erythro-dextrin gives a reddish brown colour. Another dextrin (achroo-dextrin) gives no colour with iodine. Cellulose, which is an insoluble substance, sometimes gives a faint blue colour with iodine, but this is greatly intensified by the addition of concentrated sulphuric acid. These substances can be distinguished by their relative solubilities. Starch and glycogen are colloids (see p. 50), whilst the dextrins are more like true solutions.

Returning to the structural formulæ it can be shown that when one carbon atom has four different groups united to it a three-space model can be arranged in two different ways. The projection of these models on the plane of the paper gives rise to the two following diagrams:—

```
\[
\begin{array}{c}
I \\
\mid \\
2 - C - 4 \\
\mid \\
3 \\
\end{array}
\]
```

```
\[
\begin{array}{c}
I \\
\mid \\
4 - C - 2 \\
\mid \\
3 \\
\end{array}
\]
```

In these illustrations it is seen that the one compound is related to the other, as the image of the one is to its reflection, or as the right to the left hand of a pair of gloves. This relation corresponds with the power of rotating the plane of polarised light, one to the right (clockwise) and the other to the left (anti-clockwise), and is spoken of as asymmetry.

**TABLE I. Table of carbohydrate reactions.**

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<th>Carbohydrate</th>
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<th>Trommer's test or other alkaline copper reduction.</th>
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<th>Alcohol</th>
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<td>Pentose</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>d</em>-Arabinose</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hexoses or Monosaccharides:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>d</em>-Glucose</td>
<td>205° C.</td>
<td>Red precipitate</td>
<td>Red precipitate</td>
<td>Yellow</td>
<td>Soluble</td>
<td>Soluble</td>
<td></td>
<td>Phloroglucinol or orcinol.</td>
</tr>
<tr>
<td><em>d</em>-Fructose</td>
<td>205° C.</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>Resorcinol.</td>
</tr>
<tr>
<td><em>d</em>-Galactose</td>
<td>190–193° C.</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>Mucic acid formed on oxidation.</td>
</tr>
<tr>
<td>Disaccharides:</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose †.</td>
<td>206° C.</td>
<td>Red precipitate</td>
<td>No reduction</td>
<td>Yellow</td>
<td>Soluble</td>
<td>Soluble</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose †.</td>
<td>200° C.</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
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<td>&quot;&quot; &quot;&quot;</td>
<td></td>
</tr>
<tr>
<td>Sucrose ‡.</td>
<td>None</td>
<td>No reduction</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>Mucic acid formed on oxidation.</td>
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<td>Polysaccharides§:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Acrhroodextrin</td>
<td>None</td>
<td>No reduction</td>
<td>No reduction</td>
<td>Yellow</td>
<td>Soluble</td>
<td>Partially soluble</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrodextrin</td>
<td></td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td></td>
</tr>
<tr>
<td>Glycogen.</td>
<td>&quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>Brown</td>
<td>Soluble</td>
<td>Insoluble</td>
<td>&quot;&quot; &quot;&quot;</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>&quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>Blue</td>
<td>Sometimes faint blue</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td></td>
</tr>
<tr>
<td>Cellulose.</td>
<td>&quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td>&quot;&quot; &quot;&quot;</td>
<td></td>
</tr>
</tbody>
</table>

* On hydrolysis yields two molecules of glucose.  
† On hydrolysis yields one molecule of glucose and one of galactose.  
‡ On hydrolysis yields one molecule of glucose and one of fructose.  
§ On hydrolysis yield reducing sugars.  
|| From W. D. Halliburton's *Essentials of Chemical Physiology*.  
¶ From R. H. A. Plummer, *Practical Organic and Biochemistry*.  
\[ [a]^p = \frac{+a \times 100}{e \times l} \]
The carbohydrates contain several asymmetric carbon atoms and therefore they rotate the plane of polarised light. The extent and direction of rotation is used as a means of identification. The specific rotatory power of a substance is the rotatory power of one gramme of substance in one cubic centimetre of liquid in a layer one decimetre long.

\[ [\alpha]_b = \pm \frac{a \times 100}{c \times l} \]

in which

\([\alpha]_b = \text{Specific rotation in wave length of light from sodium vapour.}\]

\(a = \text{observed rotation.}\)

\(c = \text{concentration in percentage.}\)

\(l = \text{length of tube in decimetres.}\)

The measurement of the rotation of light depends upon the fact that when a ray of light passes through a prism of Iceland spar it is deflected into two portions, the ordinary ray and the polarised ray. By splitting a prism in such a direction that the ordinary ray is totally reflected, only the polarised ray can pass through. This is known as a Nicol's prism. The polarised ray is a ray of light so altered that instead of the light waves vibrating radially like the spokes of a wheel they vibrate in the form of an ellipse. If a second Nicol prism is placed behind the first, we speak of the front one as the polarising prism and the back one as the analysing prism. If the axes of the two prisms are parallel all the polarised light can pass through the analysing prism, but if they are not parallel the amount of light passing through is diminished. In various instruments different methods are used to make this passage of light more obvious, such as splitting the field of view into two or more portions which can be matched only when the axes of the prisms are parallel. If after matching the field of view a solution of sugar is placed between the polarising and analysing prisms the analysing prism must be rotated in order to make the different parts of the field match once more. The angle of rotation is the measure of the rotatory power of the sugar.

The alcohol hydroxyls of the sugars enable them to form ester-like compounds with other substances. For instance the glucosides consist of sugar united with other groups.

Methyl glucose is the compound of glucose with a methyl group, and it can be shown to exist in two forms, the \(a\) and \(\beta\) varieties. These two differ in their relation to enzymes and in the direction in which they rotate polarised light. To
explain this difference another form of asymmetry is supposed to occur, namely, that a ring containing oxygen is formed.

\[ \text{CH}_3\text{O} - \text{C} - \text{H} \quad H - \text{C} - \text{OCH}_3 \]

\[ \text{CHOH} \quad \text{CHOH} \quad \text{CHOH} \quad \text{CHOH} \quad \text{CHOH} \quad \text{CH}_2\text{OH} \]

\[ \text{a-Methyl glucoside} \quad \beta-\text{Methyl glucoside.} \]

Glucose is assumed to occur in these two forms, an hydroxyl group replacing the methoxy (\text{CH}_3\text{O}) group. Glucose solutions show a variable effect on the rotation of polarised light (muta-rotation), which is due to the inter-conversion of the \(\alpha\) and \(\beta\) varieties. The stable condition is an equilibrium condition between the two varieties. The equilibrium varies with the concentration and temperature, hence these must be kept constant in making measurements. A trace of alkali is frequently added to the solution to hasten the condition of equilibrium. Other forms of glucose, such as \(\gamma\)-glucose are important in biological processes.

For further information the reader must be referred to the monographs on carbohydrates.

**Fats**

As already mentioned the oxidation of a primary alcohol produces an aldehyde and then an acid. The oxidation of the marsh gas series produces what is termed the fatty acid series. The fatty acids consist of a chain of carbon atoms; each succeeding compound containing one \(-\text{CH}_2-\) group more than its predecessor. As the number of carbon atoms increases the acids become less soluble in water and their melting points rise. Formic and acetic acids are liquid at moderate temperatures, and they mix in all proportions with water, but the higher fatty acids which occur in the usual fats are solid and do not dissolve in water.

\[
\begin{align*}
\text{H-COOH} & : \text{Formic Acid.} \\
\text{CH}_3\text{COOH} & : \text{Acetic Acid.} \\
\text{CH}_3(\text{CH}_2)_10\text{COOH} & : \text{Stearic acid.} \\
\text{CH}_3(\text{CH}_2)_{14}\text{COOH} & : \text{Palmitic Acid.} \\
\text{C}_{n-1}\text{H}_{2n-1}\text{COOH} & : \text{General formula.}
\end{align*}
\]
pounds of galactose with fatty acids and with the nitrogenous base sphingosine.

Instead of three molecules of fatty acid uniting with one molecule of glycerine, there are compounds in which two molecules of fatty acid unite with glycerine and the third molecule of fatty acid is replaced by phosphoric acid. Attached to the phosphoric acid is a nitrogenous base: in lecithin the base is cholin.

\[ \begin{align*}
CH_2O & - OCC_{17}H_{35} \\
CHO & - OCC_{17}H_{35} \\
& \quad \text{(Palmityl groups)}
\end{align*} \]

\[ \begin{align*}
CH_2O - P = O & \\
\text{Phosphoric group.}
\end{align*} \]

\[ \begin{align*}
\text{OH} & \quad \text{OC}_2H_4 \quad \text{N} \quad \text{CH}_3 \\
\text{HO} & \quad \text{N} \quad \text{CH}_3 \\
& \quad \text{CH}_3 \\
& \quad \text{CH}_3 \\
& \quad \text{Cholin}
\end{align*} \]

Lecithin.

These phosphorised fats are probably essential for living cells as they are found in all cells. They are classified according to the relative number of atoms of nitrogen and phosphorus in their molecules.

<table>
<thead>
<tr>
<th>Character.</th>
<th>Shows the amount of</th>
<th>Method used.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid value</td>
<td>Free fatty acid</td>
<td>Direct titration with alkali. Saponify and measure the amount of alkali that combines with the fatty acid during saponification.</td>
</tr>
<tr>
<td>Saponification value</td>
<td>Total fatty acid</td>
<td>Saponify, render acid and distil, neutralise distillate with alkali.</td>
</tr>
<tr>
<td>Reichert-Meissl value</td>
<td>Volatile fatty acid</td>
<td>Measure amount of iodine absorbed under standard conditions.</td>
</tr>
<tr>
<td>Iodine value</td>
<td>Unsaturated fatty acid</td>
<td></td>
</tr>
</tbody>
</table>

The first two are expressed as the amount of potassium hydroxide in milligrams necessary to neutralise the fatty acid in one gram of fat.

The third is expressed as the number of cubic centimetres of O:N alkali required for the volatile fatty acids from five gram of fat.

The last is expressed as the amount of iodine in grams absorbed by 100 grams of fat.
AROMATIC AND HETEROCYCLIC COMPOUNDS

The compounds already described have consisted of open chains of carbon atoms (aliphatic compounds), but there are carbon compounds in which the carbon atoms are arranged in a ring. These are known as aromatic compounds, and the simplest of them is benzene, containing six carbon and six hydrogen atoms. When the carbon atoms close up to form the benzene group one valency of the carbon atom remains unaccounted for and it behaves as a trivalent atom.

The spare valency has been assumed to be distributed in one of two ways as shown in the following structural formulæ.

\[ \text{According to Claus.} \quad \text{According to Kekulé.} \]

For practical purposes we frequently represent the benzene group as a hexagon, each angle of which represents one \( \equiv \text{CH} \) group.

Any group which replaces a hydrogen atom can be placed alongside of the angle corresponding to the carbon atom from which the hydrogen atom has been displaced. The displacement of one hydrogen atom from benzine gives rise to the phenyl group.

FIG. 3.—Cholesterol crystals. (Redrawn from a photomicrograph.)

**Cholesterol**

Cholesterol is frequently classified with fats under the general term "Lipoid," which also includes the phosphorised fats. The excuse for this classification is that they all dissolve in ether and other solvents for fats and on extracting tissues with fat solvents they are all extracted together.

Cholesterol is really a secondary alcohol with a ring formation and the properties of an unsaturated substance. It forms the bulk of the so-called unsaponifiable matter associated with fats: it belongs to the terpene series.

Cholesterol can combine with fatty acids to form cholesterol esters. It crystallises in characteristic flat plates with an indented corner and can be estimated quantitatively by the
precipitation of an insoluble compound with digitonin.*
Certain colour reactions are characteristic,† such as a red colour given by its solution in chloroform on the addition of concentrated sulphuric acid.

Ring compounds which contain other elements in the ring nucleus in addition to carbon are called heterocyclic compounds. The pyrrol group consisting of four carbon atoms and one nitrogen occurs in several important compounds.

Several benzene nuclei or benzene nuclei and heterocyclic rings may occur united in various degrees, for example the indole group occurs in tryptophane.

\[
\begin{align*}
\text{Pyrrole} & : & \text{Indole} \\
\text{HC} & \text{CH} & \text{CH} \\
\text{HC} & \text{CH} & \text{CH} \\
\text{NH} & \text{NH} & \\
\end{align*}
\]

**Compounds containing Nitrogen**

The nitrogenous compounds of biological interest are mainly derivates of ammonia. On replacing the hydrogen atoms of ammonia (NH\(_3\)) by other groups substituted ammonia are formed.

If one of the hydrogen atoms is replaced by a group so that the nitrogen is linked directly to the carbon the compound is called an amine, but if the nitrogen and carbon are united, through the intermediation of an oxygen atom, the compound is called an amide.

\[
\begin{align*}
\text{NH}_3 & \quad \text{R—NH}_2 & \quad \text{RCONH}_2 \\
\text{Ammonia} & \quad \text{Amine} & \quad \text{Amide} \\
\end{align*}
\]

These two types of substances differ in their reactions. The latter yields ammonia on treatment with either acid or alkali whilst the former is not decomposed by such treatment.

Amines are basic and form salts with acids.

Compounds consisting of a sugar and an amine are occasionally found: the simplest of which is the amine of glucose, glucosamine. Other basic nitrogen compounds have been mentioned under the heading of the fats as forming part of the phosphorised fats.

Urea

is the di-amide of carbonic acid and it can be considered as formed from ammonium carbonate by the removal of two molecules of water. The removal of one molecule of water from ammonium carbonate produces the intermediate substance ammonium carbamate. The chemical characteristics of urea will be discussed later (p. 182).

\[
\begin{align*}
\text{OH} & \quad \text{ONH}_4 & \quad \text{ONH}_4 & \quad \text{NH}_2 \\
C & = O - 2\text{NH}_3 & \quad C & = -\text{H}_2\text{O} & \quad C & = O \\
\text{OH} & \quad \text{ONH}_4 & \quad \text{NH}_2 & \quad \text{NH}_2
\end{align*}
\]

Carbonic acid. Ammonium carbonate. Ammonium carbamate. Urea

Other nitrogen-containing compounds such as uric acid, creatine and creatinine will be considered later.

Amino-acids,

as their name suggests, consist of fatty acids to which an amine group is attached. The amine group is attached to the carbon atom next to the carboxyl group (α position). The acidity of the carboxyl group is decreased by the presence of the amino group and the alkalinity of the amino group is decreased by the presence of the acid group. Thus we have formed neutral substances which can unite with bases by their acid group and with acids by their amine group. They are therefore called amphoteric substances.

The simplest amino acid is derived from acetic acid; the second member of the series is derived from propionic acid and is of importance because of the many compounds into which it enters and its relation to many other substances (see Chapter VII).

\[
\begin{align*}
\text{CH}_3\text{COOH} & \quad \text{CH}_2(\text{NH}_2)\text{COOH} & \quad \text{CH}_3\cdot\text{CH}_2\text{COOH} \\
\text{Acetic acid.} & \quad \text{Amino-acetic acid or glycine.} & \quad \text{Propionic acid.} \\
\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH} & \quad \text{Amino-propionic acid or alanine.}
\end{align*}
\]

Unfortunately these simple formulae do not suffice for all purposes. The presence of other groups complicates the subject to a certain extent. Two amine groups occur in some amino acids and an amino acid may be formed from an acid containing two carboxyl groups. In the former case the substance is more basic and in the latter case more acid than the simple amino acids.

Substituted alanines are formed by slight alterations in the
compound. By oxidising the end carbon atom to a primary alcohol serine is formed and by replacing the hydroxyl group (OH) of the primary alcohol by a sulphhydrate (SH) group cysteine is formed. Two molecules of cysteine united through their sulphur atoms form cystine.

The union of alanine with aromatic and heterocyclic compounds gives rise to important amino acids. Phenylalanine is formed by the union of benzene and alanine. On oxidising benzene phenol or carbolic acid is formed; this united with alanine forms tyrosine. By uniting alanine with indole tryptophane results.

**Proteins**

The proteins are formed by chains of these amino acids. The amino acids are united to each other by the NH$_2$ and COOH groups with the loss of one molecule of water.

$$\text{RCOOH} + \text{NH}_2\text{R} = \text{RCO-NHR} + \text{H}_2\text{O}.$$  

By repeated linkages of this sort long chains have been produced which behave like some of the simpler proteins, so there is small doubt that the more complicated proteins will be synthesised in the same way.

$$\text{NH}_2\text{CH}_2\text{COOH} + \text{NH}_2\text{CH}_2\text{COOH} = \text{Glycine.}\text{Glycine.}$$

$$\text{NH}_2\text{CH}_2\text{CO-NHCH}_2\text{COOH} + \text{H}_2\text{O}$$

Glycyl-glycine.

The colour tests for proteins depend firstly on the presence of—CONH—groups which occur whenever two amino acids unite with each other and secondly on the chemical nature of the amino acids contained in the molecule.

**Biuret Reaction.**—To a solution of protein add a trace of copper sulphate, then make the solution alkaline with sodium or potassium hydroxide. A purple or pink colour is produced, depending on the nature of the protein. This test is due to the presence of a number of—CONH—groups united to each other in various ways.* Owing to the pink colour given by biuret with copper sulphate and alkali this test is called the biuret test.

**Xanthoproteic reaction.** On heating a protein solution with a few drops of concentrated nitric acid a yellow colour is produced. This test is due to the nitric acid forming nitro-compounds with the benzene groups of the aromatic amino acids.* The addition of alkali causes the colour to become

orange. Compare this test with the yellow colour of picric acid, an aromatic nitro compound, which becomes orange on rendering alkaline.

Tyrosine Reaction. On heating the protein solution with a few drops of Millon's reagent the precipitate formed by the addition of the reagent turns brick red in colour. This reaction is due to the phenol group in tyrosine.*

Tryptophane Reaction. A drop or two of glyoxalic acid solution is added to the protein solution and the mixture is floated on strong sulphuric acid. A purple ring at the junction of the watery mixture with the strong sulphuric acid indicates the presence of the amino acid, tryptophane.†

Sulphur Test. To a solution of protein some lead subacetate is added and then just sufficient alkali to redissolve the precipitate that forms at first. If the solution becomes brown or black on heating the test indicates the presence of loosely combined sulphur which is removed by the alkali in the form of sulphide and forms black lead sulphide.

The reaction is probably due to cystine in the molecule.

Molisch reaction (p. 8) shows the presence of a carbohydrate group in protein.

Other reactions of proteins are not so strictly chemical in nature. They depend mainly on the ease of precipitation of the proteins from their solutions. We can distinguish two separate processes, reversible precipitation and irreversible precipitation or coagulation. In the case of the former there is a precipitate which will redissolve on removal of the precipitant and in the case of the latter there is a precipitate which must be chemically altered before it can be made to redissolve.

Many of the substances which cause precipitation will cause coagulation if their action is allowed to continue; thus the distinction between a precipitant and coagulant is partly a matter of the length of time that the reagents are allowed to act on the protein.

Protein precipitants and coagulants.—

Strong mineral acids.
Alcohol.
Alkaloidal reagents (hydroferrocyanic acid, picric acid, potassio-mercuric iodide, trichloracetic acid, tannic acid, phosphotungstic acid and bromine water).—

Excess of neutral salts of alkalies and alkaline earths.

Salts of heavy metals. Heating to various temperatures above 30° C.
The following classification indicates the nature of the various protein substances.*

1. Protamines. Simple basic substances mainly derived from fish-sperm.
2. Histones. Slightly more complex substances precipitated by ammonia. This and the preceding class merge into each other.
3. Albumins. Proteins which dissolve in distilled water and are coagulated by heating.
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   (c) Peptones. These give a pink colour with copper sulphate and alkali, but are not precipitated from their solution by salts.
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| Protein Source          | Ox Muscle | Keratin from Ox horn (α-scleroprotein) | Gelatin (α-scleroprotein) | Caseinogen from Cow’s milk (α-phosphoprotein) | Zein from Millet (α-gliadin) | Edestin from Horse seed | Serum globulin from Horse’s blood | Serum albumin from Horse’s blood | Globin from Horse’s blood (α-hemoglobin) | Salmine (α-prolamine) | Glycine | Alanine | Valine | Leucine | Phenylalanine | Tyrosine | Serine | Cystine | Proline | Oxyproline | Aspartic Acid | Glutamic Acid | Tryptophane | Arginine | Histidine | Ammonia | Total |
|------------------------|----------|----------------------------------------|--------------------------|-----------------------------------------------|----------------------------|--------------------------|--------------------------|-------------------------------|----------------------------------|---------------------|----------|--------|--------|--------|---------|-----------|---------|--------|--------|---------|------------|-------------|-------------|-------------|-----------|---------|--------|-------|
|                        |          | 2.1                                   | 0.4                      | 0.4                                           | 0.0                        | 0.0                      | 0.0                      | 0.0                           | 0.0                               | 0.0                 | 4.2      | 0.5    | 0.7    | 0.8    | 0.5     | 0.5       | 0.5     | 0.4    | 0.5    | 0.4     | 0.4        | 0.5          | 0.5         | 0.5         | 0.5      | 0.5    | 0.5   |
|                        |          | 3.3                                   | 0.8                      | 1.2                                           | 1.0                        | 1.2                      | 1.0                      | 1.0                           | 1.0                               | 1.0                 | 1.0      | 1.0    | 1.0    | 1.0    | 1.0     | 1.0       | 1.0     | 1.0    | 1.0    | 1.0     | 1.0        | 1.0          | 1.0         | 1.0         | 1.0      | 1.0    | 1.0   |
|                        |          | 1.2                                   | 0.8                      | 1.2                                           | 1.0                        | 1.2                      | 1.0                      | 1.0                           | 1.0                               | 1.0                 | 1.0      | 1.0    | 1.0    | 1.0    | 1.0     | 1.0       | 1.0     | 1.0    | 1.0    | 1.0     | 1.0        | 1.0          | 1.0         | 1.0         | 1.0      | 1.0    | 1.0   |
|                        |          | 3.3                                   | 0.8                      | 1.2                                           | 1.0                        | 1.2                      | 1.0                      | 1.0                           | 1.0                               | 1.0                 | 1.0      | 1.0    | 1.0    | 1.0    | 1.0     | 1.0       | 1.0     | 1.0    | 1.0    | 1.0     | 1.0        | 1.0          | 1.0         | 1.0         | 1.0      | 1.0    | 1.0   |
|                        |          | 2.1                                   | 0.4                      | 0.4                                           | 0.0                        | 0.0                      | 0.0                      | 0.0                           | 0.0                               | 0.0                 | 4.2      | 0.5    | 0.7    | 0.8    | 0.5     | 0.5       | 0.5     | 0.4    | 0.5    | 0.4     | 0.4        | 0.5          | 0.5         | 0.5         | 0.5      | 0.5    | 0.5   |
|                        |          | 3.3                                   | 0.8                      | 1.2                                           | 1.0                        | 1.2                      | 1.0                      | 1.0                           | 1.0                               | 1.0                 | 1.0      | 1.0    | 1.0    | 1.0    | 1.0     | 1.0       | 1.0     | 1.0    | 1.0    | 1.0     | 1.0        | 1.0          | 1.0         | 1.0         | 1.0      | 1.0    | 1.0   |
|                        |          | 1.2                                   | 0.8                      | 1.2                                           | 1.0                        | 1.2                      | 1.0                      | 1.0                           | 1.0                               | 1.0                 | 1.0      | 1.0    | 1.0    | 1.0    | 1.0     | 1.0       | 1.0     | 1.0    | 1.0    | 1.0     | 1.0        | 1.0          | 1.0         | 1.0         | 1.0      | 1.0    | 1.0   |
|-----------|------------------|----------------|---------------------------------------------|----------------------------------------|--------------------------------------------|-------------------------------------------------|-------------------------------------------------|---------------------------------|-------------------------------------------------|-------------------------------------------------|
| Albumin   | Soluble          | Purple         | Insoluble                                   | Insoluble                              | Insoluble                                   | Insoluble                                       | Insoluble                                       | Insoluble                        | Insoluble                                       | Insoluble                                       |
| Globulin  | Insoluble        |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Gliadin   | Soluble          |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Glutenin  | Insoluble        |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Gelatin   | Soluble          |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Metaprotein| Insoluble        |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Heteroproteose |     |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Protoheteroproteose | |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Deuteroproteose |     |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Peptone   | Insoluble        |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Polypeptides | Insoluble      |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |
| Amino Acids| Soluble          |                |                                             |                                        |                                            |                                                 |                                                 |                                 |                                                 |                                                 |

1 and 2 are coagulated by heat. The colour reactions depend on the nature of the constituent amino acids.
This brief outline of the nature of chemical substances obtained from cells covers the greater number of substances found in living structures.

In closing this chapter it is important to emphasise the fact that the living cell requires a supply of available energy and that this supply is obtained from organic compounds. Whatever may be the functions of the various groups of substances described above they are all capable of furnishing a supply of energy. This energy supply and the relations of these groups of compounds to each other will be considered in later chapters.

For fuller information on the subjects of this chapter see Monographs on Bio-chemistry (Longmans, Green & Co.).

E. F. ARMSTRONG: The Simple Carbohydrates and the Glucosides.
J. B. LEATHES: The Fats.
H. MACLEAN: The Lipins.
T. B. OSBORNE: The Vegetable Proteins.
R. H. A. PLIMMER: The Chemical Constitution of the Proteins. Parts I and II.
S. B. SCHRYVER: The General Characters of the Proteins.
CHAPTER II

REACTIONS IN HOMOGENEOUS SYSTEMS

Living cells are not uniform in structure and the different parts are distinguishable from one another. Therefore we must study how chemical reactions proceed in a uniform medium and how surfaces of separation influence these reactions.

A mass of material of uniform composition is known as a phase and reactions in such a medium are known as reactions in a homogeneous system, but when the material consists of parts with different compositions we have to deal with surfaces of separation: reactions in a system of more than one phase are known as reactions in a heterogeneous system.

The organic chemical compounds described in Chapter I are a source of energy which can be set free by oxidation and used in cells for carrying out their various processes.

Transformation of chemical energy into mechanical action is brought about in two ways.

The first way is by producing pressure which can be used to produce movement. For biological purposes this process is best understood by studying reactions that occur in a single phase, and any separation to form a closed space may be regarded as an artificial barrier. In the present chapter we will discuss the nature of chemical reactions that take place in a single phase.

The second way in which chemical energy can be transformed into mechanical activity is by altering the tension or pull at a surface of separation. This process involves the presence of separate phases and we shall deal with the reactions in heterogeneous systems in Chapter III.

As living cells are composed of watery solutions we can confine our study of reactions in homogeneous systems to reactions in solutions in water. In order to do so we can make use of the hypothesis of Van't Hoff that substances in solution behave as if they were gases occupying the same volume as the solution.*

Diffusion

The first process to which we must refer is that of diffusion; that is the tendency for a dissolved substance (solute) to become equally distributed throughout the solvent. The molecules pass from the parts of the solution where they are more concentrated towards those parts where they are less concentrated.

Diffusion is a slow process and is effective in living organisms as a factor in the exchange of materials only when the distances are short such as those of cellular dimensions.*

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**Osmosis**

If a semi-permeable membrane, that is one which allows the solvent to pass through but not the solute, separates the solvent from the solution the result is that the solvent passes through the membrane to dilute the solution. This obviously

REACTIONS IN HOMOGENEOUS SYSTEMS 29
produces the same result as diffusion, namely that after all the solvent has passed through the membrane to dilute the solution there is one solution of uniform concentration. This process is called osmosis.

DIALYSIS
A mixture of substances, some of which pass through the membrane and others which do not, can be separated by means of diffusion through a membrane. This process is known as dialysis and it is made use of to separate diffusible from non-diffusible substances.*

Osmotic Pressure
If the solution inside the semi-permeable membrane is enclosed in rigid walls the passage of the solvent inwards will produce a pressure. As the pressure inside increases the solvent is prevented from entering. When the pressure inside the membrane balances the osmosis a steady pressure is maintained which is called the osmotic pressure of the solution.

Vapour Pressure.
As it is not an easy matter to obtain good semi-permeable membranes the osmotic pressure is usually measured by some indirect method. These indirect methods depend on the decrease of the vapour pressure of the solvent by the dissolved substance and we shall now study the reason why the decrease of the vapour pressure is proportional to the osmotic pressure and finally how to measure it by freezing point or boiling point determinations.

In order to understand the relation of these indirect methods to the direct measurement of osmotic pressure, it is necessary to refer to the kinetic theory of gases. The gas molecules are conceived as being in constant oscillating motion and collisions occur between the various molecules and between the molecules of the gas and the walls of the containing vessel. The pressure of the gas is dependent on the number of molecules striking the wall at any one time. The number of collisions with the wall depends on the rate of movement and the number of molecules in a given volume. The rate of movement is a function of the temperature. Hence equal volumes of different gases at the same temperature and pressure contain the same number of molecules.†

† The mean kinetic energy of the various kinds of molecules is the same. For further information on this Law of Avogadro see textbooks of physical chemistry.
The process of diffusion depends on the collisions of the molecules, and as the number of collisions depends on the concentration of the solution the rate of diffusion is proportional to the differences of concentration.

Evaporation is produced by the movement of some of the molecules out of a fluid into the vapour space above. When the vapour space becomes saturated, i.e. the same number of molecules pass from the vapour space to the solution as pass from the solution to the vapour space, a steady condition results, which is called the vapour pressure of the solution. The vapour pressure of a solution depends, like the gas pressure, on the temperature and the number of molecules in a given space. In a solution the molecules of the solvent are fewer per unit space because the solute molecules lie between them, hence the vapour pressure of a solvent is decreased by dissolving something in it.

![Diagram](image-url)

**Fig. 5.**—Diagram illustrating the relation of concentration (osmotic pressure) to vapour pressure, depression of freezing point and rise of boiling point.

The upper curve represents the pure solvent and the lower one the solution. The line across the diagram shows the atmospheric pressure of 760 mm. of mercury. When the vapour pressure reaches this pressure the solution boils. The actual differences are exaggerated, but the real points are freezing point of water at 0°C. and 4.58 mm. Hg. pressure. A molecular solution causes a depression of 1.85°C. in the freezing point and a rise of 0.515°C. in the boiling point.
The decrease of the vapour pressure is therefore proportional to the osmotic pressure as both depend upon the number of molecules in the solution. The decrease of the vapour pressure is difficult to measure, but the rise of boiling point or fall of freezing point can be used to calculate the osmotic pressure.

By measuring the vapour pressure of a pure substance at different temperatures and by plotting the results so that the abscissae represent degrees Centigrade and the ordinates the vapour pressure in millimetres of mercury pressure, a temperature-pressure diagram is produced. Below the freezing point the curve falls more steeply and represents the vapour pressure of the solidified solvent. When the vapour pressure of a liquid is equal to the pressure of the atmosphere the solution boils.

If on the diagram representing the vapour pressure curve of water we draw a curve of vapour pressure of a solution we see that at any given temperature the vapour pressure of the solution is always less than the vapour pressure of the pure solvent. The curve for the solution cuts the curve of the solidified solvent at a temperature below the freezing point of the solvent; this represents the freezing point of the solution. The decrease of vapour pressure depends upon the concentration of the solution and the point at which the vapour pressure curve of the solution cuts the vapour pressure curve of the solidified solvent depends on the decrease of the vapour pressure, hence the depression of the freezing point can be used to calculate the osmotic pressure (molecular concentration) of the solution.

On following the vapour pressure curve upwards it is seen that in order to raise the vapour pressure of the solution until it is equal to the pressure of the atmosphere, the temperature must be higher than that which would raise the vapour pressure of the pure solvent to the same pressure: that is the boiling point is raised. As the rise in the boiling point depends upon the concentration of the solution, the rise can be used to calculate the osmotic pressure of the solution.

**Calculation of Molecular Concentration of a Solution from the Freezing Point Determination**

For any solvent the freezing point and boiling point are altered to a known extent for corresponding concentrations. In water a solution containing one molecular weight in grams in a litre of solution decreases the freezing point by 1.86°C,
and raises the boiling point by 0.515°C. For acetic acid the corresponding figures are 3.9°C and 3.0°C, respectively.

Let us take as an example an experiment in which a solution in water was cooled to 1.4°C below the freezing point of water, the temperature rose quickly to 0.135°C below the freezing point, remained steady there for a short time, then began to cool down slowly. The solution was supercooled 1.265°C (1.4 − 0.135) below the observed freezing point. As ice was separating heat was given off so that the solution warmed up to the observed freezing point, then as more ice separated the solution became more concentrated and the temperature began to fall slowly.

In order to obtain the true freezing point it is necessary to correct the observed freezing point because of the concentration brought about by the separation of ice. To do this we make use of the latent heat of formation of ice, which is 80 calories per gram. Thus every degree of supercooling causes the solution to be concentrated by $\frac{1}{80}$ because $1^\circ$ per cubic centimetre is equal to $1$ calorie, which is $\frac{1}{80}$ of the heat given off when 1 gram of water is converted into ice.

In the example the supercooling was 1.265°C so that the observed freezing point was that of a solution concentrated by $\frac{1.265}{80}$, i.e. to $\frac{78.735}{80}$ of its original volume and the observed freezing point was $\frac{80}{78.735}$, the value that it ought to have been. Therefore the true freezing point was $\frac{78.735}{80} \times 0.135 = 0.133$.

As the depression of the freezing point of water for a molecular solution is 1.86°C, the concentration of the solution used was $\frac{0.133}{1.86} = 0.071$ M.

For biological purposes the boiling point method is not used so much as the freezing point method, because proteins are coagulated by heating, hence the conditions of the solution are altered. Numerous precautions must be taken in measuring the freezing and boiling points, but the details must be sought in a book on methods of Physical Chemistry.

In watery solutions a depression of the freezing point by 0.001 of a degree Centigrade is equivalent to a pressure of 9 millimetres of mercury. The error of the freezing point
method is about 0.005 of a degree corresponding to 45 millimetres of mercury, hence wherever possible, as for example in measuring the osmotic pressure of protein solutions, the direct method should be employed rather than the indirect.

**IONIC DISSOCIATION**

Certain substances in watery solution show marked departures from the above generalisations. It has been found that although many substances behave normally in regard to osmotic pressure measurements, other substances give either too low or too high results. The low results are ascribed to association, in which two or more molecules unite to form single particles; thus there are fewer particles in the solution and a lower osmotic pressure than should correspond to the number of molecules dissolved.

The substances which give too high results are those which aid the passage of the electric current. These are assumed to break up into two or more particles which are electrically charged (ionic dissociation). These substances which conduct electricity are called electrolytes, and those which do not conduct electricity are called non-electrolytes.

The Ionic Dissociation Theory* can be illustrated by the behaviour of sodium chloride solution. Measurements of osmotic pressure show that a certain proportion of the molecules break up into two ions, sodium and chlorine, so that there are more particles than correspond to the number of molecules of the salt in the solution but less than twice that of the number of molecules. The sodium chloride dissociates completely into positively charged sodium ions (Na\(^+\)) and negatively charged chlorine ions (Cl\(^-\)) only in infinitely dilute solution. The total number of molecules being represented by unity the value two is found in very dilute solution. In more concentrated solution the dissociation is partial and it is represented by \(a\). The number of particles formed by complete dissociation of a molecules is \(2a\) (\(a\) sodium ions and \(a\) chlorine ions) and there are \(1 - a\) molecules undissociated; hence the total number of osmotically active particles in the solution will be \(1 - a + 2a = 1 + a\). This figure indicates the osmotic pressure of a solution of a binary electrolyte which dissociates to a certain extent.† We shall now show that measurements of electrical conductivity give the same value of \((a)\) for the degree of dissociation.

† Salts which decompose into more than two ions would give more particles in the solution.
The electrical conductivity of a solution is the reciprocal of the resistance of a column of liquid 1 square centimetre in area and 1 centimetre long. The conductivity decreases as the solution is made more dilute, due to the diminution in the total number of ions in the solution, but the relative or molecular conductivity increases owing to the greater dissociation of those molecules which are present in the solution.
In other words the electrical conductivity does not diminish so rapidly as the concentration of the solution, therefore the ratio given by the electrical conductivity divided by the number of molecules in the solution increases as the solution is made more dilute. The molecular conductivity at a given concentration divided by the molecular concentration at infinity, that is when complete dissociation occurs, gives the same value that is obtained by calculating the degree of dissociation from the osmotic pressure of the solution, \( \frac{\lambda_x}{\lambda_\infty} = \Lambda_x \).

The ionic dissociation theory thus outlined explains many facts both qualitatively and quantitatively which are difficult to explain on any other hypothesis.

It has been found that different ions conduct electricity to a greater or less extent and the difference is ascribed to the rate of movement of the various ions. The conductivity of a solution is the algebraical sum of the conductivity of the various ions in the solution.

**Table VII.**

*Table of Ionic Velocities.*

<table>
<thead>
<tr>
<th>Substances</th>
<th>H⁺</th>
<th>K⁺</th>
<th>Na⁺</th>
<th>OH⁻</th>
<th>Cl⁻</th>
<th>( \frac{1}{4} )SO₄⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>18°</td>
<td>313</td>
<td>64.5</td>
<td>42.7</td>
<td>174</td>
<td>65.2</td>
<td>67</td>
</tr>
<tr>
<td>25°</td>
<td>340</td>
<td>73.8</td>
<td>50.5</td>
<td>196</td>
<td>75.2</td>
<td>77</td>
</tr>
</tbody>
</table>

Substances which in moderately concentrated solution are nearly completely dissociated into ions are called strong electrolytes whilst those which are only slightly dissociated are called weak electrolytes. The similar behaviour of different substances which by dissociation can set free a common ion is thus explained: all chlorides set free chlorine ions and all acids hydrogen ions. The reactions of a given substance may depend on the ions formed from it or on the undissociated molecules.

The strength of an acid will depend upon the degree of its dissociation into ions as the more dissociated the acid the greater the number of hydrogen ions (H⁺) for the same con-
centration of acid. Hence the true acidity must always be expressed in terms of the concentration of hydrogen ions.

**LAW OF MASS ACTION**

In order to follow this subject further we must divert our attention to the Law of Mass Action.* This law states that the rate of a chemical reaction depends upon the effective† concentration of the reacting substances. The law can be explained on the Kinetic Theory mentioned above as the rate of reaction depends upon the number of collisions and the latter depends upon the number of molecules per unit volume of reacting substances.

Let us consider a dilute solution in which a reaction takes place between A and B with the formation of C and one molecule of water. The reaction can also be considered as reversible so that on the one hand we have a dehydration, and on the other an hydrolysis.

\[
\text{dehydration} \quad A + B \xrightleftharpoons{} C + \text{water.}
\]

\[
\text{hydrolysis}
\]

The rate of formation of C depends on the molecular concentration of A multiplied by the molecular concentration of B multiplied by a factor \(k\) called the velocity constant. Therefore \(\frac{dx}{dt} = k'C_A'C_B\) where \(C_A\) = molecular concentration of A and \(C_B\), the molecular concentration of B.

The rate of formation of A and B from C can be expressed in a similar way as \(\frac{dx}{dt} = k'C_C'C_{H_2O}\) but as we are dealing with a dilute solution and the concentration of water is so great that small changes in the amount of water make no

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† The law of Mass Action deals with the number of reacting particles in unit volume. In the case of substances which exist in solution as single molecules it is the molecular concentration, *i.e.* the molecular weight in grams in a litre. In the case of substances which dissociate in solution such as the complete dissociation of a dibasic acid it is the number of hydrogen ions that pass to be considered; the normal solution is the half of the molecular concentration as each molecule yields two hydrogen ions. If a substance associates in solution the effective concentration is some sub-multiple of the molecular concentration. The correct application of the law therefore depends on a knowledge of the actual reacting particles.
difference to the rate of reaction, we can write the equation as
\[ \frac{dx}{dt} = k''C. \]
In other words the concentration of water is constant and this constant figure is included in \( k''. \)

During the union of A and B to form C the rate of reaction decreases because the concentrations of A and B are diminishing and the reaction in the opposite direction is accelerated as the amount of C is increasing.

At the equilibrium point the rate of formation of C exactly equals that of its decomposition and the composition of the mixture is shown by the equations
\[ k' C_A \cdot C_B - k'' C_o = 0 \quad \text{or} \quad k' C^*_A \cdot C_B = k'' C_o \]

Thus \( \frac{C_A \cdot C_B}{C_o} = \frac{k''}{k'} = K \) and this means that the composition at the equilibrium point is proportional to the ratio of the velocity constants of the reaction and this is expressed by the symbol K or equilibrium constant.

These simple equations are of immense importance in chemistry as they can be used to express quantitatively the chemical changes which follow the mixing of the substances in varying proportions. A proper appreciation of their value will help the biological chemist to understand much that is otherwise difficult and it is necessary that every biological chemist should be able to deal with his subject by means of this law.

The constants \( k' \) and \( k'' \) depend upon the nature of the reaction, but once the reaction has been studied and the constants determined it is possible to foretell the result of mixing the substances in any proportions.

The reaction of breaking up of C is called a mono-molecular reaction as only one variable is concerned, and the combination of A and B is called a bi-molecular reaction as two variables are concerned. More complicated reactions occur but they are unusual and can be dealt with by introducing extra terms into the equations. If two molecules of the same substance are required, the corresponding power of the concentration must be used. For instance, if two molecules of

* For reasons which depend upon the mathematical treatment of the subject the rate of reaction is represented as a differential and the symbol \( \frac{dx}{dt} \) is used. \( \frac{dx}{dt} \) means the small change \( dx \) which takes place in the quantity of \( x \) during the short interval of time, \( dt \).
A were necessary, the equation would contain $C_A \cdot C_A$ or $C_A^2$.

Applying these equations to the ionic dissociation of electrolytes we find that the dissociation of weak electrolytes conforms to the law of mass action: the dissociation is an equilibrium condition. Thus the weak acid carbonic acid ($H_2CO_3$) breaks up into a hydrogen ion ($H^+$) and a bicarbonate ion ($HCO_3^-$). Hence

$$\frac{C_{H^+} \cdot C_{HCO_3^-}}{C_{H_2CO_3}} = K$$ or $$C_{H^+} \cdot C_{HCO_3^-} = K C_{H_2CO_3}.$$ 

We note that a further dissociation is possible, namely, that of $HCO_3^-$ into $H^+$ and $CO_3^{2-}$ which gives the equilibrium equation

$$C_{H^+} \cdot C_{CO_3^{2-}} = K' C_{HCO_3^-}.$$ 

The constant $K'$ is $1.291 \times 10^{-11}$, and therefore $CO_3^{2-}$ can occur in appreciable quantity only when the concentration of $HCO_3^-$ is very high, or when the concentration of $H^+$ is very low, which as we shall see below is in alkaline solution.

Water can split into two ions, hydrogen ($H^+$) and hydroxyl ($OH^-$) which gives the equilibrium condition, $C_{H^+} \cdot C_{OH^-} = K C_{H_2O}$. The concentration of the water is relatively so great and uniform that we can simplify the equation to $C_{H^+} \cdot C_{OH^-} = a$ constant. This constant has been determined in several different ways and is equal to $0.72 \times 10^{-14}$ at $18^\circ$ Centigrade.

**HYDROGEN ION CONCENTRATION**

The result mentioned in the preceding paragraph is extremely important as the hydrogen ion is the characteristic of an acid and the hydroxyl ion that of a base. An increase in acidity must be accompanied by a reciprocal decrease in alkalinity, therefore it is possible to express the various acidities and alkalinites in terms of the concentration of hydrogen ions. A truly neutral solution, such as pure water, is one in which the concentration of hydrogen ions equals that of the hydroxyl ions.

The hydrogen ion concentration at the neutral point is calculated as follows:

$$C_{H^+} \cdot C_{OH^-} = K = 0.72 \times 10^{-14}$$

$$C_{H^+} = C_{OH^-}.$$ 

$$C_{H^+} = 0.72 \times 10^{-14}$$ and $$C_{H^+} = \sqrt{0.72 \times 10^{-14}} = 0.85 \times 10^{-7}.$$ 

Sørensen has suggested the use of the symbol $p_H$ to represent the negative exponent of the hydrogen ion concentration.†

As \( p \) is generally used to represent pressures a better symbol would be the term \(-\log[H^{+}]\) as that is the correct mathematical symbol for the values expressed by Sørensen's \( p_H \). The following table shows the acidity of some well-known solutions expressed in the various ways:

**Table VIII**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Normality of hydrogen ions ([H^{+}]) (-\log[H^{+}]) or (p_H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 n Hydrochloric Acid</td>
<td>(0.91 \times 10^{-1}) or (1.04)</td>
</tr>
<tr>
<td>0.1 n Acetic Acid</td>
<td>(0.13 \times 10^{-2}) or (2.89)</td>
</tr>
<tr>
<td>Neutral solution</td>
<td>(0.85 \times 10^{-7}) or (1.29)</td>
</tr>
<tr>
<td>0.1 n Ammonia solution</td>
<td>(0.52 \times 10^{-11}) or (11.29)</td>
</tr>
<tr>
<td>0.1 n Sodium hydrate</td>
<td>(0.88 \times 10^{-13}) or (13.06)</td>
</tr>
</tbody>
</table>

**Electromotive Force**

One of the methods for measuring the concentration of hydrogen ions is an electrical one.

A plate of metal dipped into water gives off positively charged ions of the metal used and the plate becomes negatively charged. If a salt of the same metal is present in the solution the positively charged metallic ions decrease the ease with which the ions are given off by the plate, therefore the electrical potential at the plate is decreased. If the concentration of the metallic ions is high enough the potential may be reversed and the plate becomes positively charged.

Platinum electrodes saturated with hydrogen gas behave as if they were a metallic form of hydrogen and the electrical potential varies with the concentration of hydrogen ions in the solution. The electrical potential at platinum electrodes in an atmosphere of hydrogen can be used therefore as a measure of the concentration of hydrogen ions in the solution.*

In order to measure the electrical potential due to the ions in a solution two electrodes are required, one the electrode at which the potential is being measured and the other a constant electrode the electrical potential of which is known. Frequently a calomel electrode is used for the electrode with known potential and the arrangement of an experiment is as follows:

Instruments required for measuring the electrical potential.

Fig. 7.—Diagram to illustrate the method for measuring electrical potentials.

A known potential from the galvanic cell B is present at the two ends of the wire XY, the moving contact Z can give a potential proportional to the distances. The potential to be measured is led through the electrometer E in the opposite direction to that of the known potential. When the electrometer shows no current the two potentials are in the ratio of XZ:XY.

In the diagram a calomel electrode C, consisting of mercury covered by mercurous chloride in contact with a standard chloride solution is connected through a concentrated potassium chloride solution in vessel V with a hydrogen electrode H. The hydrogen electrode shows the platinum plate P dipping into a solution. The hydrogen enters through the lower tube marked with arrows and escapes through the small mercury seal S.

Instead of the combination represented here other combinations may be used and the electrical potentials in living tissues can be measured by two calomel electrodes connected by means of the tissue instead of a vessel as represented by V in the diagram.

There are electrical potentials at the contact of platinum with solution under investigation, at the contact of the solution under investigation with the standard chloride solution and at the contact of the standard chloride solution with mercurous chloride and mercury.

The chloride solution is of constant concentration and it is saturated with mercurous chloride; thus the potential between this solution and mercury does not vary. This potential can be measured and deducted from that found for the whole system.
The potential at the contact of the solution under investigation with the standard chloride solution is discussed in the following paragraphs.

**Concentration Cells**

At the contact of the solution under investigation with the chloride solution there is a potential due to the diffusion of the ions. As shown in the table on page 35, the rate of diffusion of the different ions varies, and if one of the ions diffuses more rapidly than the others the solution into which it diffuses acquires the charge of the more rapid ion. This difference of diffusion sets up an electrical potential.

As the positive and negative ions attract each other the oppositely charged ion is dragged after the faster one; the one is held back and the other pulled onwards so that the rate of movement of the two becomes equal. Nevertheless the more rapid ion confers its charge on the solution into which it is diffusing.

It has been shown that if two different concentrations of the same salt are in contact the electrical potential is

\[ 0.0235 \frac{u-v}{u+v} \log \frac{C_1}{C_2} \]

where \( u \) is the rate of diffusion of the positive ion, \( v \) that of the negative ion. \( C_1 \) and \( C_2 \) are the concentrations of the two solutions.*

From this equation we see that if a salt is used, with ions which diffuse at about the same velocity the electrical potential becomes very small. Therefore a strong solution of potassium chloride or ammonium nitrate is frequently interposed between the solution under investigation and the chloride solution in the calomel electrode.

Electrolytes in solution by acting as conductors short circuit the potential between the two solutions, therefore the concentrated solution increases the short circuiting because of the large number of ions in the solution.

The potential due to the contact between the two solutions is reduced to a minimum by the short circuiting and by the nearly equal rate of diffusion of the two ions.

Differences in potential in the schema shown above are by these means limited to the contact between the hydrogen electrode and the solution under investigation. The potentials have been measured with a series of solutions of known hydrogen ion concentration. The formula for the electromotive

force at the contact of an electrode with a solution containing an ion of the metal is \( \pi = \frac{RT}{NF} \log \frac{P}{C} \), where \( \pi \) is the electromotive force in volts. \( \frac{RT}{NF} \) is a factor which expresses the relation of the ions to the absolute temperature, the valency of the ions, etc., \( P \) the solution pressure of the metal, \( i.e. \) it represents the tendency of the metal to give off ions and \( C \) the concentration of the corresponding ion.

If a series of measurements are made with the same electrode \( P \) remains constant and cancels from the equation, thus the differences of electromotive force are \( \pi_2 - \pi_1 = \frac{RT}{NF} \log \frac{C_1}{C_2} \) and for hydrogen at 18°C. \( \log \) transferred from natural to Napierian logarithms and using 0.1N potassium chloride in the calomel electrode \( \frac{RT}{NF} \) is equal to 0.0577.*

**Calculation of the Concentration of Hydrogen Ions from Measurement of Electromotive Force**

By this equation we can calculate the concentration of hydrogen ions in the solution if we measure the electromotive force in the system given above, using a calomel electrode containing 0.1N potassium chloride. The electromotive force of a solution in which the molecular concentration of hydrogen ions is equal to 1† is found to be 0.3377 volts. This substituted in the equation makes it \( \pi_2 - 0.3377 = 0.0577 \log_{10} \frac{1}{C_2} \). As \( \log_{10} \frac{1}{C} \) is the same as \( -\log C \) we can put \( \pi_2 - 0.3377 = 0.0577 \times -\log [H^+] \). In an example in which the electromotive force is equal to 0.540 volt this substituted in the formula becomes \( -\log [H^+] \times 0.0577 = 0.540 - 0.3377 = 0.2023 \), whence \( -\log [H^+] = 3.50 \) and the concentration of hydrogen ions is \( 3.2 \times 10^{-4} \).‡

This method requires great care and a certain amount of apparatus, thus it cannot be carried out away from a laboratory.

During saturation of the electrode with hydrogen volatile

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† The concentration of hydrogen ions can be measured by other methods such as the saponification of esters and where possible the electrometric method should be controlled by some other method.
substances such as carbon dioxide may be driven out of the solution with the result that this concentration of hydrogen ions in the solution will be altered.*

The loss of carbon dioxide can be minimised by the use of special forms of electrodes.†

**COLORIMETRIC MEASUREMENT OF HYDROGEN ION CONCENTRATION**

A simpler method for measuring the hydrogen ion concentration is by means of indicators. These are coloured acids or bases. The free acids or bases are very slightly ionised but when neutralised to form salts they ionise strongly. The ion and the undissociated substance have different colours, hence a change of colour occurs when the weak acid or base is neutralised. There is probably a tautomeric change in many indicators when the reaction changes.

The ionisation constant (K in the equations given on p. 37) varies in different indicators and according to the law of mass action the amount of ion and unionised substance changes over a certain range of concentration of hydrogen ions. Therefore the colour change takes place over a restricted range of hydrogen ion concentrations and the limit of this range varies with different indicators.

Friedenthal ‡ and Salm § classified indicators according to the range of hydrogen ions at which the colour change occurred.

Sørensen has elaborated this indicator method for measuring hydrogen ion concentrations.|| He uses mixtures of solutions which are so selected that any desired hydrogen ion concentration can be produced. These mixtures have the property of neutralising a certain amount of acid or alkali without an appreciable change in their hydrogen ion concentration. These mixtures are called “buffer” or “stabilising” solutions.

To equal quantities of an unknown solution and one of these standard solutions the same amount of indicator is added. The colours are compared and if the colours match the concentration of hydrogen ions in the unknown solution equals that in the standard solution. If the colours do not match a


† For inaccuracy due to catalytic alteration of carbon dioxide at the platinum surface see C. L. Evans, *Journ. Physiol.*, 1921, vol. 54, p. 353.


A fresh standard solution is prepared with a higher or lower concentration of hydrogen ions depending on whether the colour with the first standard solution showed less or more acid than the unknown solution. For the colours and ranges of change of colour see figure 8.

The shaded areas are left without boundaries to indicate that the transition is not abrupt.

This colorimetric method is extremely easy and convenient. It requires very little apparatus and can be used for field work away from large laboratories. It suffers from the disadvantage that the presence of neutral salts interferes with the accuracy of the measurements.*

Electrochemical methods can be used to determine the concentrations of ions other than hydrogen ions. The electrical potential at the surface of a metal depends on the concentration of the ions of the metal in the solution. This is called an electrode of the first order. This form of electrode is exemplified by the one described for the measurement of hydrogen ions by platinum saturated with hydrogen gas.

Another form of electrode is called an electrode of the second order. The principle on which it is based is as follows:

A solution of an "insoluble" salt gives a constant figure for the product of its constituent ions (solubility constant). By adding a soluble salt with one ion the same as one of the ions of the "insoluble" salt the concentration of the other ion of the "insoluble" salt must be inversely decreased if the product of the ions of the "insoluble" salt is to remain constant.

To make use of this type of reaction an "insoluble" salt of a metal is used with an electrode of the metal; the anion (acidic ion) of the insoluble salt is the same as the ion to be measured. Variations in the concentration of the soluble salt produce variations in concentration of the anion with inverse changes in the concentration of the cation (metallic ion). As has been pointed out above the potential at the metal varies with the concentration of the metallic ion in contact with it, therefore variation in concentration of the anion produces changes in potential at the metal electrode.

In the case of the calomel electrode the potential at the mercury surface is kept constant by the presence of the insoluble mercurous chloride so long as the concentration of chloride ions in the solution is kept constant. A standard potassium chloride solution is used when the calomel electrode is used as a constant electrode but the concentration of chlorine ions can be measured by this electrode owing to variations in potential when chlorine ions are varied in the solution as increase in chlorine ions must cause changes in the concentration of mercurous ions.

\[ C_{Hg} \times C_{Cl^-} = K \]

Electrodes of the second order may be used to measure changes in the concentrations of ions in tissues.*

Salts formed from a weak base or a weak acid are dissociated in solution. The dissociation is governed by the equations,

\[ C_{H^+} \times C_{\text{acid}} = K_{\text{undissociated acid}} \]
\[ C_{\text{base}^+} \times C_{\text{OH}^-} = K_{\text{undissociated base}} \]

where \( C_{H^+}, C_{\text{acid}^-}, C_{\text{base}^+} \) etc., stand for the corresponding concentrations and \( K \) is the equilibrium constant.

All salts are highly ionised, therefore a solution of a salt containing a weak base must show a decrease in the concentration of hydroxyl ions or an increase in the concentration of undissociated base; as a rule both these take place to maintain the equilibrium condition. Therefore the solution of a salt of a weak base with a strong acid is acid in reaction, e.g. aniline hydrochloride. Conversely the solution of a salt of a weak acid with a strong base has an alkaline reaction, e.g. sodium carbonate.

Removal of the products of dissociation, for instance by dialysis, may lead to a complete dissociation of the salt.

To summarise we find that diffusion, osmotic pressure and vapour pressure all depend upon the concentration of the substances in solution. In the case of non-electrolytes the effective concentration usually corresponds to the number of molecules in solution but in the case of electrolytes, owing to electrolytic dissociation, the increased number of particles in solution increases the effect on the vapour pressure, etc.

Chemical reactions are affected by the number of particles of reacting substances in solution and these effects can be expressed by simple mathematical equations.

Electrolytes cause electrical phenomena which can be measured and the measurements used to calculate the number of the various ions in the solution.

These generalisations will be applied frequently in later chapters of this book.

Volume changes due to variations in vapour pressure may produce some forms of movement and may thus be one of the means by which chemical energy can be converted into movement.

**GENERAL REFERENCES.**


CHAPTER III

REACTIONS IN HETEROGENEOUS SYSTEMS

The outstanding feature of heterogeneous mixtures is that there are surfaces separating the various homogeneous substances or phases. These surfaces show peculiar characteristics which are termed surface tension phenomena. They are best exemplified by liquids and owing to their influence on the ascent of liquids in capillary tubes they are often spoken of as capillary phenomena. We must examine the influence of these surface phenomena and compare them with the equilibrium conditions in the various phases separated by the surfaces.

**DISTRIBUTION BETWEEN TWO PHASES**

The solubility of a gas is generally proportional to the concentration (pressure) of the gas. This is known as Henry’s Law* and is applicable only when the gas is in the same molecular condition in the solution and in the space above the liquid.

If the dissolved gas forms double molecules the concentration in the solution is proportional to the square root of the pressure, and if the gas is combined with anything in the solution the total amount dissolved may not bear any simple relation to the gas pressure above the solution. That is a departure from Henry’s Law indicates that the gas is not dissolved in the same molecular condition in which it occurs in the gas phase.

The distribution of substances between various phases follows the same law, hence any departure from direct proportionality between the concentrations in the various phases requires investigation to discover the cause of the discrepancy.

**SURFACE TENSION**

The cause of surface tension is the attraction of the molecules in the bulk of the liquid on the molecules in the surface. These surface molecules are attracted on one side only as

* W. Henry, *Phil. Trans.*, 1803, p. 29.
there is no attraction from outside, hence the layer of liquid is slightly more condensed on the surface. This condensation produces a stress across the surface of the liquid. Most observations have been made on air-liquid surfaces, but the liquid-liquid surfaces are of greater importance in Biology.

Owing to the stress in the surface there is a tendency to reduce the amount of surface. Hence a liquid suspended in another liquid of about the same specific gravity assumes the shape of a sphere as this has the smallest surface for a given volume.

Substances which decrease the surface tension accumulate on the surface whilst substances which raise the surface tension become less concentrated on the surface. This is known as the Gibbs-Thompson law. The differences in concentration at the surface are spoken of as adsorption.

The spherical form and the changes in concentration are both explained by the Law of Minimum Energy which states that a system assumes the condition of least available energy. Thus by reducing the extent of surface or by decreasing the surface tension, the total available energy (surface × surface tension) is decreased.

Owing to the surface tension a pressure is produced. It is well known that the pressure inside a soap bubble is greater than that of the surrounding air because the tension of the two surfaces (one inside and one outside) of contact between the soap film and the air causes the soap film to contract on the contained air. The pressure on the concave side of a surface is greater than that on the convex side by \[ \frac{T + \frac{T}{r_1} + \frac{T}{r_2}} \]
where \( T \) = surface tension and \( r_1 \) and \( r_2 \) are the radii of curvature in two planes at right angles to each other.* Thus in a sphere with uniform internal pressure a local change of surface tension will cause a change of curvature. If the pressure inside is to remain the same, a decrease in surface tension must be accompanied by a decrease in the radii of curvature, hence a projection or pseudopodium is produced.

An electrical charge on the surface lowers the surface tension. This is explained as follows:—If the particles of the surface are charged they repel each other owing to the mutual repulsions of similarly charged bodies. This repulsion counteracts the attraction between the molecules, hence the surface tension is less.

The electrical charge on the surface also affects adsorption

because the charge on the adsorbed substance will either increase or decrease the adsorption, depending on whether the charge on the adsorbing surface is of opposite or of the same sign as the adsorbed material.

The less the surface tension the easier it is to produce an increase in the extent of surface. For instance solutions with a low surface tension, such as soap solution, can easily be made to froth. Hamburger * finds that substances which decrease the surface tension increase the phagocytic power of leucocytes.

When the surface tension is reduced to zero the solutions mix and the surface between them becomes irregular as the liquids diffuse into each other.

**TWO IMMISCIBLE SOLUTIONS**

In speaking about osmotic pressure we mentioned a semi-permeable membrane, that is one which permits the passage through it of the solvent but not of the solute. As cells are frequently said to be surrounded by a semi-permeable membrane we must examine some of the phenomena of membranes.

Before dealing with membranes, however, we must consider the case in which we have two immiscible liquids with a surface of separation. Water and phenol below 68.4°C. will not mix in all proportions but form two solutions, one of phenol in water and the other of water in phenol. As these are in equilibrium the vapour pressure of water must be the same in each. On adding some substance which dissolves in water and not in phenol the vapour pressure of the watery phase is decreased and water passes out of the phenolic phase until equilibrium is once more established; therefore the volume of the phenolic phase is decreased. Further equimolecular solutions will cause equal changes in vapour pressure and therefore equal degrees of shrinking.

A salt the ions of which differ in solubility in the two phases will be distributed so that the phase in which the positive ion is more soluble will be positively charged and the other phase in which the negative ion is more soluble will be negatively charged, therefore there will be an electrical potential at the surface of separation.

**Membranes**

When two liquids or two portions of the same liquid are demarcated by a solid material we say that the phases are

separated by a membrane. In this case the behaviour of the two liquids is complicated by the nature of the membrane. If all the substances can pass through the membrane the behaviour is the same as it would be without the membrane. A difference in vapour pressure (concentration) on the two sides of the membrane will produce a temporary osmotic pressure which falls again when the materials are equally distributed.

If, on the other hand, a substance is present which will not pass through the membrane a permanent osmotic pressure is produced.

The permeability of a membrane is determined firstly by the solubility of a substance in it, secondly by the molecular size of the substance, and thirdly it is said that the electrical charge on the membrane is also important. If the substance is not soluble in the membrane it cannot pass through; thus a rubber membrane is permeable to ether but not to water. The molecular weight also has some influence as the substances with high molecular weight do not readily pass through membranes.

The influence of the electrical charge on the membrane is probably unimportant. It is stated that a positively charged membrane will repel positively charged ions. The negatively charged ions will, however, be attracted and as they cannot escape at the opposite side owing to the electrical attraction between the positively charged membrane and negatively charged ion, the charge on the membrane will be neutralised. After the charge on the membrane is neutralised positive ions will pass through as easily as negative ones, thus it follows that the charge on the membrane cannot control the passage of materials through it.

**Colloids**

The substances which do not pass through ordinary membranes such as parchment or parchment paper have been termed colloids because of their similarity to glue,* whilst substances that do diffuse through membranes are called crystalloids.

There seems to be every stage between a liquid containing visible particles in suspension and a true solution. The colloidal solutions are those that present properties intermediate between microscopic particles and true solution. A

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FIG. 10.—Illuminated field as seen in ultramicroscope

The rays converge to a focus in the centre and then diverge again, \( a \) and \( b \). Note that the greatest number of particles is rendered visible in the most brightly illuminated spot \( c \). This is due to the fact that the more intense the illumination the smaller are the particles that it is possible to observe. The particles which are too small to be seen outside the focus of the beam are obvious under the more brilliant light at this focus (Zsigmondy).

*From "Principles of General Physiology," W. M. Bayliss (Longmans).*
colloidal solution may be regarded as a solution containing a solute of high molecular weight, or an aggregate of molecules, so that each particle represents a large total molecular weight or, on the other hand, a colloidal solution may be regarded as a suspension of one phase in another so that there are surfaces of separation and the phenomena of surface tension hold at these interfaces.

The ultra-microscope shows that colloidal solutions consist

Fig. 9.—Diagram of the course of the rays of light in the ultramicroscope.

From “Principles of General Physiology.” W. M. Bayliss (Longmans).

of particulate matter. This instrument makes use of the Tyndall phenomenon that a bright beam of light causes diffraction haloes around small particles so that viewed at right angles to the beam of light objects too small to be seen by the eye can be detected. By using a microscope at right angles to the beam of light smaller diffraction haloes can be detected, the particles that cause them not being visible under a microscope.*

The suspension of one phase in another is spoken of as a

disperse system, therefore we can regard the colloidal solution as a disperse system with surfaces of separation between the particles and the solution. The suspended phase is spoken of as the disperse phase.

"SOLS" AND "GELS"

Colloids can exist in solution as liquids, when they are called sols, or as jellies, when they are called gels. Sometimes a gel can be turned into a sol, for example gelatine, and such a colloid is called a reversible gel, this is called solation. Some gels cannot be turned into sols; these are called irreversible gels. The reverse process of turning a sol into a gel is called gelation or coagulation.

We have already mentioned that colloids will not diffuse through membranes. The other characteristics of colloids are their viscosity and the ease with which they are precipitated from their solution.

**Viscosity of Colloids**

The high viscosity of colloids can be explained by the hypothesis of Hatschek * that when the suspended particles occupy a large volume of the solution they come into contact and the viscosity is raised by the force required to overcome the friction of the particles against each other.

If this be so we must believe that the influence of certain reagents on the disperse phase causes a swelling of that phase. For instance, Pauli and Handovsky have found that acid or alkali added to protein solution up to a certain concentration causes an increase in viscosity, thus indicating that the acid or alkali causes the protein phase to absorb more

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* E. Hatschek, _Zeit. f. chem. u. industrie d. koll._, 1911, vol. 8, p. 34.
water, therefore the total volume of the disperse phase is increased.*

The protein solution probably consists of two phases, one a dilute solution of protein in water and the other a concentrated solution of protein in a little water. It is an increased osmotic attraction by the protein for water that causes the concentrated protein phase to increase in volume.†

**Precipitation of Colloids**

The precipitation of colloids depends on at least two factors. (1) The surface tension which tends to cause them to run together because larger particles have relatively less surface than smaller particles. (2) The electrical charge on the particles which tends to keep them separated. The less the electrical charge the more easily the particles run together.‡

The cause of the electrical charge on suspended particles cannot be explained in all cases. In some cases it appears to depend on the chemical nature of the colloid; thus ferric hydroxide (basic) has a positive charge and silicic acid (acidic) has a negative charge. By decreasing the electrical charge the particles run together more easily, therefore oppositely charged colloids precipitate each other.

The influence of electrical charges in precipitating colloids is well shown by the action of monovalent, divalent and trivalent ions. A negatively-charged colloid is precipitated by positive ions in the ratio of the molecular concentrations of \( x \sqrt{x} \) \( \sqrt{x} \) of mono- di- and tri-valent ions respectively. This ratio is explicable by the kinetic theory in this way. To furnish two positive charges it requires two monovalent ions and only one divalent ion. The probability that one divalent ion will come into contact with a colloidal particle is proportional to the reciprocal of the molecular concentration, that is \( \frac{1}{x} \), whilst for two monovalent ions the probability is

\[
\frac{1}{x} \times \frac{1}{x} = \frac{1}{x^2}
\]

Similarly the probability that three positive charges

\[
\frac{1}{x} \times \frac{1}{x} \times \frac{1}{x} = \frac{1}{x^3}
\]


The total surface tension of both sides of the boundary must be positive, otherwise the phases will mix.
will come into contact with a colloidal particle is \( \frac{1}{x} \) for trivalent ions and \( \frac{1}{x^3} \) for monovalent ions. Therefore to have equal effects the monovalent ions must be present in proportion to the square of the divalent ions and to the cube of trivalent ions which is expressed by the ratio \( x : \sqrt{x} : \sqrt[3]{x} \).

**Cataphoresis**

Owing to the electrical charge colloidal particles, placed in an electrical field, travel to either one pole or the other. This movement is spoken of as cataphoresis.

Proteins behave in a peculiar manner. In alkaline solution they behave like negatively charged colloids and in acid solution like positively charged colloids. They behave in fact like colloidal amino acids. The amino acids can act either as acids by combining with bases, in which case the amino acid exists as a negatively charged ion or as bases by combining with acids, in which case the amino acid exists as a positively charged ion.

According to the Law of Mass Action these two conditions are represented by the equations

\[
K_a = \frac{C_H \times C_x}{C_{1-x}} \quad \text{and} \quad K_b = \frac{C_{OH} \times C_x}{C_{1-x}}
\]

where \( K_a \) is the dissociation constant of the amino acid acting as an acid and \( K_b \) that of the amino acid acting as a base and \( C_H, C_{OH}, C_x, \) and \( C_{1-x} \) are the concentrations of hydrogen ion, hydroxyl ion, amino acid ion and unionised amino acid respectively.

**The Isoelectric Point**

The least amount of dissociation occurs near the neutral point either slightly to the acid or basic side. At this point there is the least amount of movement in the electrical field, and it is spoken of as the isoelectric point.* The isoelectric point is determined by the ratio \( K_a / K_b \) so that by adjusting the hydrogen ion concentration to the isoelectric point the minimal ionisation of the protein can be obtained.

The isoelectric point is said to be the point at which proteins are most easily precipitated from their solutions† but Walpole finds that this does not always hold.‡

The isoelectric point is also the point of minimum viscosity* and of the minimum osmotic pressure, † both of which probably depend upon the least amount of ionically dissociated protein. 

The classification of colloids and their properties are summarised in the following table.

| TABLE IX |
|-----------------|-----------------|
| **EMULSOID.**   | **SUSPENSOID.** |
| Like emulsions these consist of liquid particles in the disperse phase. | Like suspensions these consist of solid particles in the disperse phase. |
| They are lyophile, that is, they have an affinity for the solvent. | They are lyophobe, that is, they do not seem to have an affinity for the solvent. |
| The solution is less than the combined volume of solute and solvent, that is, contraction takes place. | The solution is the sum of the volumes of the solute and solvent. |
| Surface tension is less than that of the solvent. | Surface tension practically the same as that of the solvent. |
| The viscosity is greater than that of the solvent. | The viscosity is almost the same as that of the solvent. |
| The solutions give good osmotic pressures. | The solutions give very little or no osmotic pressure. |
| They are in many ways like true solutions, being, for instance, less readily precipitated by salts. | They are much less like true solutions, being, for instance, very easily precipitated by salts. |

**Protective Effect of Emulsoïd Colloids on Precipitation of Suspensoid Colloids**

Emulsoïd colloids are not only less easily precipitated by salts but they hinder the precipitation of suspensoid colloids. This protective influence is measured by its influence in preventing the precipitation of a gold suspensoid and is expressed as the gold value.‡

**Size of Colloidal Particles**

The size of colloidal particles has been determined by centrifugalisation§ and by ultrafiltration||. The latter consists of forcing the solution through filters of different degrees of fineness so that only particles less than a certain size can pass through the filter. The colloidal particles vary in size from 6 μm to 250 μm or 6 to 250 mm. × 10⁻⁶.

* W. Pauli and H. Handovsky, loc. cit.
Limitation of diffusion of colloids by membranes may lead to electrical changes. If the colloid is one that forms salts and one of the ions is diffusible through membranes the partial separation of the diffusible from the non-diffusible ion causes an electrical potential in the same way that differences in the solubility of ions in two immiscible liquids causes an electrical potential.

The ion that tends to pass through the membrane confers a charge of the same sign as itself on the outside of the membrane, whilst the inner side of the membrane possesses a charge corresponding to the colloidal ion.

The two ions are kept, however, from separating by their opposite charges, and unless some secondary process, such as hydrolysis, occurs the separation of ions is infinitesimal; the electrical potential is a stress and only a minimal amount of separation occurs.*

**Adsorption**

Owing to the large amount of surface possessed by colloidal solutions condensation on surfaces by surface tension effects become of considerable magnitude. This process of adsorption can be expressed by a formula $\frac{x}{m} = ac^n$ where $x$ is the amount adsorbed by the surface $m$, from a solution whose final concentration is $c$, $a$ and $\frac{1}{n}$ being constants for a particular surface and solution.

The importance of the points mentioned in this chapter consists in the fact that cells have surfaces, therefore surface phenomena must be considered in biological processes. Moreover cells contain colloidal substances; the behaviour of colloids has therefore an important bearing on the behaviour of cells.

The phenomena that occur across surfaces of separation are unequal distribution of substances, electrical potential and surface tension. The last of these can be used to produce mechanical effects by which chemical energy may be transformed into movement.

**GENERAL REFERENCES**


CHAPTER IV

CATALYSIS AND ENZYME ACTION

Chemical reactions brought about by living cells are characterised by the ease with which apparently inert substances are altered. Reactions which usually require strong acids or alkalis and prolonged heating are produced rapidly by extracts obtained from living tissues. Hence we must devote further attention to the means by which the rate of chemical reactions can be affected.

In studying the rate of chemical change we saw that the rate of change depended upon the concentration of the reacting substances and on the velocity constant, $k$. Thus the equation, \[ \frac{dx}{dt} = kC_1C_2, \] where $k$ = the velocity constant and $C_1$ and $C_2$ the concentrations of the reacting substances, indicates that if the concentration of the reacting substances is the same, a change in the velocity constant, $k$, will cause a change in the rate of reaction. Such an alteration in rate is known as a catalytic action and the change may be either an increase (positive catalysis) or a decrease (negative catalysis) in the rate of reaction.

A catalyst does not add to or subtract energy from the reacting system, thus as the equilibrium constant $K$ of a reversible reaction is not altered* the rate of the reverse change must be equally accelerated. This is shown by the equations on p. 37 where $K = \frac{k'}{k''}$ and if $K$ remains the same and $k'$ is altered $k''$ must be altered to the same extent.

In the above the assumption is made that all reactions that can be brought about by catalytic activity are proceeding in the absence of a catalyst, but, in some cases, infinitely slowly. This assumption is not universally granted as some workers believe that the enzyme is capable of a trigger-like action in releasing a chemical change which will not occur until the

* The catalyst may unite with one of the reacting substances and by altering the active mass cause a slight change in the equilibrium conditions.
catalyst is added. Bayliss has compared these two views by means of a mechanical simile. A weight placed on an inclined plane may move slowly downwards or remain at rest. On adding oil the slowly-moving weight moves more quickly and the weight that was at rest may commence to move. The catalyst may be compared to the oil and it may be said to decrease the resistance of the reaction.

ENZYMES

It is generally assumed that most chemical transformations in cells are controlled by enzymes, and as enzymes seem to be present in all cells the study of enzymes is of immense importance in Biological Chemistry. In what follows the enzymes will be discussed on the assumption that they act as catalysts by increasing the rate of chemical reactions.

The chemical composition of the various enzymes is unknown and we can demonstrate the presence of an enzyme only by its effect on the rate of reaction. The nomenclature of enzymes depends upon the kind of chemical change that is accelerated. Certain names which were introduced in the early period of enzyme studies are so well known in the literature that they are retained, but now names are made by adding the suffix -ase to the name of the substance acted upon (substrate). This method does not give any indication of the nature of the chemical change which is accelerated, but this disadvantage is not great as the chemical nature of the substrate usually indicates the nature of the reaction; in some cases the ending -ase is qualified by some other term.

The enzymes show several well marked groups. The first group consists of those which act by adding a molecule of water with the decomposition of the substrate into two substances. This is the group of the hydrolytic enzymes. The changes produced involve very little energy change, hence they are easily reversible and these are the enzymes that are used as a preliminary to the transference of material from one place to another, e.g. in the process of digestion.

The hydrolytic enzymes can be subdivided according to the nature of the materials upon which they act; for example, amyloclastic or those which act upon starch, steatoclastic or those which act upon fats and proteoclastic or those which act upon proteins.*

The second group is that of the oxidising enzymes which act by accelerating the rate of oxidation of the substrate. This type of reaction is accompanied by energy changes and these

enzymes are associated with energy transformations in living cells.

Owing to the energy changes the reversible action of oxidising enzymes is not easy to demonstrate. To illustrate this point we can refer to the union of hydrogen and oxygen to form water. At ordinary temperatures hydrogen and oxygen unite very slowly, but if a catalyst (spongy platinum) is present the union occurs with the liberation of much energy in the form of heat. It is obvious that if the action is to be reversed that energy must be supplied, and we can supply this energy by heating the water vapour produced by the first action. This is expressed by Le Chatelier's Theorem, which in its broadest form states that if one of the factors determining the equilibrium of a system is changed the change that takes place in the system is such as would tend to annul the alteration in the factor.

Union of hydrogen and oxygen to form water liberates heat, therefore decomposition of water into hydrogen and oxygen absorbs heat. Thus a rise in temperature tends to be annulled by the decomposition of water vapour.*

At 1000° C. and atmospheric pressure water vapour is \(3 \times 10^{-5}\) per cent. dissociated and at 2500° C. and the same pressure the dissociation is 3.98 per cent., or the dissociation is increased one hundred thousand times by a rise of temperature from 1000°—2500° C.† We thus see that if we wish to reverse the action of an oxidising enzyme we must furnish a supply of energy. In the case of what are called reducing enzymes the reversible reaction is produced by a suitable arrangement so that the energy of one reaction is made to carry out another (linked reactions).

We also have a miscellaneous group of enzymes, some of which are hydrolytic and others oxidising. They carry out such reactions as deamidisation and the conversion of \(\alpha\)-ketonic aldehydes into \(\alpha\)-hydroxy acids. They are kept in a separate group as their main interest is independent of their hydrolytic or oxidising activity.

As the study of enzymes deals mainly with the effect of various conditions on their activity we must first of all consider the effect of various conditions on enzymes. The description will refer in the first instance to hydrolytic enzymes and be extended to the other forms afterwards.

BIOLOGICAL CHEMISTRY

INFLUENCE OF TEMPERATURE

The rate of most chemical reactions increases with rise of temperature and the increase is an exponential function of the temperature; that is if we plot the rate of reaction against the temperature we obtain a curve as shown in the diagram which is convex towards the axis of the ordinates. This means that the rate of reaction becomes of explosive rapidity at high temperatures. The usual way to express the increase in rate is the temperature coefficient of Arrhenius which signifies the ratio of the two rates at temperatures ten degrees Centigrade apart.* Most chemical reactions have a coefficient between two and three at moderate temperatures and physical processes have a coefficient outside this range. The diagram gives two curves showing temperature coefficients of two and of three.

For a temperature interval of ten degrees the rate is doubled or trebled, for twenty degrees it is four or nine times as fast, and for thirty degrees eight and twenty-seven times as fast.

**FIG. 13.** Effect of temperature on rate of reaction (carbon assimilation of cherry laurel leaf).

The increase of rate is shown by the dotted line. This is calculated from the rate of assimilation at 9° and 19°C, 3.8 and 8°o mg. respectively, a coefficient of 2.1 for ten degrees Centigrade.

The full lines show the falling off in assimilation with maintained temperatures of 30'5°, 37'5° and 40'5° C. These are plotted with the baseline as representing times, so that extrapolated to zero time the curves cut the dotted curve. This diagram illustrates the falling off inactivity at higher temperatures due to the destructive action of high temperatures on the tissues of the leaf.

respectively. In agreement with this rule enzyme action shows a temperature coefficient between two and three at moderate temperatures.

At higher temperatures a complicating factor occurs. Enzymes are destroyed at high temperatures and it is one of the criteria of an enzyme that its action is abolished by heating to 100° C. The destruction commences at a lower temperature and the rate of destruction increases as the temperature rises. In order to show the activity of an enzyme it is necessary for the enzyme to act upon the substrate for a definite time. For this reason we introduce what is termed the time factor.*

Thus the rate of reaction increases as the temperature rises, and if we could measure the rate of reaction instantaneously it should continue rising, but at higher temperatures the rate of destruction becomes so great that the enzyme is destroyed before it can produce any measurable change in the substrate. We therefore find that there is a range of temperature at which the enzyme is most efficient. This temperature is spoken of as the “optimum” temperature. The shorter the time interval of the measurement the higher is the optimum temperature as the increased activity due to rise of temperatures occurs and owing to the shorter time there is less destruction of the enzyme.†

INFLUENCE OF ACID AND ALKALI

Enzymes are extremely sensitive to the presence of acid and alkali. Some act best in acid; others in alkali and others still near the neutral point. During certain enzyme actions the concentration of hydrogen ions tends to change owing to the formation of substances which can neutralise acid or alkali. For this reason recent experiments have been carried out using stabilising solutions.‡ In this way the reaction is steadied and the results easier of interpretation. Without the use of the stabiliser the reaction changes all the time, hence the effect of acid and alkali is less clear.

† The rate of destruction may have a very high temperature coefficient, hence the optimum temperature would not be much affected by a shorter time interval. Compare H. Chick and C. J. Martin. Journ. Physiol., 1910, vol. 40, p. 404.
‡ In a previous chapter the real acidity has been shown to be the hydrogen ion concentration, so we will here deal with the effect of the concentration of hydrogen ions on enzyme action. The symbol \(-\log [H^-]\) is sometimes used to express the negative exponent of the logarithm to the base 10 of the concentration of hydrogen ions.
Proteoclastic enzymes can be divided into three groups according to their behaviour at different concentrations of hydrogen ions. First there is the group of pepsin-like enzymes which act best in a decidedly acid medium \((C_H \approx 10^{-2})\) and are destroyed by weak alkali. Next there is the group of trypsin-like enzymes which act best in an alkaline medium \((C_H \approx 10^{-9})\); finally there is the group of erepsin-like enzymes which act best near the neutral point \((C_H \approx 10^{-7})\).

All enzymes are destroyed by marked acidity or alkalinity. Associated with these relations to the concentration of hydrogen ions are differences in the substances attacked and the products produced by their activity. The latter we shall defer to a later chapter (p. 120).

Proteoclastic enzymes are not the only ones affected by varying reaction. Other enzymes show an optimum activity at definite concentrations of hydrogen ions near the neutral point but the range of their action is not so extended.

The influence of acidity and alkali on enzymes has been attributed to a reaction with the enzyme. Presuming that the enzyme is an acid, a base, or an amphoteric substance we can trace out varying relations between the enzyme and the concentration of hydrogen ions. Suppose that the enzyme is active only in the form of an ion, then at some point near the neutral point, the isoelectric point of an amphoteric substance, the minimum amount of ions will be present and the activity least.*

Trypsin, for instance, has been stated to behave like a dibasic acid.

To make the matter clearer we can refer to the dissociation of the known dibasic acid, carbonic acid. This according to the law of mass action occurs in two stages as shown by the following equations:

\[ K_1 \frac{C_{\text{H}_2\text{CO}_3}}{C_{\text{H}^+}C_{\text{HCO}_3^-}} \text{ and } K_2 \frac{C_{\text{HCO}_3^-}}{C_{\text{H}^+}C_{\text{CO}_3^-}} \]

in which \( K_1 = 3.04 \times 10^{-7} \) and \( K_2 = 1.291 \times 10^{-11}.\)

We see that the following molecular and ionic species occur, hydrogen ion, undissociated carbonic acid, bicarbonate ion, and carbonate ion, and in the following table are given the amounts of these substances when the total amount of carbonic acid is equal to unity.

TABLE X.

Products of Dissociation of $H_2CO_3$.

<table>
<thead>
<tr>
<th>$C_H$</th>
<th>$pH$ or $-\log [H^+]$</th>
<th>$C_{H_2CO_3}$</th>
<th>$C_{HCO_3}$</th>
<th>$C_{CO_3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-5}$</td>
<td>5</td>
<td>0.9704</td>
<td>0.0296</td>
<td>$-$</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>6</td>
<td>0.768</td>
<td>0.232</td>
<td>$-$</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>7</td>
<td>0.248</td>
<td>0.752</td>
<td>$-$</td>
</tr>
<tr>
<td>$10^{-9}$</td>
<td>9</td>
<td>0.005</td>
<td>0.985</td>
<td>0.01</td>
</tr>
<tr>
<td>$10^{-11}$</td>
<td>11</td>
<td>0.003</td>
<td>0.437</td>
<td>0.56</td>
</tr>
<tr>
<td>$10^{-12}$</td>
<td>12</td>
<td>0.002</td>
<td>0.075</td>
<td>0.923</td>
</tr>
</tbody>
</table>

From the above table we see that if the active portion were the undissociated acid the greatest activity would be in acid solution ($C_H = 10^{-5}$ or more); if the active portion were the first dissociation product, corresponding to $HCO_3^-$, the greatest activity could be slightly on the alkaline side of the neutral point ($C_H = 10^{-9}$) and if the active portion were the second dissociation product, corresponding to $CO_3^{2-}$, the greatest activity would be in alkaline solution ($C_H = 10^{-12}$ or less).

If the action of trypsin depended upon the first dissociation product, corresponding to the $HCO_3^-$ ion, we see that the activity of trypsin would increase, as the alkalinity increases, up to a certain alkalinity and then decrease as the second dissociation, corresponding to the formation of the $CO_3^{2-}$ ion, becomes more important.*

Invertase acts like an amphoteric electrolyte with an acid dissociation constant of $10^{-6.7}$ and a basic dissociation constant of about $10^{-12}$. The hydrolysis of sugar is said to be due to the undissociated invertase hence the optimum activity corresponds to the isoelectric point (or zone in this case). The negative ion is inactive; the positive ion is also inactive and is in addition easily destroyed.†

Other enzymes have been studied in a similar way and deductions have been made as to the nature of the active portion. It is interesting to have some method of explaining the influence of hydrogen ion concentration on enzyme activity and also on enzyme destruction.

INFLUENCE OF THE CONCENTRATION OF THE ENZYME

We commenced this chapter by stating that the enzyme may be said to alter the constant $k$ in the velocity equation. We have already seen that the rate of enzyme action is affected by the temperature and acidity of the medium in

* S. P. L. Sørensen, loc. cit. p. 463. It is possible that the decrease in activity might be due to a decrease in ionisation owing to the excess of base decreasing the ionisation of the salt (see p. 36).
† S. P. L. Sørensen, loc. cit. p. 460.
which it acts. Therefore in studying the effect of the concentration of the enzyme on the rate of reaction all other factors must be kept uniform.

Let us introduce some factor $\varphi$ into the law of mass action to represent the amount of active enzyme in the solution.

Thus \[ \frac{dx}{dt} = kC_x \] becomes \[ \frac{dx}{dt} = k'\varphi C_x. \] We see that there are two extreme cases. The first in which $\varphi$ is very small, therefore the enzyme causes a slow change of concentration of $x$. Thus $C_x$ is almost constant, and the rate of change will be proportional to the amount of enzyme added. The second case is that in which $\varphi$ is relatively large, whence the concentration of $x$ changes rapidly. The rapid alteration in $C_x$ will be the predominant factor, and the rate of reaction with relatively large amounts of enzyme will be apparently independent of the amount of enzyme added.

Between these two extremes we find that the rate of change depends partly on the amount of enzyme and partly on the concentration of the substrate. These various relations may exist in the same experiment. In the early stages the rate of reaction may be proportional to the amount of enzyme: as the concentration of the substrate diminishes the rate of reaction is dependent on both the concentration of the enzyme and the substrate. Lastly, in the final stages the rate of reaction may become independent of the amount of enzyme added.

Moore has worked out an equation which covers all possible relations of enzyme to substrate, and also takes into account the reverse reaction.

As shown in the preceding section $\varphi$ will depend on the condition of the enzyme, i.e. the acidity or alkalinity of the solution will produce more or less active enzyme from the same amount of added enzyme.

**Specific Relation Between Enzyme and Substrate**

Unlike many other catalysts the enzymes show a capriciousness which is sometimes extremely surprising. Hot acid or hot alkali can hydrolyse all the substances that can be hydrolysed by enzymes, and the products of the reaction are the simplest compounds possible. The enzymes, on the other hand, act only on certain definite substances and the products of the reaction are often only stages in the complete hydrolysis.

For instance, acids hydrolyse starch, glycogen, maltose, lactose, cane sugar, proteins, etc., but separate enzymes are
required for most of these substances. The enzyme which hydrolyses starch and glycogen does not hydrolyse the other substances. Different enzymes are required for each of maltose, lactose and cane sugar.

A careful examination of the various substances acted on by enzymes shows a definite relation between the structural formulæ of the substances attacked by the same enzyme. The analogy has been made that the enzyme fits the substrate like a key fits a lock,* or as a hand fits a glove.†

One example will suffice. The glucosides can be divided into two groups, according to whether they have an α or β configuration (p. 11).

The enzymes that hydrolyse the α forms will not attack the β forms and the converse.‡

<table>
<thead>
<tr>
<th>Glucoside</th>
<th>Maltase α-Glucose</th>
<th>Emulsin β-Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Methyl-α-Glucoside</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>β-Methyl-α-Glucoside</td>
<td>o</td>
<td>+</td>
</tr>
<tr>
<td>α-Methyl-β-Glucoside</td>
<td></td>
<td>o</td>
</tr>
<tr>
<td>β-Methyl-β-Glucoside</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Ethyl-α-Glucoside</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>β-Ethyl-α-Glucoside</td>
<td></td>
<td>o</td>
</tr>
<tr>
<td>β-Phenol-α-Glucoside</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Methyl-α-Galactoside</td>
<td>o</td>
<td></td>
</tr>
<tr>
<td>β-Methyl-α-Galactoside</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl-α-Mannoside</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl-β-Mannoside</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Methyl-α-Xyloside</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Methyl-α-Xyloside</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl-α-Arabinoside</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl rhamnoside</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl glucopentoside</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A similar relation is found between the nature of the amino acid linkages in protein and the enzymes that loosen these linkages.

The specific relationship of enzymes is frequently used for analytical purposes, and examples will be given later (see p. 182).

**MODE OF ACTION OF ENZYMES**

There are two views as to the manner in which enzymes produce acceleration of the reaction. One is that the enzyme

forms an intermediate compound with the substrate, and that this breaks down with the formation of the products of the reaction and the setting free of the enzyme. The other is that the enzymes are colloids and the reacting substances are condensed on their surface: the rate of reaction is increased owing to the increased concentration of the reacting substances.

Formation of Intermediate Compounds.—In order that the reaction velocity may be increased it is necessary that the combined times for the formation of the intermediate compound and for its destruction must be less than the time for the reaction without the formation of an intermediate compound.

The usual example of catalysis, by means of the formation of an intermediate compound is the oxidation of sulphur dioxide to sulphur trioxide by means of nitrogen peroxide. The nitrogen peroxide thus acting as an oxygen carrier in the same way that a hydrolytic enzyme may be considered to act as a water carrier. The nitrogen peroxide forms a compound with the sulphur dioxide; this decomposes, forming nitric oxide and sulphur trioxide; then the nitric oxide takes up oxygen from the air and nitrogen peroxide is again formed. This process can go on indefinitely, so that a small amount of nitrogen peroxide can transform a large amount of sulphur dioxide and the nitrogen peroxide is found unchanged at the end of the experiment. The following equations illustrate the reaction.

\[
\begin{align*}
SO_2 + NO_2 & = SO_3 + NO \\
2NO + O_2 & = 2NO_2
\end{align*}
\]

We have seen that according to the Law of Mass Action the accumulation of the products of the reaction decreases the rate of the reaction owing to the reverse reaction becoming more rapid. There is another way in which the rate of reaction can be diminished by the product of the reaction. If the enzyme action, as stated previously, is related to the structural formula of the substrate, the amount of enzyme united with the various substances in the solution will depend on the similarity of their structural formulae to that portion of the substrate to which the enzyme becomes attached. The structural formula of one more than another of the products of the reaction may be similar to spacial relations of the atoms in that portion of the substrate which is related to the enzyme, hence the enzyme will unite with this substance more readily than with the other products of the reaction. Such substances, by removing the enzyme from the solution, will have a more
marked retarding influence than the other products of reaction. The following table shows that such does occur.*

**Table XII**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Hydrolyte</th>
<th>Effect of Hexose on Rate of Change.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose.</td>
</tr>
<tr>
<td>Lactase</td>
<td>β-Galactosides, e.g. Lactose</td>
<td>No influence</td>
</tr>
<tr>
<td>Emulsin</td>
<td>β-Glucosides, e.g. most natural glucosides</td>
<td>Retards</td>
</tr>
<tr>
<td>Maltase</td>
<td>α-Glucosides, e.g. Maltose</td>
<td>Retards</td>
</tr>
<tr>
<td>Invertase</td>
<td>Fructosides, e.g. Sucrose</td>
<td>No influence</td>
</tr>
</tbody>
</table>

The combination of enzyme with the substrate explains the relation of the concentration of enzyme and substrate (p. 65). When the enzyme is present in excess the amount of combination will depend on the concentration of the substrate, but when the substrate is present in excess the amount of combination will depend on the concentration of the enzyme.

**Surface Condensation**

It is well known that finely divided platinum condenses hydrogen, so that one volume of platinum absorbs many volumes of hydrogen. The concentration of hydrogen must therefore be equivalent to that of many atmospheres. Under the influence of platinum hydrogen unites with oxygen, even at moderate temperatures, so rapidly that the platinum soon becomes heated to a red heat.

If the enzyme causes a similar concentration of substrate and water the rate of reaction may be considerably increased. The increase in the rate of reaction will depend upon the degree of concentration on the surface of the enzyme.

The extent of surface on which condensation occurs will be the factor that regulates the effect of concentration of the enzyme on the rate of catalysis.

The enzymes form colloidal solutions, thus the extent of their surface is very great. In illustration of this we can quote the following:—“A sphere of gold one-tenth of a

centimetre radius has a surface of 0.126 sq. cm., while the surface of the same mass, if subdivided to the above colloidal dimensions, would have a surface of about 100 sq. m., or be multiplied by ten millions.”*

At present we cannot definitely decide between the above hypotheses. The specific relation of the enzyme to the substrate and the specific inhibiting effect of some of the products of reaction seem to point to the formation of intermediate compounds. On the other hand, it is said that “specific adsorption” can occur, but the nature of this “specific adsorption” is difficult to understand. Electrical effects can act only when the particles are positively or negatively charged. Possibly the “specific adsorption” is a link between the purely physical adsorption and chemical combination as specific relations are more in the nature of chemical reactions.

The two views may be united by saying that the adsorption brings the substances together, and then some loose chemical union occurs which breaks down with the formation of the products of reaction.

**DIRECTIVE ACTION OF ENZYMES**

In many cases a chemical substance may be altered by several different paths giving rise to different end products. In organic synthesis the methods chosen are those which give the greatest yield of the desired product. The acceleration of one reaction will produce a greater yield of the product formed by that reaction, hence enzymes are useful for the formation and isolation of some substances.

**CO-ENZYMES**

Enzymes are sometimes said to be composed of two substances, enzyme and co-enzyme. Hydrochloric acid is stated to be the co-enzyme for pepsin. The discussion on p. 63, in which the possibility is pointed out that alkali increases the action of trypsin by producing an active ion, is applicable to the case of hydrochloric acid and pepsin.

Lipase, or fat splitting enzyme, is composed of two parts, one soluble in water and passing through filter paper, the other removed by filtration. Both are present in the crude extract obtained by treating an organ with glycerine.†

Oxidising Enzymes

are divided into three classes. Peroxidases, which accelerate oxidation in the presence of hydrogen peroxide. Oxidases, which accelerate oxidation in the presence of molecular oxygen. Catalase, which accelerates the decomposition of hydrogen peroxide.

Peroxidases

are widely distributed throughout the animal and vegetable kingdoms. They are classified according to the nature of the substance that they oxidise into polyphenol, tyrosin, alcohol, uric acid, etc., peroxidases. These names indicate the substances that are used in vitro, as indicators for the presence of these peroxidases.

Oxidases

are not so widely distributed as the peroxidases. They are less stable, and it is claimed that they are really a mixture of a stable peroxidase with an unstable substance called oxygenase. The oxygenase is capable of taking up molecular oxygen with the formation of a peroxide-like substance. Iron, manganese, etc., have been found associated with oxidases, and they may be the peroxide forming substance, but in some cases the peroxide forming substance is probably of organic nature; for instance something allied to catechol.*

Catalases

are not true oxidising enzymes, as they decompose hydrogen peroxide with the liberation of molecular oxygen. This process does not accelerate oxidation, and it is believed that the catalase is a protective mechanism to prevent the poisonous action of hydrogen peroxide on protoplasm. It may be that the decomposition of hydrogen peroxide is merely a surface effect of the colloids in the tissues and fluids as even the inorganic colloids accelerate the decomposition of hydrogen peroxide.

Reducing Enzymes

have been described. These cannot be enzymes in the sense of catalytic agents as described in this chapter, because they would then necessarily be oxidising enzymes as well. Oxidation leads to the liberation of energy, but reduction causes an accumulation of energy. Therefore reduction can take place only when energy is supplied from some other source. The so-called reducing enzymes may be oxidising enzymes that accelerate oxidation, but take the oxygen from

some substance which serves as an indicator and thus they appear to be reducing enzymes.

**Deamidising Enzymes**

The removal of NH$_2$ groups from amino acids can be brought about by enzymes. The removal of NH$_2$ groups may be the result of hydrolysis or of oxidation, so we shall not deal with these further as they would come under the hydrolytic or oxidising enzymes already described. Dakin states that the usual fate of amino acids is that they are oxidised to $\alpha$-ketonic acids.*

**Anti-Enzymes**

These are substances that paralyse the action of enzymes, thus preventing their action.

**Coagulating Enzymes**

*Coagulation of Milk.*—Milk when treated with a neutral extract of the stomach of a calf sets to a solid mass which is termed junket. After some hours the junket separates into a liquid called whey, and a solid portion which forms cheese.

The process that takes place is the conversion of the soluble protein caseinogen into insoluble casein by the action of the enzyme rennin. The casein entangles the fat of the milk so that cheese consists of fat and protein.

Coagulation of milk requires the presence of calcium salts. If all the calcium is removed from milk by the addition of an oxalate the milk will not clot, but it can be shown that the rennin has acted on the caseinogen by boiling the solution to destroy the rennin, and then adding calcium salts when a precipitate of casein results. The process is said to be that rennin converts caseinogen into casein, and casein forms an insoluble precipitate with calcium.

*Coagulation of Blood.*—When blood is shed it becomes viscid, and ultimately sets to a jelly occupying the same volume as the original blood. In the absence of this process of coagulation all surgery would be impossible, as death from haemorrhage would occur as the result of the slightest wound. This process also requires the presence of calcium salts, as the addition of oxalates to blood prevents its coagulation, however we shall see the relation of calcium to blood coagulation is different from its relation to milk coagulation.

The essential change in blood coagulation is the formation of fibrils of a material termed fibrin. These fibrils form a network, which contracts after it is formed. The network, by

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its interlacing, gives the rigidity to the clot, and all the cells of the blood are entangled in the interstices of the network. As the network shrinks the liquid portion is squeezed out, forming serum, and the clot shrinks with its entangled cells.

Fibrin is formed from a protein, fibrinogen, in solution in the blood. Under the influence of an enzyme called thrombin the fibrinogen is converted into fibrils of insoluble fibrin.

Fibrinogen can be prepared from the liquid portion of the blood (plasma) by precipitation with sodium chloride added to half saturation. The serum obtained from clotted blood is plasma minus fibrinogen.

**Table XIII**

*Substances that affect coagulation of the blood.*

<table>
<thead>
<tr>
<th>Coagulation retarded by</th>
<th>Probable mode of action</th>
<th>Corresponding method of hastening coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooling . . . . .</td>
<td>Slows chemical reactions and retards destruction of blood cells . .</td>
<td>Keeping warm at about 40°C.</td>
</tr>
<tr>
<td>Oxalates, citrates, fluorides . . .</td>
<td>Removes calcium from the active system . . .</td>
<td>Calcium salts within limits.</td>
</tr>
<tr>
<td>Leech extract . .</td>
<td>Antithrombin . .</td>
<td>Thrombin such as venom of Echis carinatus.</td>
</tr>
</tbody>
</table>

Excess of neutral salts Inhibits formation of thrombin.

Prevent blood from contact with foreign substances by using paraffined vessels.

Peptone injected into blood but not outside the body . . . . . .

Probably produces an ( ? Injection of gelatine.)

**Milk Coagulation**

Caseinogen

*Calcium ions*

Casein (Soluble)

Calcium Caseate (Insoluble)

**Blood Coagulation.**

Thrombokinase

Calcium ions

Thrombogen

Thrombin

Fibrinogen

Fibrin.
Thrombin is not present in circulating blood, but is found as thrombogen, which can be converted into thrombin. In order to convert thrombogen into thrombin, two substances are required, namely calcium salts and thrombokinase. The calcium is required in blood coagulation for the formation of thrombin, not for the actual clotting as in milk.

Thrombokinase is obtained from tissue cells, white blood corpuscles and platelets, and it is not until these are damaged that coagulation occurs. This is why coagulation does not occur in the blood vessels, but only after the blood is shed, when it comes in contact with tissues, or the contained corpuscles break down.*

In the actual plugging of blood vessels adhesion of blood cells to the edges of the injured vessel helps to stop the bleeding, but that is a process which is outside of the scope of this book.

**Zymoids**

If the enzymes consist of two parts, one which combines with the substrate and the other that produces the reaction, it is possible to stop the reaction by altering the second part and leaving the combining part unchanged. Zymoids are enzymes that have been altered so that not only are they inactive, but they prevent the action of fresh enzyme.† It has been suggested that the zymoids combine with the substrate, thus preventing the enzyme from attacking the substrate, yet do not act upon the substrate itself: in fact they play the rôle of a dog in the manger.

Biologically, enzymes are important, as they enable cells to perform reactions which without the presence of enzymes require agencies which destroy all living structures. Temperature and acidity affect the rate of enzyme action, as do also the presence or absence of co-enzymes or anti-enzymes.

**GENERAL REFERENCES**


CHAPTER V

CONVERSION OF CHEMICAL ENERGY INTO
MECHANICAL ACTIVITY

PART of the energy that is obtained by oxidation of the organic materials described in Chapter I is transformed by the cells into various biological processes. The function of the cell is to act as an energy transformer.*

Many of these transformations cannot yet be given concretely enough for discussion in this book but the simpler transformations of chemical energy into mechanical action can be briefly described.

There are two ways in which mechanical action can be produced. The first is by change in osmotic pressure transferring water from one place to another. The transference of water causes a change in pressure (or volume) with the result that some form of movement takes place. The second method is by a change of surface tension at surfaces of separation so that the surface is pulled on with greater or less force which in turn may produce movement.

Cells are distinctly demarcated from their surroundings, hence one can apply the laws described in Chapters II and III to the osmotic movement of water between the cell and its surrounding medium or to the changes in surface tension between the same two structures.

PHYSICO-CHEMICAL RELATIONS OF CELLS TO THEIR SURROUNDINGS

It is true that the cells themselves and sometimes their surrounding media are heterogeneous systems, but for purposes of description we can simplify the subject by treating them as separate phases, that is we can consider the reactions between the cells and their surroundings as the interchange between two separate fluids.

The points to be considered are four in number:

1. What are the chemical differences between cells and their surroundings?

2. Why do cells not mix with the liquid in contact with them?

3. What exchanges take place between cells and extracellular solutions?

4. How do changes in the surrounding media affect cells?

These problems are of great importance because they apply to all kinds of cells whether they are those of unicellular or multicellular organisms. The difference between unicellular and multicellular organisms is that, as a rule, the media surrounding the former are more variable whilst those surrounding the latter are approximately uniform in composition and there is a circulatory mechanism to renew the solution surrounding the cells.

**Chemical Differences Between Cells and their Surroundings**

The chemical differences between cells and their surroundings vary with the kind of cells and the nature of their habitat. A characteristic difference is that between red blood corpuscles and the plasma in which they float.

**Table XIV**

*Showing Analysis of Horse’s Blood.*

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Contained in 1,000 parts of Serum</th>
<th>Contained in 1,000 parts of Corpuscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>920.5</td>
<td>613.15</td>
</tr>
<tr>
<td>Solids</td>
<td>97.95</td>
<td>386.84</td>
</tr>
<tr>
<td>Proteins (not haemoglobin)</td>
<td>84.24</td>
<td>56.78</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td></td>
<td>315.08</td>
</tr>
<tr>
<td>Sugar</td>
<td>1.176</td>
<td>—</td>
</tr>
<tr>
<td>Cholesterin</td>
<td>0.298</td>
<td>0.388</td>
</tr>
<tr>
<td>Lecithin</td>
<td>1.720</td>
<td>3.973</td>
</tr>
<tr>
<td>Fat</td>
<td>1.300</td>
<td>—</td>
</tr>
<tr>
<td>Phosphorus as nuclein</td>
<td>0.020</td>
<td>0.095</td>
</tr>
<tr>
<td>Sodium</td>
<td>4.434</td>
<td>—</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.263</td>
<td>4.935</td>
</tr>
<tr>
<td>Ferric oxide</td>
<td></td>
<td>1.563</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.1113</td>
<td>—</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.045</td>
<td>0.0809</td>
</tr>
<tr>
<td>Chlorine</td>
<td>3.726</td>
<td>1.949</td>
</tr>
<tr>
<td>Phosphoric Acid</td>
<td>0.240</td>
<td>1.901</td>
</tr>
<tr>
<td>Inorganic Phosphoric Acid</td>
<td>0.0715</td>
<td>1.458</td>
</tr>
</tbody>
</table>

**Table XV**

*Showing the difference between the composition of the unicellular plant Asterionella and the water in which it lives.*

As no analysis is given of the fresh plant it has been assumed that the solid matter forms ten per cent. of the fresh

---

material; it probably forms a higher percentage of the weight than this. If the solids do form a larger proportion than ten per cent. the contrast between the amounts of the various constituents would be more pronounced.

Comparison of Inorganic Constituents of Asterionella and its Surroundings.

<table>
<thead>
<tr>
<th>Asterionella assuming that 90% of the organism is formed of water.</th>
<th>Composition of Brooklyn water.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter</td>
<td>per cent.</td>
</tr>
<tr>
<td>Silica (SiO₂)</td>
<td>4.248</td>
</tr>
<tr>
<td>Iron Oxide (Fe₂O₃)</td>
<td>4.948</td>
</tr>
<tr>
<td>Lime (CaO)</td>
<td>0.232</td>
</tr>
<tr>
<td>Magnesia (MgO)</td>
<td>0.145</td>
</tr>
<tr>
<td>Potash (K₂O)</td>
<td>0.126</td>
</tr>
<tr>
<td>Soda (Na₂O)</td>
<td>0.122</td>
</tr>
<tr>
<td>Manganese Oxide (Mn₂O₃)</td>
<td>0.084</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.067</td>
</tr>
<tr>
<td>Sulphate</td>
<td>0.038</td>
</tr>
<tr>
<td>Per cent.</td>
<td></td>
</tr>
<tr>
<td>0.001516</td>
<td></td>
</tr>
<tr>
<td>0.000034</td>
<td></td>
</tr>
<tr>
<td>0.004770</td>
<td>0.002432</td>
</tr>
<tr>
<td>0.000184</td>
<td></td>
</tr>
<tr>
<td>0.001390</td>
<td></td>
</tr>
</tbody>
</table>

It is interesting that sodium is not mentioned in the analysis of the plant, yet it is more abundant than potassium in the water.

The chemical differences shown in the above tables are characteristic. They are seen to be differences in organic and in inorganic material.

In respect to the organic material the red blood cells are surrounded by a solution containing protein but the plant cells are surrounded by a solution without protein.

The difference in organic compounds between the cells and their surroundings is easily explained. The organic constituents of cells are mainly colloidal or insoluble substances such as proteins, carbohydrates and lipoids, the diffusion of which is easily limited. These substances can be hydrolysed to simple diffusible substances such as amino acids, hexoses and soaps, so that the cells can obtain their supplies by diffusion. The simple substances are converted into more complex compounds, thus the accumulation of organic material is possible.

The difference in composition of inorganic materials is less easily explicable. The inorganic ions cannot be decomposed into simpler substances, so that one must discuss the way in which these differences can be produced. Some substances such as sulphur and phosphorus may exist partly as organic compounds which form inorganic ions (sulphates and phosphates) on oxidation in the cells, but we cannot claim the same form of union for such ions as those of potassium.
The first step in the discussion is to examine the way in which cells are prevented from mixing with their surroundings.

**Demarcation of Cells from their Surroundings**

There are two physico-chemical conceptions which may represent the conditions which prevent cells from mixing with the liquids in contact with them. They are:

1. That the cell consists of a surface membrane in which the cell contents are contained in the same way as the contents of an osmometer are held inside its semi-permeable membrane.
2. That the cell consists of a fluid which is immiscible with its surroundings just as a solution of water in phenol does not mix with a solution of phenol in water.

Apart from these two conceptions the only other possibility is that the cells are expending energy in a continual struggle to maintain their integrity, an idea which is acceptable only after the two physico-chemical schema have been found untenable.

The first supposition implies that the exchanges between cells and their surroundings depend on the nature of the substances that can pass through the membrane: that is that the texture of the membrane is the main factor in regulating the income and output of the cell.

The second supposition implies that the exchange of material between cells and their surroundings depends on the nature of the cell contents and the relative solution pressure of a substance in the cells and their surrounding solutions. Any substance which is more soluble in the cell contents or is removed in some way from solution in the cell contents will accumulate inside the cells; that is, the main factor in regulating cell processes is the nature and behaviour of the cell contents.

This as we have stated above is the explanation offered for the accumulation of organic compounds in cells.

The second step is to examine the evidence as to the nature of the exchanges between the cells and their surroundings.

**Exchanges between Cells and Extra Cellular Solutions**

Increase in the active concentration of a substance inside a cell will cause a rise in osmotic pressure with an attraction of water into the cell and a tendency for the substance under consideration to escape through the surface of the cell. If this substance cannot escape the rise in osmotic pressure will persist, but if it can escape the osmotic pressure will fall as the
equilibrium between the concentration of the substance inside and outside the cell is restored.

The osmotic activity will cause the phenomena which are described in connection with diffusion either in a homogeneous or heterogeneous system.

Increase in the active concentration outside a cell will cause similar changes, only the osmotic movement of water will be in the reverse direction.

As it is easier to vary the concentration of substances outside of the cell, the simplest experimental procedure is to subject isolated cells to solutions containing different strengths of various substances.

If the total active osmotic concentration is greater outside the cell water will pass out of the cell and the cell will shrink. In the case of cells surrounded by some rigid framework the shrinkage of the cell will cause the protoplasm to separate from the framework leaving a gap between the two; this is called plasmolysis (Fig. 14).

If, on the contrary, the osmotic concentration is less outside water will pass into the cell, causing the cell to increase in size. Naked cells will swell but cells surrounded by a framework will entirely fill the spaces allotted to them, thus rendering the structure rigid; this is called turgor.

These two changes can be well exemplified by strips of raw potato about five centimetres long and five millimetres square. When placed in five per cent. solution of sodium chloride the strips become soft and limp but in distilled water

**Fig. 14.** Drawing of plasmolysis.

In A the cell is distending its cell wall. In B the cell is less distended owing to a stronger salt solution outside. The cellulose wall has contracted, but the cell contents are still in contact with the cellulose framework. In C the cell has commenced to shrink from the cellulose framework and in D the cell has shrunk well away from the framework. *(After de Vries. Copied from Algemeine Physiologie Verworn (Fischer, Jena).)*
they become hard and rigid. These changes are reversed by transferring the potato strips from one solution to the other. The first quantitative investigations of these phenomena were carried out by de Vries, who determined the strengths of solutions which just failed to produce plasmolysis.*

Hamburger made corresponding observations on red blood corpuscles,† but as there is no rigid cell wall changes in volume were observed and not plasmolysis. Under certain conditions (usually preceded by swelling of the corpuscles), the haemoglobin escapes from the corpuscles; the bright red opaque suspension of corpuscles becomes a dark red transparent solution of haemoglobin and the framework of the corpuscles (stroma) remains as semi-opaque bodies called ghosts. This process is called laking or haemolysis.

By comparing the concentrations of solutions that just failed to produce haemolysis and those that maintained the red blood corpuscles at the same volume that they occupy in the blood, it has been found that in many cases these concentrations are such that they all possess the same osmotic concentration. In other words the effects on the cells correspond to measurements of osmotic pressure as made by freezing point or other determinations.

**Table XVI**

*Showing the strengths of solutions that cause haemolysis of red blood corpuscles.‡*

<table>
<thead>
<tr>
<th>Substance</th>
<th>No haemolysis</th>
<th>Slight haemolysis</th>
<th>Average</th>
<th>Depression of freezing point *</th>
<th>Osmotic pressure in millimetres of mercury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium nitrate</td>
<td>1.04</td>
<td>0.96</td>
<td>0.99</td>
<td>0.3314°C.</td>
<td>3050</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.60</td>
<td>0.56</td>
<td>0.62</td>
<td>0.345°C.</td>
<td>3150</td>
</tr>
<tr>
<td>Potassium sulphate</td>
<td>1.16</td>
<td>1.06</td>
<td>1.11</td>
<td>0.293°C.</td>
<td>2680</td>
</tr>
<tr>
<td>Cane sugar</td>
<td>6.29</td>
<td>5.63</td>
<td>6.09</td>
<td>0.345°C.</td>
<td>3150</td>
</tr>
<tr>
<td>Potassium acetate</td>
<td>1.072</td>
<td>1.003</td>
<td>1.037</td>
<td>0.342°C.†</td>
<td>3130</td>
</tr>
<tr>
<td>Potassium oxalate</td>
<td>1.27</td>
<td>1.18</td>
<td>1.22</td>
<td>0.32°C ‡</td>
<td>2920</td>
</tr>
</tbody>
</table>

* Calculated from figures given in Landolt and Bernstein.
† Using the figures for the dissociation of sodium acetate.
‡ Assuming the same extent of dissociation as in potassium sulphate.

In the preceding table it is seen that certain substances seem to act by a purely physico-chemical osmotic effect. There are, however, substances such as urea and ammonium

chloride which do not prevent hæmolysis. Thus the assumption is made that the cell wall is impermeable to the former but permeable to the latter.

The difference between such salts as potassium chloride and ammonium chloride has been explained by the assumption that it is the potassium ion to which the cell wall is impermeable, while the chlorine ion can pass through, but it may possibly be due to a difference in the unionised salts.

**TABLE XVII**

*Showing types of substances that do and do not enter cells*

<table>
<thead>
<tr>
<th>Substances that do not seem to enter cells</th>
<th>Substances that enter cells slowly</th>
<th>Substances that do seem to enter cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium, Strontium</td>
<td>Amino acids</td>
<td>Ammonia</td>
</tr>
<tr>
<td>Barium, Magnesium</td>
<td></td>
<td>Free Acids and Alkalis</td>
</tr>
<tr>
<td>Sugars (cane, grape milk)</td>
<td></td>
<td>Alcohols</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aldehydes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ketones</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urea</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bile salts</td>
</tr>
</tbody>
</table>

By comparing the substances that accumulate inside the cells with the above table we see that so far as the organic materials, except the sugars, are concerned they may enter as simple diffusible molecules, be built up into complex colloidal substances and thus removed from the active physico-chemical system as described earlier in this chapter.

In the case of the inorganic material we must look further for the manner in which they have been accumulated.

An accumulation, for instance, of potassium, might be brought about by active cellular processes accompanied by an expenditure of energy. After the accumulation has taken place it might be maintained by the membrane becoming impermeable or by a continual activity of the cell to maintain the difference in concentration.

On the other hand an accumulation may occur by some means by which the ion in question is removed from the active system so that there is an apparent increase in solubility. This may take place in one of three ways.

1. The potassium may be adsorbed on the surface of some colloidal particles. This is the way in which potassium is supposed to be retained in the soil whilst sodium is washed away in the drainage water.
2. The potassium may be precipitated as an insoluble substance.
3. The potassium may form an unionised substance.

Whatever explanation is found for the accumulation of potassium in cells may be applied to explain the differences in other ions.

Although Höber has found that the contents of cells show an electrical conductivity * we do not know which substances are the conducting materials. On the other hand Moore and Roaf have haemolysed red blood corpuscles, dialysed them for forty-eight hours and they found that haemolysis and dialysis do not remove all the inorganic salts from red blood corpuscles.†

Effect of Changes in the Surrounding Medium on Cellular Activity

Under usual conditions it is necessary that any substance that is to exert an effect on cellular activity must reach the cells through their surrounding media. There is no difficulty in the case of those substances that can enter the cells, but there are substances which affect the activity of cells but do not themselves enter the cells.

The observations of Warburg on sea-urchin eggs stained with neutral red show that the cell contents are acid to this indicator. On putting the eggs into dilute ammonium hydroxide the cell contents become alkaline without an increase in the rate of oxidation but in dilute sodium or potassium hydroxides the cell contents remain acid, yet the rate of oxidation is increased.‡

Thus we see that it is possible to influence the activity of cells by substances that apparently do not enter into the cells.

The above outline of the interaction of cell surroundings on cells leads to a discussion of the deductions to be drawn from the experimental results and these hinge mainly on whether the cells are or are not surrounded by a semi-permeable membrane.

The doctrine that cells are surrounded by semi-permeable membranes was at one time widely accepted and the membrane was said to be of fat-like material because those substances which affected the activity of cells are all soluble in fats.§

Although many facts agree with the supposition that cells possess semi-permeable membranes there are some facts which

directly negative the impermeability of the membrane. If the red blood corpuscles are impermeable to potassium how is it that potassium has accumulated inside the cell so that its concentration in the corpuscles is many times that of its concentration in the surrounding plasma?* One can assume that the potassium entered the cell during its growth and that the cell membrane only became impermeable at a later stage, but Hamburger and Bubanovic have shown that red blood corpuscles are permeable to potassium and to sodium.†

Changes in cell permeability are said to occur; thus the escape of liquid from the pulvinar cells when a leaf petiole droops have been ascribed to an increase in permeability. On the other hand, the escape of liquid has been ascribed to changes in the osmotic concentration of the cell contents as the solution that escapes is too dilute to be some of the cell contents which have filtered through a more permeable wall.‡

The absence of a true membrane round some cells is proved by the process of amœboid movement which cannot take place if there is a fixed structure such as a membrane. Also the fact that it is possible to pass a glass capillary into the cells and to inject substances into the cells without a leak from the cell surface§ shows that the integrity of the cell does not depend on an intact membrane.

The second view that the cells consist of a fluid immiscible with the surrounding fluid leads to certain definite comparisons between cells and the behaviour of two immiscible liquids.

At the junction of the two liquids there is a surface tension, and if the specific gravity of the two liquids is nearly the same the suspended liquid assumes that shape which has the least surface for the given volume, namely, it becomes a sphere, and this is the resting shape of a naked isolated cell such as an amœeba.

Owing to the pull of the surface tension there will be a slight pressure inside the sphere according to the formula \[ P = \frac{T}{r_1} + \frac{T}{r_2} \]

where \( P \) is the pressure, \( T \) = surface tension, and \( r_1 \) and \( r_2 \) are the radii of curvature of two sectors at right angles to each other (p. 48).

If one part of the surface of the sphere has a lower surface tension than the rest of the surface a projection will occur at that part because to balance the same pressure a lower surface tension will require a smaller radius, or, what is the same thing, a projection from the surface of the sphere.

A local change in surface tension may be brought about either by the action of some substance in the surrounding medium or by some local chemical change inside the cell itself. Such local chemical change may be a liberation of ions, causing a change in electrical charge which as described on p. 48 will produce a change of surface tension.

Under the influence of surface tension any substance which can lower the surface tension will accumulate at the surface of separation between the two liquids, no matter whether it comes from the cell contents or the surrounding medium. Surface condensation may produce such a concentration that precipitation may occur with the production of a surface layer.*

Salts in the surrounding medium may act on the colloids at the surface producing gelation or the reverse.† Such precipitation and resolution is quite a different matter from the passive semi-permeable membrane that was previously considered to be the distinguishing feature of cells because it is instantly renewable and it is easily altered by cellular activity.

The comparable effects on cells of similar osmotic concentrations can be explained by comparing the vapour pressure of the two phases. A solution of water in phenol has the same vapour pressure of water as the solution of phenol in water in equilibrium with it. By adding salts to the watery solution the vapour pressure of water is decreased, hence water will pass from the phenol phase until the vapour pressures are equal. Therefore the phenol phase will decrease in volume and the change in volume will be in proportion to the osmotic concentration of the surrounding salt solution.

The cell colloids exert osmotic pressure and these colloids are affected by various physico-chemical conditions; thus it is possible that they may have an influence in the water exchange of cells.‡

Differences in concentration between the cell and its surroundings might be accounted for by differences in solubility in the two, but Kite has shown that dyes which do not

pass into the cell when present in the surrounding medium are soluble in the cell contents when passed directly into the cell.*

The experimental evidence indicates that in many ways the cells react as if they were surrounded by semi-permeable membranes but that some cells at least do not possess a limiting membrane.

Amœboid movement is better explicable on the assumption that the cell is a fluid immiscible with its surroundings. The surface condensation of fatty materials produced by the influence of surface tension does not explain the ease or difficulty with which some substances enter the cells because there are fat soluble substances that do not enter cells and there are substances not soluble in fat that do enter the cells.

The ingestion of solid particles by one liquid suspended in

![Diagram](image)

**Fig. 15.—Diagram to illustrate formation of a pseudopodium.**

With even surface tension a spherical form exists. (A) By the action of some substance outside the cell a local decrease in surface tension causes a projection as shown at B. It is possible that the same result may be brought about by some local change inside the cell; this is indicated by the second sphere of influence. The internal change may be either a rise in osmotic pressure or a decrease in surface tension, probably the latter.

another depends on the relative surface tensions between the object and the two solutions. A drop of chloroform in water will absorb a piece of glass coated with shellac and extrude it after the shellac has been dissolved from the surface of the glass.†

Destruction of cells by substances which decrease the surface tension indicates that the cells behave like immiscible fluids, so that when the surface tension is decreased the two fluids mix.

Czapek has shown that when the air–water surface tension is reduced to 0.68 to 0.69 of that of pure water, the cell contents escape.‡

When a phenol–water mixture is warmed to 68.4°C the constituents mix in all proportions. Red blood corpuscles haemolyse when warmed, but unlike the phenol–water mixture separation does not occur again when the solution is cooled.

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The conditions of membrane equilibrium described on p. 56 can all occur in a system of two immiscible fluids, as can also the high resistance to the electrical current.

Whether cells are surrounded by semi-permeable membranes or not, the following conclusions are warranted in regard to the subject:

1. The unequal distribution of substances between cells and their surroundings is explicable on either assumption, but the production of the unequal distribution is not possible if the membrane is impermeable to the substance that accumulates inside.

2. Plasmolysis and Turgor may be produced by osmotic pressure acting on a semi-permeable membrane or by an effect on the vapour pressure of an immiscible liquid.

3. Hæmolysis or Cytolysis will occur by rupture of a semi-permeable membrane or by decrease of surface tension allowing two immiscible fluids to mix.

4. The high electrical resistance of cells may be due to a membrane impermeable to ions or to two liquids that do not exchange ions with each other.

5. Amœboid movement and cell division can be explained by surface tension changes at the junction of two immiscible liquids but amœboid movement cannot occur if a membrane is covering the cells.

Different conditions may hold in different cells. An amœba has no surface membrane, but red blood corpuscles must have some structure because their shape is impossible if surface tension is allowed to act on a free surface, and there are ghosts left when the contents of the corpuscles have escaped.

The surface membrane of the red blood corpuscle may be merely a permanent modification of the temporary gelation produced on the surface of free cells.

The presence of a membrane does not necessarily mean that the membrane is impermeable to inorganic ions because it may act merely by retaining cell colloids and the inorganic constituents may be unequally distributed according to some process associated with the application of the phase law to the system of colloid cell contents and inorganic solution outside the cell.

It must not be forgotten that cells are living structures and capable of acting as energy transformers. The characteristic property of living organisms is their regulative action on chemical processes.
The observation of Warburg that sodium and potassium hydrates do not change the reaction of the cells of sea-urchin eggs yet they cause an increased oxidation* may indicate that the cell prevents their entry by expending chemical energy.

The discussion on the exchange between cells and their surroundings applies just as well to surfaces of separation inside the individual cells. Such surfaces are present and they may be of considerable importance for biological processes.

**FIG. 16.**—Drawing showing an amoeba moving in the direction of the arrow. The round anterior end clear of granules flows out whilst the posterior end is drawn in as the animal moves on-wards (after Verworn).

**FIG. 17.**—Pulvinus of *Mimosa.*

If the cells at B increase in size, the leaf is raised, but if they shrink the leaf falls. In some cases, the cells at A change reciprocally to those at B. The vascular tissue is narrowed at the pulvinus to allow greater flexibility.

(From "Vegetable Physiology." J. R. Green (Churchill).

TRANSFORMATION OF CHEMICAL ENERGY INTO MECHANICAL ACTIVITY.

Returning to the problem of the production of mechanical energy by cells we believe that amœboid movement and cell division are probably due to changes in surface tension.

A localised change in surface tension produced either by some localised change inside the cell or by the action of some substance in the vicinity of the cell produces amœboid movements. It must not, however, be forgotten that localised changes in osmotic pressure inside the cell can produce and may aid such movements. A zone of increased surface tension will cause a constriction which may go on to complete separation of the cell into two daughter cells.

Movements of leaves are produced by turgor or plasmolysis of the cells of the pulvinar region. Drooping of the petiole is accompanied by an escape of liquid from the cells; the most probable explanation of the escape of liquid is a change in the osmotic concentration of the cell contents.

Contraction of striated muscle is explicable by a change in vapour pressure (osmotic pressure) in the anisotropic material

of the fibrillae and the hypothesis based on this assumption explains all the facts of muscular contraction.*

The discussion of this subject is confusing because the term permeability is frequently used as being equivalent to penetrability. The former implies a membrane surrounding the cells whilst the latter merely states that the substance in question does or does not enter the cell.


It has been stated that muscle contraction cannot be due to changes in osmotic pressure because preliminary extension of a muscle increases the force liberated by muscle and that surface tension is the only force which, like muscle contraction, has a negative temperature coefficient. The first reason is invalid because an extension of the model described elsewhere will cause a squeezing of liquid from the anisotropic material so that the concentration in it will be greater. Therefore a greater tension will be exerted by the stretched muscle when it contracts. The second reason is also invalid because, as described on p. 59, the combination of hydrogen and oxygen to form water has a negative temperature coefficient, i.e. it is reversed at high temperatures. From the last statement it follows that the hydrolysis of salts of weak acids and bases is increased at higher temperatures and if muscle contraction be due to such salts the contraction may be weaker at higher temperatures.
A membrane round living cells cannot be a fundamental requirement of the cells as amœboid cells cannot have a membrane. The surface condensations due to surface tension phenomena are quite distinct from a membrane as they are due to equilibrium conditions of the cell contents in relation to their surrounding medium. That permanent structures corresponding to membranes may be present in some cells is not unlikely.

When a membrane is present it is the servant, not the master, of the cell.

GENERAL REFERENCES
A SUPPLY of available energy is necessary for both plants and animals. As we shall see later a few organisms can obtain energy by the oxidation of inorganic substances, such as sulphur (see p. 211), but the main source of energy is the radiant energy of the sun. All living cells can synthesise but not all cells can store radiant energy, and in this chapter we shall confine our attention to the storage of radiant energy.

The process of storing light energy as chemical energy is called photosynthesis.

Photosynthesis is most marked in cells which contain the green colouring matter chlorophyll, and we shall apply the chemical principles described in the preceding chapters to the process of photosynthesis.

PHOTOSYNTHESIS

The fundamental points are as follows: (1) The plant absorbs various inorganic salts and water by its roots. (2) When exposed to light the green leaves absorb carbon dioxide and set free oxygen. (3) A plant grown under proper conditions can be shown to increase in weight, and the increase is accompanied by an accumulation of carbon compounds. The carbon is furnished by the carbon dioxide of the atmosphere and a process of reduction occurs with the liberation of oxygen.

The first stage of the synthesis is the production of carbohydrate.

\[ 6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \]


These points can be proved experimentally, and we shall examine them in this chapter, leaving the fate of the carbohydrate to be discussed later.
In studying this process we can begin with comparatively simple observations. A leaf taken from a plant, which has been kept in the dark, after decolorisation and on treatment with iodine shows very little or no starch. After exposure to light most leaves show an accumulation of starch. Those leaves that do not form starch show the presence of other carbohydrates, mainly d-glucose. This illustrates the necessity of light for the energy accumulation, and it can be shown that only the green parts of plants accomplish this synthesis in a measurable degree.

If, however, the leaf be exposed to light in an atmosphere free from carbon dioxide no formation of carbohydrate will occur. If the leaf be kept in a closed space containing a known amount of carbon dioxide the amount of carbon dioxide in the space decreases and the amount of oxygen increases. The reverse process occurs when the leaf is kept in the dark: the carbohydrate is used up, oxygen disappears and carbon dioxide accumulates. The latter is the process of respiration, and it will be studied in a later chapter. The processes of photosynthesis and respiration may not proceed by the same intermediate steps and they cannot be said to be strictly reversible, but the energy exchanges are reversible.

\[
6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow C_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 + 738,000 \text{ calories}^* \\
by \text{synthesis}
\]

This reaction shows an accumulation of energy equivalent to 4100 calories for every gram of carbohydrate formed, and a similar amount can be liberated by the decomposition of the carbohydrate. As already mentioned this energy comes from the sunlight.

In studying this process we can gain some insight into the mechanism by finding out what conditions affect the change, and to what degree they affect it. By altering one factor at a time and by keeping the others constant the influence of the various factors can be determined. We shall consider the following factors: (1) Concentration of substrate; (2) Intensity of illumination and quality of the light; (3) temperature.†

* A calorie (c) is the amount of heat required to raise 1 g. of water from 15° C. to 16° C. A large calorie (C) = 1000 c. 

\[
C_6\text{H}_{12}\text{O}_6 = 180 \text{ g. glucose} \cdot \cdot \cdot \text{for 1 g. glucose the energy value is} \\
\frac{738000}{180} = 4100 \text{ calories or 4.1 C.}
\]

The effects of concentration of substrate are practically confined to one substance. As there is always an excess of water, we can devote our attention to the concentration of carbon dioxide. In land plants we can study the effect of changes in the volume percentage of carbon dioxide in the surrounding atmosphere, and in water plants changes in the concentration of carbon dioxide in the solution. Even in land plants the carbon dioxide must dissolve in the liquids of the cell, hence we are ultimately reduced to the consideration of a solution of carbon dioxide.

The concentration of carbon dioxide in solution is proportional to the volume (partial pressure) of the carbon dioxide in the atmosphere in equilibrium with the solution, hence the concentration of carbon dioxide in solution is frequently expressed as tensions meaning the pressure in millimetres of mercury percentage by volume of the carbon dioxide in the gaseous mixture in equilibrium with the solution. Thus if the total pressure of gas is 760 millimetres of mercury and 2 per cent. by volume of the gas is carbon dioxide the tension is 15·2 millimetres.

Thus we have to consider the behaviour of a solution of carbon dioxide.

Carbon dioxide unites with water to form carbonic acid, and the latter dissociates in two stages, giving rise to hydrogen ions, bicarbonate ions and carbonate ions. We cannot distinguish between the carbon dioxide in solution and the carbonic acid formed from the carbon dioxide, but we do know that the sum of the concentrations of these two is proportional to the tension of carbon dioxide.

Ewart has shown that both acid and alkali inhibit photosynthesis;* thus it is not possible to draw any conclusion as to which of the above dissociation products obtained by dissolving carbon dioxide in water is used as the raw material for photosynthesis.

We require observations in which the hydrogen ion concentration is kept constant. If stabilising mixtures are used the hydrogen ion concentration can be kept constant, then the concentrations of bicarbonate ion and of carbonate ion and of carbonic acid will each be proportional to the carbon dioxide tension. By adding bicarbonate to a solution it is possible to keep the carbon dioxide tension constant with a

fall on hydrogen on concentration, and possibly in some such way as this it will be possible to discover which is the important factor for photosynthesis. Provisionally we can consider that the photosynthesis depends upon the carbonic acid in solution.

The rate of reaction can be measured by the rate of disappearance of carbon dioxide, or from the rate of appearance of oxygen. In land plants we rely on gas analysis, but in water plants the rate can be measured by the number of bubbles of oxygen escaping from the cut end of the stem. In the latter case great care must be taken to ensure accuracy as the size of the bubbles may vary and the rate of solution of oxygen in the surrounding water must be borne in mind.

![Diagram to show effect of limiting factors.](image)

**Fig. 19.**—Diagram to show effect of limiting factors.

Carbon assimilation increases from A to B, but with increasing carbon dioxide runs from B to C, showing no further increase in rate of assimilation. If some limiting factor is raised the increase now goes to D and increase of some further limiting factor allows the assimilation to rise to F.


The plant is always decomposing food material, using up oxygen and producing carbon dioxide. This process of respiration is the reverse of photosynthesis, and we really measure the excess of one process over the other. The process of respiration by itself can be measured in the dark and, assuming that the rate of respiration is the same in the light, the figures can be corrected to show the total photosynthesis. Unfortunately this assumption may not be correct.

At low concentrations of carbon dioxide the photosynthesis is found to be proportional to the tension of carbon dioxide. When the tension of carbon dioxide rises above a certain value there is no further increase in the rate of synthesis. This limit to the rate of synthesis shows what is termed a
limiting factor, namely some factor which interferes with the expected increase in rate. The limiting factors are illumination and temperature, as an increase in either of these allows the rate of synthesis to continue rising as the concentration of carbon dioxide increases.

**Effect of Intensity of Illumination.**—In a similar way it can be shown that with weak illumination the rate of synthesis is proportional to the intensity of the light, but above a certain intensity of light the synthesis does not increase with the increase in illumination. The limiting factors in this case are concentration of carbon dioxide and temperature, as a rise in either of these factors allows photosynthesis to rise to a still greater rate with a further increase in the intensity of light.

**Effect of Temperature.**—The temperature effect is less simple than the preceding factors. With increasing temperature the rate of reaction is more than doubled by each rise of ten degrees Centigrade. This is well seen at medium temperatures, but at low or high temperatures the rule does not hold. At temperatures near the freezing point photosynthesis practically ceases, so there is a high temperature coefficient, as the temperature rises to temperatures at which photosynthesis is appreciable. This effect must be due to some process with a high temperature coefficient which cuts short the synthesis at low temperatures.

Likewise at high temperatures the rate decreases. This decrease is due to a destructive action on the cell colloids. The whole process is paralleled by the action of temperature on enzymes. As the temperature rises the rate of destruction increases, and owing to the time required to produce photosynthesis the cell is killed before a measurable amount of photosynthesis has occurred.

Blackman has estimated the initial rate of synthesis in the following way. He measured the rate of photosynthesis at different time intervals, from which he constructed a curve for each temperature, showing the falling off in photosynthesis with the duration of the experiment. By extrapolating back to zero time the initial rate of reaction for each temperature was estimated* (see Fig. 13).

The initial rates thus obtained showed a fair agreement with what one would expect by calculation from the rate of reaction and the temperature coefficient, each measured at lower temperatures.

Even in spite of these corrections there is a slight decrease in the temperature coefficient as the temperature rises, but such a slight departure from a mathematical exactitude is not unusual in many physico-chemical processes.

The rate of photosynthesis depends upon the presence of the above mentioned limiting factors. Thus the rate increases as the carbon dioxide increases, until a certain concentration is reached, when the photosynthesis remains uniform in rate, even if the concentration of carbon dioxide is increased further. By using weaker light the maximum is reached with a lower

![Graph showing the relation of carbon assimilation to carbon dioxide supply, temperature, and illumination in Elodea.](image)

**Fig. 20.**—Relation of carbon assimilation to carbon dioxide supply, temperature and illumination in Elodea.

By following the horizontal lines the minimal carbon dioxide supply, temperature and illumination can be found for any rate of carbon assimilation, or given the amount of carbon dioxide supply, the temperature and the illumination, the carbon assimilation will be the lowest value on the horizontal line opposite one of these factors.


concentration of carbon dioxide, and by increasing the intensity of illumination the rate rises *pari passu* to a still higher concentration of carbon dioxide.

When the intensity of illumination is being tested a similar inter-relation between it and the concentration of carbon dioxide can be demonstrated.*

Photosynthesis is related to the presence of the green colouring matter called chlorophyll. Therefore it is necessary to examine the relation of pigments to light, after which we can return to the subject of photosynthesis.

The colour of an object depends upon the absorption and reflection of the light of different wave lengths. The study of coloration has a much wider scope than the chemical discussion of pigments, as some colours are due to structural arrangements whereby light is reflected in such a way that interference of one wave with another produces colours. The significance of coloration is a biological problem of great interest.

White light can be decomposed by means of prisms into a series of coloured lights forming the spectrum. The instrument by which this is done is called a spectroscope, and it is used for the study of chlorophyll and other pigments.

The spectrum of sunlight shows narrow dark lines parallel to the coloured bands. These lines are called Fraunhofer lines, and they are useful for measurement of other lines: they are due to absorption of the light of certain wave lengths by the vapour, in the sun, of the same elements which furnish those wave lengths when incandescent.

Newton described seven colours in the spectrum, but most people can recognise only six.

Absorption of light may take the form of diffuse absorption at the ends of the spectrum, or of dark bands crossing the coloured area. Absorption occurs also in the invisible spectrum, either in the infra red or ultra violet region: the former being recognised by its heating effect and the latter by its effect on the photographic plate.

It is only the energy of light that is absorbed that can be used to add energy to the system.

Colours in plants and animals are useful, in two ways, in addition to the process of photosynthesis, namely, for protection and for sexual selection. It is characteristic of the economical working of biological processes that waste products are sometimes used for purposes of coloration.*

In plants the colours are largely contained in the specialised leaves which form the flowers and they are therefore of sexual importance. The colours of flowers are developed by a process of oxidation. By studying extracts of flowers it is

possible to show that in general there is an oxidase associated with an oxidisable substance.

White flowers are due to the absence of either the oxidase or oxidisable substance. There are thus two possible forms of whiteness, and these can be shown to exist. Some white flowers become coloured when treated with an oxidase: that is, they contain oxidisable substance but no oxidase. Other white flowers do not become coloured when treated with an oxidase, that is, they do not contain the oxidisable substance, but an oxidase can be extracted from them.

Turning now to the chemical nature of the plant pigments we find that they are derivatives of cyclic compounds combined with sugars to form glucosides called anthocyanins.

Anthocyanins are hydrolysed by an enzyme into sugar and anthocyanidine. The anthocyanidine is a reduction product of flavonol, and it can exist as colourless or coloured tautomeric modifications.*

Animal pigments may serve the purpose of concealment. Thus we find animals with pigmentation resembling their background. Some of these pigments are possibly derived from the plants on which they feed, but in other cases the pigments are contained in special cells called chromatophores. The chromatophores by movements of expansion or contraction can alter the colour of the surface so that the animal can change its colour, as in the case of the chameleon, fish, amphibia, etc.

We find, however, certain animal pigments which are of special importance. The black pigment which lines the eye is required for optical purposes, so that light can neither diffuse through the wall of the eye, nor is it reflected inside the eye. The black pigment of the negro’s skin is a protection to the deeper layers. The sunlight is absorbed on the surface, and the heat produced got rid of by evaporation of sweat, but if the rays penetrated to the subcutaneous tissues, where arrangements for heat loss are less efficient, overheating and destruction would occur, followed by toxæmic symptoms.

These two black pigments are composed of melanin, which is a black insoluble substance derived from tyrosin by the action of an oxidising enzyme tyrosinase.

The browning of the human skin when exposed to the sun and the increase in pigmentation in some diseases is not yet understood.

FIG. 21.—Spectra of certain plant pigments

Absorption spectra of chlorophyll and associated pigments. Scale of wave lengths at top and bottom. Fraunhofer lines marked at the top. The colours of the regions of the spectrum indicated at the bottom.

2. a Chlorophyll in ether (do., p. 170).
3. β-Chlorophyll in ether (do., p. 171).
5. Xanthophyll in alcohol (do. p. 246).

From "Principles of General Physiology." W. M. Bayliss (Longmans).
The development of colour in the skins of animals is possibly due to the action of an oxidase.*

Rhodopsin, the visual purple, is bleached by light and this bleaching may be of fundamental importance in the photo-chemistry of vision.

**Extinction Coefficient**

Absorption of light by a solution is proportional to the thickness of the solution and to the strength of light falling on it. As the strongest light falls on the surface the outer layers absorb most of the light. The extinction coefficient is the reciprocal of the thickness of solution required to reduce the light to one-tenth of its incident value.

**Chlorophyll**

The chlorophyll is contained in small oval bodies called chloroplastids. As it occurs largely on the surface of these plastids the absorption of light is more efficient than if the chlorophyll were evenly distributed throughout the plastids.

Chlorophyll can be extracted from leaves by means of alcohol or other solvents. The crude extract contains two forms of the green pigment, namely, α and β chlorophyll, with two yellow pigments, carotin and xanthophyll. The solution in alcohol has a dark green colour by transmitted light, but by oblique illumination it appears red: that is, the solution is fluorescent.

The red fluorescence of chlorophyll indicates that the solution absorbs shorter wave lengths near or beyond the blue end of the spectrum, and gives out waves nearer the red end of the visible spectrum.

Examined by means of the spectroscope a solution of chlorophyll in acetone shows an absorption band in the red near the Fraunhofer line C, three absorption bands with decreasing intensity towards the violet, and a second absorption maximum which completely absorbs the blue and violet end of the spectrum. By filling the air spaces of a leaf with water it becomes transparent, and the spectrum is seen to correspond to a colloidal solution in one per cent. acetone.

On extraction of chlorophyll by alcohol the enzyme chlorophylase acts upon the chlorophyll, splitting it into the unsaturated alcohol phytol and a crystalline substance, chlorophyllin, which is the magnesium salt of a carboxylic acid. Borodin’s crystals are the ethyl ester of chlorophyllin,

formed by treating chlorophyll with ethyl alcohol so that the phytol is replaced by an ethyl group.

Chlorophyll contains magnesium and by treating it with acid magnesium is split off, leaving a phytol ester called phæophytin. Phæophytin is an acid substance, and by combining it with metals, such as zinc, iron, copper, etc., the green colour and fluorescence of chlorophyll are restored.

Further decomposition of chlorophyll with alkali at increasing temperatures leads to the production of a series of products ending in porphyrins, which are allied to the derivative of hæmoglobin, called hæmatoporphyrin, in that they contain pyrrol groups.

**Stages in the Decomposition of Chlorophyll a**

\[
\begin{align*}
\text{Mg} + \text{phæophytin} & \xrightarrow{\text{By acid}} \text{Chlorophyll a} \\
& \xrightarrow{\text{By MgO}} \text{chlorophyllin a} \\
& \xrightarrow{\text{By alkali}} \text{phytol} \\
& \xrightarrow{\text{By alkali in cold or by chlorophyllase}} \text{phytol.}
\end{align*}
\]

A series of changes by alkali at increasing temperatures.

\[
\begin{align*}
\text{Phytol} + \text{phytochlorin} & \xrightarrow{\text{By acid}} \text{isochlorophyllin a} \\
& \xrightarrow{\text{By MgO}} \text{chlorophyllin a} \\
& \xrightarrow{\text{By alkali in cold or by chlorophyllase}} \text{phytol.}
\end{align*}
\]

Decomposition of chlorophyll b follows very much the same series of changes to give the same final end products.

*(Abbreviated from Willstätter and Stohl.)*

**The Energy Supply for Photosynthesis**

The supply of energy for the photosynthesis of carbohydrates comes from the sun, and in order that the energy can be used the light must be absorbed.

By absorbing all the light heat is produced, thus the total energy of the light can be measured. The distribution of energy in the various regions of the spectrum of sunlight is known, and by measuring the amount of the various coloured lights absorbed the energy of the absorbed light can be calculated. The chlorophyll absorbs most energy from the portion of the spectrum corresponding to the absorption band in the red, and this is the region of the spectrum which
Fig. 22.—Effect of spectral colours on starch production

Hydrangea leaves, still attached to the plant, have been deprived of starch by keeping in the dark. They have then projected upon them a small solar spectrum for five to six hours. Subsequent treatment with iodine, in the usual way, shows a picture of the absorption spectrum of chlorophyll in the blue "compound" of iodine and starch. The lower piece of leaf has been partially covered with a screen, represented below it, in such a way that the wider part of the aperture corresponded with the region of the spectrum between the lines B and C.

(From "Proc. Roy. Soc.," Timiriazeff.)
is most efficient in photosynthesis. If a leaf which readily deposits starch is exposed to the light of a spectrum, it is found that the starch is deposited first in the region corresponding to the absorption band in the red and then to the other absorption bands.* Another method of showing the relation of photosynthesis to absorbed light is to place a filament of spirogyra in a culture of bacteria which become active only in the presence of oxygen. On exposing the filament to a spectrum the bacteria first become active in the neighbourhood of the absorption band in the red.†

The latter method can also be used to show that the chlorophyll is the portion of the cell which absorbs the energy for the photosynthesis. If light is allowed to fall on a spirogyra filament so that the chlorophyll is illuminated in parts and some parts of the cells free from chlorophyll are also illuminated, the bacteria become active only near those points where the light falls on the chlorophyll (Engelmann).

We have therefore found that the photosynthesis depends upon the light being absorbed by chlorophyll, and that the maximum effect corresponds to the absorption bands of chlorophyll. The efficiency of the absorbed light does not depend upon the region of the spectrum from which it comes, as careful measurements indicate that the photosynthesis in the regions where no absorption bands occur is proportional to the energy of the small amount of light absorbed. Therefore the greater synthesis is not due to the greater efficiency of the absorbed rays, but merely to the fact that more rays are absorbed, hence more energy is available for photosynthesis.

The chlorophyll acts as an energy trap.

On p. 59 we mentioned Le Chateliers theorem, and the action of chlorophyll in photosynthesis seems a similar case. The photosynthesis is an endothermic reaction, and the equilibrium should be shifted in the direction of carbohydrate formation by a rise of temperature. It is clear that the temperature cannot be raised in the leaf to an extent which would cause a marked shift of the equilibrium point, but if we use the term addition of energy for rise of temperature the cases are parallel. The only condition required is that the energy be furnished in a form which will be taken up by the endothermic reaction, and this seems to be the function of the chlorophyll.

Process of Synthesis

The previous facts are comparatively easy of demonstration, but when we turn to the steps of the synthesis we find more difficulty in the interpretation of results. There is some reasonable doubt whether the same intermediate stages occur in photosynthesis and respiration. We are here met by a difficulty found throughout biological chemistry, namely that the intermediate substances are present in extremely minute quantities and are thus difficult to detect. It may also happen that if these substances are present in more than minimal concentration they are toxic; thus their effect on living cells cannot be tested.

Baeyer suggested that the first stage of synthesis is the production of formaldehyde. * Usher and Priestley have made experiments in which chlorophyll was exposed to light in thin layers in the presence of carbon dioxide.† The chlorophyll was rapidly bleached, but they got over this difficulty by mixing catalase with the chlorophyll. The bleaching was due to the formation of hydrogen peroxide; in the presence of catalase it was decomposed with the production of free oxygen, and the chlorophyll was not attacked. Their experiments were made by spreading a mixture of gelatine, chlorophyll and catalase on glass plates. They were able to show the production of something which gave the reactions of an aldehyde. Schryver likewise found that on exposing chlorophyll to light a substance like formaldehyde is produced.‡

The aldehyde-like substance may be, however, a decomposition product of the chlorophyll or other cell substances under the influence of light.

Assuming that formaldehyde is produced we can examine the mechanism of this reaction. As already mentioned, a change in a chemical system is accompanied by a tendency to change in the opposite direction. Thus hydrogen and oxygen unite to form water with the evolution of considerable quantities of heat. If the temperature is raised the reverse process occurs, and at high temperatures water is largely decomposed into hydrogen and oxygen.

On heating carbon dioxide it decomposes into carbon monoxide and oxygen, so that if we heat a mixture of carbon dioxide and water the two are changed, and if the first

stage, as indicated, is accompanied by a loosening of bonds
the union of carbon monoxide and hydrogen to form formaldehyde seems fairly simple.

\[ 2\text{CO}_2 \rightarrow 2\text{CO} + \text{O}_2 \]
\[ 2\text{H}_2\text{O} \rightarrow 2\text{H}_2 + \text{O}_2 \]
\[ 2\text{H}_2\text{O} + 2\text{CO} \rightarrow 2\text{HCOH} + 2\text{O}_2. \]

Not only by heat, but also by exposure to ultra violet light
the union of carbon monoxide and hydrogen to form formaldehyde seems fairly simple.

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Not only by heat, but also by exposure to ultra violet light
the union of carbon monoxide and hydrogen to form formaldehyde seems fairly simple.

Not only by heat, but also by exposure to ultra violet light can carbon dioxide and water be split in the manner indicated. Therefore it seems probable that formaldehyde was produced before chlorophyll-bearing organisms were evolved.* The formaldehyde can polymerise, in the way to be mentioned shortly, to form carbohydrate. This carbohydrate may have been the source of energy for primitive non-chlorophyll cells. The chlorophyll seems therefore to be a mechanism for absorbing the energy, but it is not necessary for the formation of formaldehyde.

Formaldehyde, especially in the presence of alkali, readily condenses to form acrose, a hexose sugar, from which other hexoses can be derived. In living organisms the synthesis is asymmetrical, which is probably due to the presence of enzymes. The enzyme seems so closely associated with the protoplasm that it has not yet been made to act outside the cell. The condensation of formaldehyde is slower than the formation of formaldehyde. The formation of formaldehyde probably represents a reversible reaction so that the presence of formaldehyde inhibits its formation. Thus the rate of condensation of formaldehyde regulates the rate of photosynthesis.

The further transformations of carbohydrate are interesting. It has been suggested that the first product of synthesis may be cane sugar or starch, but it is more logical to believe that a hexose is formed first, and that this condenses to form di- and poly- saccharides.

Although formaldehyde is poisonous to cells, experiments have been carried out in which leaves and other portions of plants have been treated with very low concentrations of formaldehyde. Spirogyra is found to assimilate formaldehyde with the formation of sugar, even if it is kept in the dark, but in higher plants light seems to be required for this reaction.†

The formation of carbohydrate does not entirely depend upon photosynthesis, but it can be brought about by the use

of other forms of energy. Some organisms can oxidise organic or inorganic substances and use the energy so derived for the reduction of carbon dioxide. This is called chemosynthesis.

Most leaves deposit the carbohydrate in the form of starch. The deposit usually occurs in concentric layers in the small oval bodies like those which contain the chlorophyll (plastids). The starch is relatively insoluble, hence it is deposited as soon as a small amount is formed. As this deposit gives rise to a new phase the concentration of starch in solution can never rise above the minute amount in equilibrium with the solid starch. Therefore, as the concentration of starch remains low the concentration of sugar remains low, and the condensation of formaldehyde is not inhibited by the accumulation of sugar in solution. On the other hand, removal of sugar will cause the starch to be hydrolysed to sugar, and as the starch in solution disappears more must dissolve to maintain equilibrium, hence the starch granules dissolve and are used by the cells.

This outline indicates the usual principle involved in the storage of food materials. A relatively insoluble substance is formed, hence the concentration can never rise above that of its saturated solution, and as the substance is used more dissolves to keep the solution saturated.

The further transformations of the carbohydrate will be considered in the next chapter.

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CHAPTER VII
INTER-CONVERSION OF CARBOHYDRATES, FATS AND PROTEINS

IN Chapter VI we discussed the reduction of carbon dioxide and water to form carbohydrate. From carbohydrate plants obtain sufficient energy to build up fats and, with the addition of nitrogen, to form protein. We know these facts because plants can be grown without a supply of fat or protein, yet these substances are found in the grown plant in larger amounts than were present in the seeds.

In the case of animals the experiments of Lawes and Gilbert proved that fat can be formed from the carbohydrate of the food.* The formation of protein requires a supply of amino acids, so we have to deal with the problem of how amino-acids can be formed from carbohydrate. Experimental evidence to be given later (Chap. X) shows that mammals cannot synthesise certain amino acids, and that these must be furnished ready-made in the food, but that other amino acids may be formed in the body. We do not know whether all animals are incapable of synthesising the same amino acids which cannot be formed in mammals, but for the present we need consider only the synthetical problem in its broadest aspect.

By the conversion of carbohydrate into fat a more concentrated supply of energy is produced. One gram of carbohydrate furnishes 4.1 Calories, whilst one gram of fat furnishes 9.3 Calories. Therefore, as a minimum, 2.27 grams of carbohydrate are required to furnish the energy value of one gram of fat or

\[ 2.27 \text{ gr. carbohydrate} + \text{oxygen} = 1 \text{ gr. fat} + \text{carbon dioxide and water}. \]

It is not impossible that other forms of energy might be used, but carbohydrate is the coin of exchange, and its value is required even if the energy for fat synthesis should be shown to be obtained directly from sunlight.

* J. B. Lawes and J. H. Gilbert, British Association Reports, 1852, pp. 323-353.
Synthesis of amino acids requires the introduction of nitrogen, but the energy value of protein is only slightly greater than that of carbohydrate.* One would expect to find that ammonia would be the most easily available supply of nitrogen for the amine group, but higher plants absorb nitrates and use them for synthesis.

The inter-conversion of carbohydrates, fats and proteins is part of the subject of metabolism which deals with the chemical changes that occur in cells. It is dealt with here because it is a synthetic process by which a higher concentration of energy is produced by the formation of fat.

Most of the evidence to be adduced is furnished from animal sources, but the steps in the process are probably alike in the animal and vegetable kingdoms.

In order to follow these changes we must know something about the intermediate stages. We can assume that the various steps are reversible, thus the changes observed in the decomposition of the different substances can be considered as steps in the processes of synthesis.

The great difficulty of the problem of metabolism is that the intermediate stages are difficult to demonstrate. The carbohydrates and fats are completely decomposed into water, and carbon dioxide and the proteins to the same substances, with the addition of nitrogenous waste products, such as ammonia.

**METHODS USED TO DETERMINE INTERMEDIATE STAGES IN METABOLISM**

The experimental methods are various, but the following are the general methods employed. Some substance is administered to an animal and the result observed. Thus feeding animals on a diet rich in carbohydrate leads to the formation of fat, hence we must look for some means by which carbohydrate can be turned into fat.

By a comparison of the structural formulae of carbohydrate and of fats, and by a study of the reactions of these substances, a chemical analogy is made out indicating some possible line of conversion of the one into the other.

After some plausible hypothesis has been developed, it is tested experimentally. If the results of these experiments are not explicable on the hypothesis which prompted the experiments the hypothesis must be altered and new experiments performed.

* 5·3 Calories as contrasted with 4·1 Calories. In animal catabolism protein yields only 4·1 Calories as it is not completely oxidised.
The experimental procedures are as follows:

(a) Various chemical substances, which form compounds with some of the possible intermediate products, are administered. The recovery of compounds of the intermediate products with the substance administered does not prove that the former is a normal stage in metabolism, but if the compound is not formed the evidence is plainly against there being any such intermediate product.

In order to favour the excretion of the compound of the intermediate product large doses of the original substance may be given along with the substance that forms the compound with the intermediate product.

(b) The supposed intermediate product may be administered to an animal and the resulting products isolated. If the same end products are produced that are yielded by the original substance the substance administered may be an intermediate stage, but if the same end products are not produced the substance administered is probably not an intermediate stage in metabolism. The only reservation is that substances administered in large doses cannot be so conveniently dealt with as when they are slowly produced in the cells. For instance, the intermediate substance may be poisonous in large doses or its metabolism may lead to some other intermediate substance which cannot be further altered with sufficient rapidity.

In other cases the supposed intermediate substance may be combined with some substance resistant to oxidation and the fate of this compound investigated.

(c) Surviving organs may be perfused with blood or saline solution containing certain substances, and the liquid, after perfusion, examined for changes in composition. Allied to this method is the preparation of extracts and testing these extracts on various substances to find out if any enzymes are present. This method is especially useful in tracing the details of metabolism and for allocating the various steps in metabolism to the different organs.

(d) Pathological conditions sometimes lead to the excretion of unusual substances, and these conditions may be useful in furnishing evidence as to the intermediate stages in metabolism.

When we turn to the results obtained we find that much more is known about the fate of fatty acids than about the fate of carbohydrates. The reason for this difference is that the products are easier to separate and identify in the former than in the latter case.
We shall first of all examine the metabolism of carbohydrates and then that of fats and proteins; finally we must look for substances which may be intermediate links for the interconversion of carbohydrates, fats and proteins.

**Metabolism of Carbohydrates**

By hydrolysis all carbohydrates yield glucose in the body, hence we must study the decomposition of glucose. The earliest observations were those of Wiedemann* and of Schmiedeberg and Meyer,† who found that when camphor was administered to animals its poisonous action was neutralised by combining it with glucuronic acid. The glucuronic acid was presumed to be the first oxidation product of glucose. After starvation, when the carbohydrate stores have been depleted, less camphor can be neutralised, but if glucose is given along with the camphor the toxic action of the camphor is more readily withstood. The further stages of decomposition should lead to the formation of $d$-saccharic acid, oxalic acid, carbon dioxide and water, but the experimental evidence is not in favour of this chain of oxidation products. It appears probable that glucose can give rise to glucuronic acid, but that under normal conditions a very small proportion of the sugar oxidised passes through the stage of glucuronic acid.

The main line of glucose destruction seems to lead to the formation of lactic acid, one molecule of glucose giving rise to two molecules of lactic acid. The intermediate products are possibly glyceric aldehyde or dihydroxyacetone. The formation of lactic acid from glucose is not improbable from the chemical point of view, as the various stages can be imitated outside the body. Glucose, when acted upon by alkali, yields pyruvic aldehyde and pyruvic aldehyde on treatment with alkali yields lactic acid. Glyceric aldehyde and di-hydroxy acetone yield lactic acid after treatment with alkali. Dakin and Dudley have recently found an enzyme glyoxalase which can convert pyruvic aldehyde into lactic acid.‡

The formation of lactic acid explains the increase in acidity so frequently found in tissues during activity. Muscle, for instance, in the resting condition contains no lactic acid, but during activity lactic acid is formed. The removal of

the lactic acid is brought about by some process requiring the presence of oxygen.* Accompanying the activity there is a decrease in the amount of glycogen in the tissue.

The removal of lactic acid requires the presence of oxygen, but it does not necessarily lead to the oxidation of lactic acid. The lactic acid may be reformed into glucose or converted into fats or amino acids. One portion may be oxidised in order to furnish the energy to convert another portion into some of the substances mentioned above.

If glycosuria is produced, as for example by the injection of phloridzin, the amount of sugar excreted in the urine can exceed by a large quantity the amount of carbohydrate stored in the body. This sugar must be formed from other substances, such as amino acids or fats.

During phloridzin glycosuria various substances can be administered. If an increase in the excretion of sugar occurs after the administration of any of these substances it is probable that sugar can be formed from those furnishing such an increase. In the case of nitrogenous substances a study of the increased nitrogen excretion furnishes a basis for the comparison of how completely the substances are converted into carbohydrate.

The results obtained from such experiments show that carbohydrate can be formed from glycerine, lactic acid, propyl alcohol, glycin, alanin and asparagin.†

Beyond the formation of lactic acid from glucose the further fate of carbohydrates has not yet been determined.

Hexoses may give rise to pentoses and pentoses may be converted into hexoses.

Metabolism of Fats

The oxidation of fats is preceded by the hydrolysis of the fats into glycerine and fatty acid. The glycerine can form carbohydrate, or it may be oxidised completely, thus we can confine our attention to the oxidation of the fatty acids. Their oxidation depends upon the chemical constitution of the acids. All the fatty acids found in nature contain an even number of carbon atoms which suggests that the building up and destruction of acids involves, as a rule, two carbon atoms at a time.

Thus, if we combine fatty acids with a phenyl group, which is not oxidised in the body, we can show the removal of two

carbon atoms at a time. The following table* illustrates this point, the excretory products being the compounds formed by uniting the aromatic acids with glycine $\text{NH}_2\text{CH}_2\text{COOH}$:

**Table XVIII**

<table>
<thead>
<tr>
<th>Acid.</th>
<th>Formula</th>
<th>Oxidation product.</th>
<th>Excreted as</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>$\text{C}_6\text{H}_5\text{COOH}$</td>
<td>Not oxidised</td>
<td>Hippuric acid</td>
</tr>
<tr>
<td>Phenylacetic acid</td>
<td>$\text{C}_6\text{H}_5\text{CH}_2\text{COOH}$</td>
<td>&quot; &quot;</td>
<td>Phenylacetic acid</td>
</tr>
<tr>
<td>Phenylpropionic acid</td>
<td>$\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>Benzoic acid</td>
<td>Hippuric acid</td>
</tr>
<tr>
<td>Phenylbutyric acid</td>
<td>$\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>Phenylacetic acid</td>
<td>Phenylacetic acid</td>
</tr>
<tr>
<td>Phenylvaleric acid</td>
<td>$\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>Benzoic acid</td>
<td>Hippuric acid</td>
</tr>
</tbody>
</table>

These results indicate that the oxidation always occurs at the second carbon atom from the carboxyl group, or in chemical nomenclature in the $\beta$ position.† Further support to the $\beta$-oxidation hypothesis is given by the formation of $\beta$-hydroxybutyric and of aceto-acetic acid in diabetes and other conditions, especially when large quantities of fat are being oxidised.

It is believed that $\beta$-hydroxybutyric acid and aceto-acetic acid are normal stages in metabolism, but that they are relatively less easily oxidised than the other stages. Hence when large quantities of fat are being used they accumulate in the cells, escape into the blood and appear in the excretions.

Their presence in large quantities is dangerous because they must be neutralised by the alkalies of the body; therefore, the body suffers from loss of alkali and coma results. Perfusion of various acids through the liver gives rise to aceto-acetic acid ($\text{CH}_3\text{COCH}_2\text{COOH}$), as shown by the following table.‡

**Table XIX**

<table>
<thead>
<tr>
<th>Normal Fatty Acid.</th>
<th>Formation of aceto-acetic acid.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid, $\text{CH}_3\text{COOH}$</td>
<td>-</td>
</tr>
<tr>
<td>Propionic Acid, $\text{CH}_3\text{CH}_2\text{COOH}$</td>
<td>-</td>
</tr>
<tr>
<td>Butyric Acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>+</td>
</tr>
<tr>
<td>Valeric Acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>-</td>
</tr>
<tr>
<td>Caproic Acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>+</td>
</tr>
<tr>
<td>Heptylic Acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>-</td>
</tr>
<tr>
<td>Octoic Acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>+</td>
</tr>
<tr>
<td>Nonoic Acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>-</td>
</tr>
<tr>
<td>Decoic Acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$</td>
<td>+</td>
</tr>
</tbody>
</table>

Removal of two carbon atoms at a time can give acetoacetic acid only in those cases where there are an even number of carbon atoms in a chain of four carbon atoms or more, that is in the cases of Butyric, Caproic, Octoic and Decoic Acids.

Aceto-acetic acid readily decomposes into acetone, and it is also reduced to β-hydroxybutyric acid. These three substances are usually found together and are spoken of as "acetone bodies."

The β-oxidation hypothesis is a curious instance where the biological chemist seems to have developed a line apart from the organic chemist. At one time objection was made to this hypothesis that in all in vitro oxidations the α or carbon atom next to the carboxyl group was the one attacked. Dakin, on the other hand, was able to show that one oxidising agent was capable of producing β-oxidation. On neutralising a fatty acid and digesting it at 37°C with hydrogen peroxide, oxidation occurred in the β-position,* thus the chemical analogy was developed by a biological chemist in support of an hypothesis which was almost discredited because it was contradictory to the chemical facts known at the time of its promulgation.

The unsaturated fatty acids probably take up water-forming hydroxy acids and these are oxidised like the hydroxy acids formed from saturated acids. The double bond is frequently shifted before oxidation occurs.

Branched chains lose their side chains and undergo oxidation along similar lines to those of the corresponding straight chain acids.

We can sum up the oxidation of fatty acids by saying that they are oxidised in the β position probably with the formation of a ketone acid. The ketone acid is reduced asymmetrically to the optically active hydroxy acid. The liver is capable of reducing the ketone acid to hydroxy acid, and of oxidising the hydroxy acid, thus indicating the probable presence of an enzyme which accelerates the two reactions.

The absence of fatty acids with an odd number of carbon atoms indicates that the synthesis as well as the oxidation involves two carbon atoms at a time.

Raper† has suggested that this synthesis is due to aldol condensation, in which two molecules of acetaldehyde condense to form aldol which, by simultaneous oxidation and reduction, yields butyric acid.

Three or four molecules of acetaldehyde give fatty acids with six or eight carbon atoms respectively, and so upwards to the higher fatty acids.

Another hypothesis for the formation of fatty acids is that of Smedley, who pointed out that pyruvic acid is decomposed to form acetaldehyde, which condenses with pyruvic acid to form an hydroxylated acid. This acid is converted into an unsaturated acid, and then reduced to a saturated acid. Removal of carbon dioxide from the above unsaturated acid gives an aldehyde with four carbon atoms which can condense with a further molecule of pyruvic acid.

(1) \[ CH_3 - CO - COOH \rightarrow CH_3CHO + CO_2 \]

Pyruvic acid

Acetaldehyde

(2) \[ CH_3CHO + CH_3COCOOH \rightarrow CH_3CHOHCH_2COCOOH \]

CH₃CH : CHCO-COOH

Pentylinic α-keto-acid.

(3a) \[ CH_3CH : CHCO-COOH + O = CH_3CH : CH'-COOH \]

Butyric Acid

(4a) \[ CH_3CH : CHCOOH + 2H = CH_3CHCH_2COOH \]

Pentylenic α-keto-acid. Compare with reaction (1.)

(3b) \[ CH_3CH : CHCOOCH = CH_3CH : CH-CHO + CO_2 \]

(4b) \[ CH_3CH : CHCHO + C - COOCH = \]

(Compare with reaction 2.)

CH₃CH : CHOH.CH₂COCOOH

and by reactions similar to (3a) and (4a) Caproic Acid is formed, reduction taking place as a final step.*

Some such method of synthesis is extremely probable as pyruvic acid is formed in the body. It should be noted that the steps of synthesis do not correspond to those of oxidation, but if the compound on the right of equation (2) were oxidised as in (3a), and then water added, we would have CH₃CHOHCH₂COOH, or β-hydroxybutyric acid from which the other "acetone" bodies can be formed.

Metabolism of Proteins

The oxidation of proteins can be treated as the oxidation of their constituent amino acids. The first stage is the

removal of their amine groups. This can be accomplished either by hydrolysis giving rise to ammonia and an hydroxy acid, or by oxidation giving rise to ammonia and a ketone acid.

\[
\begin{align*}
R & \quad \text{Hydroxy acid.} \\
\text{H} - \text{C} - \text{OH} + \text{NH}_3 & \rightleftharpoons \quad \text{H} - \text{C} - \text{NH}_2 & \rightarrow \quad \text{C} = \text{O} + \text{NH}_3 \\
\text{COOH} & \quad \text{Amino acid.} & \quad \text{COOH} & \quad \text{Ketone acid.}
\end{align*}
\]

Although these two reactions may occur it seems probable that the decomposition of amino acids gives rise to ketone acids, and any hydroxy acids are formed by reduction of the ketone-acids.

In this way the nitrogen is removed and the remainder of the molecule can be oxidised by the removal of the terminal carboxyl group giving rise to a fatty acid with one less carbon atom. The fatty acid is then oxidised in the way described above.

That the removal of nitrogen is a reversible reaction is shown by the synthesis of certain amino acids from the ammonium salt of the corresponding ketone acids.*

The removal of the carboxyl group from amino acids leaves a nitrogenous base. These bases are formed in the body as exemplified by the formation of β-imidazomethylamine from histidine.

\[
\begin{align*}
\text{CH} - \text{NH} & \xrightarrow{\text{C} - \text{N}} \text{CH} = \\
\text{CH}_2\text{CHi(NH}_2\text{)COOH} & \quad \text{CH}_2\text{CH}_2\text{NH}_2
\end{align*}
\]

The physiological action of these amines is of considerable importance.

**INTERCONVERSION OF CARBOHYDRATES, FATS AND PROTEINS**

On looking over the previous paragraphs we see certain relationships between these various classes of compounds. Glucose can be converted into pyruvic aldehyde. Pyruvic aldehyde by the action of glyoxalase can be converted into lactic acid. Alanine can be deamidised with the formation of pyruvic aldehyde and pyruvic acid has been suggested as the starting point for the synthesis of fats. We can express these relations by the following diagram†:

The horizontal line composed of lactic acid, pyruvic aldehyde and alanine is an interesting example of a reversible reaction. The formation of lactic acid causes an increase in acidity and this reaction is inhibited by acid. Thus it is necessary to neutralise the acid when one wishes to get a good yield of lactic acid by the action of glyoxalase on glyoxal (pyruvic aldehyde).

The removal of ammonia from alanine furnishes a base capable of neutralising acids. Dakin points out that these changes are of importance in maintaining the neutrality of the cells.

The principle underlying these reactions seems to be contained in Le Chatelier's Theorem (p. 59). An increase in acidity would tend to prevent a reaction which takes place with the formation of acid. We therefore see that these interconversions are governed by principles which apply in general chemistry: the actual amount of change being regulated by the law of mass action and by chemical affinity.

Not all amino acids can be shown to be formed from carbohydrate and fat. The diagram given above is an instance of one group where conversion of amino acid to fat or carbohydrate can be proved by a series of experiments.

Although we cannot say that all the reactions are reversible the subjects discussed in this chapter indicate the way in which the plant may form fats and proteins from the carbohydrates.

The carbohydrates can be turned into pyruvic aldehyde, pyruvic aldehyde can condense through pyruvic acid to form fatty acids or in the presence of ammonia it can form alanine. There is one curious point, namely, that plants absorb nitrogen in the form of nitrates and many of them cannot thrive on ammonia as a sole source of nitrogen, yet the formation of amino acids seems to require the presence of ammonia.

GENERAL REFERENCE
Section III
CATABOLISM

CHAPTER VIII
TRANSFERENCE OF FOOD MATERIALS : DIGESTION

As the result of synthesis in the plant a reserve of materials is stored in various localities. Seeds, tubers and bulbs are examples of storehouses intended to provide for the future development and growth of the plant. These storehouses are, however, often attacked by parasitic plants and by animals.

The stored materials are deposited in an insoluble non-diffusible form, but the materials are transferred from the place of formation to the storehouses and from the storehouses to the place where they are used, in the form of soluble diffusible substances. These changes involve the reversible action of enzymes and the conditions which determine the separation of a new phase. These matters and their relation to the Law of Mass Action are discussed in this and the following chapter. All classes of substances are accumulated as reserves, thus we find carbohydrates, fats and proteins in varying proportions. Foods are classified into carbohydrate, fatty and protein foods depending on the varying proportions of one or other of these classes of substances.

Carbohydrate is stored largely in the form of starch which is an insoluble substance, fats are insoluble in water and proteins form colloidal solutions which cannot diffuse through membranes. In order to transport these substances from one place to another they must be converted into simple soluble diffusible substances.

The various kinds of substances required for the maintenance of the body are best dealt with under the heading of metabolism, but in this chapter we will describe the means by which the insoluble or colloidal materials in the food are turned into soluble diffusible substances, that is the process of digestion.
A resting seed shows no enzymic activity, but when it is exposed to proper conditions of temperature and moisture, growth occurs with the utilization of the food stores. During the process of growth more enzyme can be extracted than from the resting seed. On this fact depends the process of making malt. Barley seeds are allowed to germinate and the growth stopped after several days. The extract of these germinated seeds forms the well-known malt extract which contains a large amount of enzyme together with the products of its activity.

In some seeds the enzymes are formed by epithelial cells forming what is termed the scutellum, a layer of tissue lying between the embryo and the stored food (endosperm). The manner in which the enzyme is set free from its precursor (zymogen) is not known, but it is of interest that Reychler* has found that dilute acid liberates the active enzyme.

The processes of food transference are more complicated in animals and the details are better worked out than in plants. We shall therefore devote our attention to the processes of digestion and absorption in mammals and refer to special processes in plants and other animals afterwards.

Digestion in animals exhibits one marked difference from food transference in plants. Animals use mechanical processes to reduce the coarse particles of food to a state of fine subdivision. This is a great advantage as the rate of solution of a solid is dependent on its surface and when a solid is subdivided its surface is increased, hence digestion is accelerated by the subdivision of the food. In addition, stirring and other movements occur which facilitate digestion and absorption by removing the concentrated solution from the surface of the solid particles, thus allowing more of the substance to dissolve and by promoting the contact of fresh portions of the food with the enzymes and with the wall of the alimentary canal. These movements cannot be dealt with from the chemical side, they must be studied with the allied subject of animal physiology.

The epithelial cells which produce the enzymes are collected into groups called glands. The active substances can be obtained from these glands by extraction, with glycerine, salt solution or water, and the enzymes can be purified by precipitation with alcohol or by carrying them down in absorption with certain insoluble materials. The extracts or the purified enzymes obtained from them can be studied in their relation.

to the various food substances. Where the gland is situated away from the surface of the alimentary canal and is connected with the surface by a definite tube or duct, the secretion may be collected before it is mixed with the food materials. Owing

Fig. 23.—Diagram showing the general arrangement of the Alimentary Canal.

The salivary glands open into the mouth by ducts, one pair opening at the side of the cheek above the tongue, and two pairs below the tip of the tongue. Where the duodenum turns sharply beyond the stomach the entrance of the pancreatic duct and bile duct is seen: connected with the latter is the gall bladder, which stores the bile between its periodic discharges into the intestine. After the duodenum is seen the jejunum (J) and ileum (I) which form the rest of the small intestine.

The large intestine is composed of appendix, caecum (G), transverse colon (T), sigmoid colon (S), and rectum (R).

(Redrawn from "Elementary Physiology," Huxley. Macmillan).

to the presence of definite glands and ducts the processes of solution by the enzymes are more easily studied in animals than in plants where the enzymes are not so definitely separated from the food supplies.
The first stage of digestion occurs in the mouth, where the food is mixed with the secretions of the salivary glands. In some animals (dog for instance) these secretions are merely lubricating but in others the secretions contain an enzyme called ptyalin or salivary amylase. The amylase has the power of converting the polysaccharides, cooked starch, glycogen and dextrin into simpler carbohydrates.

The changes that take place in the hydrolysis of starch are indicated in the following table:—

<table>
<thead>
<tr>
<th>Substance</th>
<th>Appearance of solution</th>
<th>Colour with iodine</th>
<th>Action of alcohol, salts, etc.</th>
<th>Effect of heating with metallic hydroxides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>Opalescent Clear</td>
<td>Blue Blue or purple Red</td>
<td>Easily precipitated Easily precipitated</td>
<td>No reduction No reduction</td>
</tr>
<tr>
<td>Soluble starch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrodextrin</td>
<td>Clear</td>
<td>Yellow</td>
<td>Less easily precipitated</td>
<td>No reduction</td>
</tr>
<tr>
<td>Achroodextrin</td>
<td>Clear</td>
<td>Yellow</td>
<td>Still less easily precipitated</td>
<td>No reduction</td>
</tr>
<tr>
<td>Maltose</td>
<td>Clear</td>
<td>Yellow</td>
<td>Not precipitated</td>
<td>Reduced</td>
</tr>
<tr>
<td>p-Glucose</td>
<td>Clear</td>
<td>Yellow</td>
<td>Not precipitated</td>
<td>Reduced</td>
</tr>
</tbody>
</table>

Glycogen undergoes a similar series of changes, passing from glycogen to erythrodextrin, etc.

Although the above represents the general outline of carbohydrate hydrolysis, we must remember that it is possible to isolate several erythrodextrins and several achroodextrins, depending on the ease with which they are precipitated by salts or by alcohol. In the actual hydrolysis all these substances occur in the mixture. It seems probable that the enzyme splits off one molecule of maltose from the starch and then a second molecule, so that one can represent the hydrolysis as taking place in a series of stages as follows.*

```
Starch
  | Soluble Starch
    | Maltose
  | Erythrodextrin I
    | Maltose
  | Erythrodextrin II
    | Maltose (and so on)
```

The formula for starch is \((C_6H_{10}O_6)_n\), where \(n\) is approximately thirty, and the formula for maltose is \(C_{12}H_{22}O_{11}\), therefore there should be about thirteen intermediate stages between starch and maltose, each varying slightly in physical and chemical properties from the stage on either side of it. By prolonged action in vitro some of the maltose is converted into glucose, but in vivo the hydrolysis is supposed to stop at the stage of maltose.

The secretion of saliva is regulated by a nervous reflex. That is, the secretion is poured out in response to an impulse passing down a nerve from the central nervous system. The impulse from the central nervous system is the result of a nerve impulse caused by stimulation of the sense organs of taste, smell, sight, etc. Nervous reflexes are used when a quick response is required. As the food does not remain in the mouth, it is obvious that the saliva is required immediately.

In the glands the constituents that are to form the saliva exist in the form of granules. The granules are formed during rest and they accumulate in the cells. As the result of activity the granules decrease in number and only a few are left close to the lumen of the duct.* The ptyalin is present in an inactive form called ptyalinogen, which can be rendered active by treatment with dilute acid. Such inactive forms are called zymogens.

**Table XXI**

The composition of saliva is †:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>99.365</td>
</tr>
<tr>
<td>Mucin</td>
<td>0.275</td>
</tr>
<tr>
<td>Other organic solids</td>
<td>0.135</td>
</tr>
<tr>
<td>Chlorine</td>
<td>0.052</td>
</tr>
<tr>
<td>(P_2O_5)</td>
<td>0.067</td>
</tr>
<tr>
<td>Ash insoluble in water</td>
<td>0.026</td>
</tr>
<tr>
<td>Other inorganic</td>
<td>0.080</td>
</tr>
</tbody>
</table>

In addition saliva contains a small and variable amount of Thiocyanate.

The rate of hydrolysis of starch can be measured in various ways. The time necessary to convert all the products to the stage where they no longer give a colour with iodine can be used for comparison‡ or the stage at which the blue colour of starch with iodine gives place to the red colour of erythrodextrin with iodine may be used as an end point. § The copper-

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reducing power of the solution and the rotation of polarised light are, however, more accurate.*

Process of Secretion.—The production of saliva by the salivary glands is an example of secretion which is well worth studying. There has been much discussion as to whether secretion can be explained on purely physical grounds. In one sense it is quite true that all processes can be explained on chemical and physical grounds. The Law of Conservation of Energy holds in biological processes, hence there must be a balance between the income and output of energy. On the other hand, a mechanical explanation of the phenomena is not forthcoming.

The production of saliva cannot be due to filtration because if the duct of a salivary gland is attached to a canula and the canula connected with a manometer, on stimulation of the nerve to the gland the pressure in the duct rises higher than the blood pressure. Nor can the secretion be due to osmosis because the concentration of the saliva is less than that of the blood. Hence the liquid should pass back from the saliva to the blood. Even if the concentration of the secretion, as happens in the case of other glands, were greater than the concentration of the blood we must still enquire how the more concentrated solution was separated from the blood. Further differences in surface tension do not give any plausible explanation of the formation of saliva.

In any case work is done and at the same time increased oxidation occurs. It is obviously futile to discuss whether this is a "vital" phenomenon or a physico-chemical process. What we have to find out is the manner in which the energy derived from oxidation is converted into the work of secretion. When we know this the problem is settled without further discussion. At the present time, the best way to deal with the process of secretion is to look upon it as a sort of pumping action by the cell; the energy for the performance of work is produced by the oxidation that occurs in the cells.

In this discussion we have not touched upon the difference in the chemical composition of the secretion from that of the blood. In some cases the gland separates some substance which is present in the blood (urea by the kidneys), and in others the gland manufactures the material of the secretion (lactose in mammary gland). Even in the former case the concentration is increased so work must be done against the osmotic pressure. Similar considerations apply to the secre-

tion of hydrogen ions by the gastric glands. The high concentration of hydrogen ions in gastric juice can be produced only by the transformation of energy and cannot be due to diffusion, osmosis, filtration or surface tension.

Root pressure seems a similar condition. The pressure produced in the stem of a plant* cannot be explained on known physical processes. It would be interesting to know whether there is increased oxidation in the root cells during the production of the flow of liquid.

It is well to bear these points in mind, because we can concentrate our attention on the behaviour of the cell and not spend our time trying to explain what is not yet clearly understood.

**Digestion in the Stomach**

The next stage of digestion occurs in the stomach. The gastric juice contains acid and this acid destroys the ptyalin. As each portion of food passes from the oesophagus into the stomach it is forced into the centre of the preceding masses of food. The result is that the food forms a series of concentric layers, the outside being the earliest swallowed and the inside the last swallowed.† The acid must diffuse through these various layers, hence the central portions do not become acid for some time. The action of ptyalin is therefore not confined to the short time that the food is in the mouth, but it may continue for from twenty to forty-five minutes, depending on the mass of food which must be rendered acid.

The chief digestive action brought about by the gastric juice is due to the enzyme pepsin. This acts in conjunction with the hydrochloric acid which causes the acidity of the secretion. The action of pepsin is to convert proteins into peptones according to the scheme shown on the next page.

In gastric digestion the metaprotein is that which exists in acid solution. The terms acid and alkali metaprotein are misleading. When the protein forms a salt with acid the protein acts as a base and acquires a positive charge, hence it is wrong to speak of the metaprotein in an acid solution as acid metaprotein. Similarly the metaprotein in alkaline solution should not be called alkali metaprotein. We ought to say metaprotein in acid solution and metaprotein in alkaline solution respectively.

The presence of acid is necessary for the action of pepsin and the acid may be regarded as a co-enzyme. The activity

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is apparently due to a positive ion formed by an amphoteric electrolyte combined with an acid. The considerations on the action of trypsin (p. 63) can be applied to the action of pepsin, only we must substitute positive for negative ions and bear in mind that increase in hydrogen ions increases the activity instead of decreasing it.

We can show in various ways that the gastric juice contains free inorganic acid. By indicators or by hydrogen electrodes the concentration of hydrogen ions can be shown to be greater than would occur with organic acids. The amount of chlorine is more than sufficient to combine with all the bases present, so the excess must exist as hydrochloric acid.

The optimum concentration of acid is about 0·2 per cent. of hydrochloric acid. The gastric juice contains when secreted about 0·5 per cent. hydrochloric acid, but some of this is neutralised by the food and if the acidity is still too high alkali passes into the stomach from the duodenum. The concentration of hydrogen ions gives the true acidity and the rate of digestion depends upon the concentration of hydrogen ions. In pathological states the total amount of hydrochloric acid is sometimes important as it indicates the amount of acid secreted by the cells. The total hydrochloric acid can be estimated by incinerating a sample of the gastric juice and also another sample to which excess of alkali has been added. The chlorides in the ash of each sample are estimated and the

<table>
<thead>
<tr>
<th>Substance</th>
<th>Colour with copper sulphate and alkali</th>
<th>Effect of Ammonium Sulphate</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>Purple</td>
<td>Precipitates globulin</td>
<td>Reactions depend upon the nature of the protein.</td>
</tr>
<tr>
<td>Metaprotein</td>
<td>Purple</td>
<td>Precipitates</td>
<td>Precipitates at neutral point.</td>
</tr>
<tr>
<td>Heteroprotose</td>
<td>Pink</td>
<td>Precipitates</td>
<td>(Insoluble in distilled water.</td>
</tr>
<tr>
<td>Protoproteose</td>
<td>Primary Proteoses</td>
<td>Not precipitated</td>
<td>Soluble in distilled water.</td>
</tr>
<tr>
<td>Deuteroprotose, secondary protease</td>
<td>Pink</td>
<td>Not precipitated</td>
<td>Does not dialyse.</td>
</tr>
<tr>
<td>Peptone</td>
<td>Pink</td>
<td>Not precipitated</td>
<td>Dialyses.</td>
</tr>
<tr>
<td>Polypeptides</td>
<td>Blue</td>
<td>Not precipitated</td>
<td>Not usually found in gastric digestion, but found in pancreatic digestion.</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Blue</td>
<td>Not precipitated</td>
<td></td>
</tr>
</tbody>
</table>

TABLE XXII
difference in the chlorides gives the amount of hydrochloric acid. In the first incineration all the chlorine not combined with fixed bases is driven off whilst in the second owing to the excess of alkali all the chlorine is retained. The difference is therefore due to hydrochloric acid free or combined with organic bases such as proteins.

The indicators used to show the presence of acid in gastric contents are numerous, but the following are good examples:

<table>
<thead>
<tr>
<th>Indicator</th>
<th>(-\log[H])</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol Blue</td>
<td>1-2-2.8</td>
<td>Does not change colour if only organic acids are present.</td>
</tr>
<tr>
<td>Methyl Violet</td>
<td>4</td>
<td>1 in 10,000 HCl, does not change colour if only organic acids are present.</td>
</tr>
<tr>
<td>Gunzberg’s Reagent</td>
<td>4</td>
<td>Acetic acid gives colour, but it disappears on warming.</td>
</tr>
<tr>
<td>Tropæolin oo</td>
<td></td>
<td>Reacts with organic acids.</td>
</tr>
<tr>
<td>Congo Red</td>
<td></td>
<td>Acetic acid gives colour, but it disappears on warming.</td>
</tr>
<tr>
<td>Phenolphthalein</td>
<td>8-9</td>
<td>Gives total acidity including carbonic acid to stage of bicarbonate.</td>
</tr>
</tbody>
</table>

The methods used to show the rate of digestion of protein are numerous. They consist in comparing the rate of digestion of a protein by an enzyme with a control containing exactly the same materials but with the enzyme destroyed (by heat) before adding it to the other ingredients. The methods depend on measuring either the rate of solution of an insoluble protein or the rate of production of nitrogenous compounds which are not precipitated by protein precipitants.

**Methods based on rate of solution of insoluble protein:**

1. Rate of solution of fibrin.*
2. Depth of colour due to liberation of a dye from stained fibrin.†
3. Length of glass tube from which coagulated egg albumin ‡ or gelatine § have been dissolved.

**Methods based on liberation of soluble nitrogenous compounds:**

1. Removal of proteins by heat coagulation and estimation of nitrogen remaining in the solution by Kjeldahl’s method.

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Removal of proteins by trichloracetic acid (or other protein precipitant) and estimation of the nitrogen remaining in the solution.

Associated with the digestive action of gastric juice is the power of coagulating milk. The coagulation is due to the enzyme rennin acting upon the caseinogen of the milk. The coagulation occurs in two stages. The first is due to the action of rennin in producing a soluble product (paracaseinogen) from the caseinogen and the second is due to the calcium of the milk forming an insoluble substance (casein) from the paracaseinogen.

There is some doubt as to whether pepsin and rennin are the same or different enzymes. Some investigators state that there are two separate enzymes, whilst others declare that there is only one. A modification of these views is that there is only one substance but that the actions are due to two different side chains in the same molecule. Rennin action is found throughout the animal and vegetable kingdoms wherever a proteoclastic enzyme occurs. The only animals that receive milk are mammals, so the presence of a milk-coagulating enzyme in other animals is difficult to explain. It seems that the coagulation of milk may be the first stage of proteoclastic activity and the coagulation is a purely accidental circumstance.* There does not seem to be any functional advantage of the coagulation as the acid of the gastric juice is capable of precipitating caseinogen from its solution. The fact that it is possible to prepare a rennin solution with little or no peptic action and a pepsin solution with little or no rennin action indicates that they are two separate enzymes.

In the gastric juice is found a Lipase which acts upon emulsified fats but not upon unemulsified fats.†

Like the salivary secretion the secretion of gastric juice is caused by a reflex consisting of impulses from the organs of sight, smell, taste, etc., to the central nervous system and from the central nervous system to the stomach by the vagus nerves. The secretion is not so prompt as the secretion of the salivary glands as stimulation of the nerves going to the stomach is followed by a latent period of five minutes before the secretion appears.

There is a second mechanism, of a more chemical nature, which aids in causing the secretion of gastric juice. Edkins has shown that by extracting the pyloric end of the stomach with solutions of meat extract, dextrin, glucose, etc., a solution is obtained which on injection into the blood stream causes a secretion of acid and pepsin.*

As the active substance is extracted by the products of digestion of starch and of protein it is not formed until digestion has commenced. The time at which it is formed is after the external stimuli of taste, smell, etc., have disappeared, hence it is well adapted to promote the later stages of gastric digestion.

Chemical substances which are formed in one part and stimulate another part to activity have been termed Hormones.† Other examples of hormones will be discussed later on. Edkins suggested the name Gastrin for the hormone found by him in the stomach.

**Table XXIV**

*Composition of Gastric Juice.*†

<table>
<thead>
<tr>
<th>Component</th>
<th>Parts per 1,000.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (Ferment, etc.)</td>
<td>15.742</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>2.022</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1.381</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.818</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.53</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>4.517</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>2.971</td>
</tr>
<tr>
<td>Magnesium phosphate</td>
<td>0.357</td>
</tr>
<tr>
<td>Ferric phosphate</td>
<td>0.257</td>
</tr>
</tbody>
</table>

of which the first two are the only important items.

The discussion on the secretion of saliva applies to the secretion of gastric juice. The histological changes, disappearance of granules, etc., are the same. The secretion of acid is confined to one portion (fundus) in the glands of which are found certain large oval cells which stain readily with acid dyes (e.g. eosin). They are called oxyntic cells and are believed to be associated with the secretion of acid. The other portions of the stomach secrete the enzymes but no acid.

The food is retained in the stomach for some time, after which the pylorus opens and allows some of the contents to escape into the first portion of the small intestine (duodenum). The mechanism that regulates the escape of the stomach

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contents is a ring of muscle round the pyloric end of the stomach. Increase of acid in the stomach tends to cause this ring to relax, whilst acid in the duodenum and solid particles in the stomach tend to cause it to contract. During digestion the solid particles diminish and the acid increases, hence we have an increasing tendency for the pylorus to relax. As soon as any of the contents of the stomach escape into the duodenum, the acid in the duodenum causes the pylorus to close and remain closed until the acid is neutralised. Boldyreff says that excess of acid will cause such a violent contraction of the duodenum that the alkaline duodenal contents will be forced into the stomach and thus prevent too great an acidity in the stomach.* (See p. 120.)

**Digestion in the Duodenum**

Two secretions are mixed with the food in the duodenum, the bile from the liver and the pancreatic juice from the pancreas. Both these are alkaline and are useful in neutralising the acid of the gastric contents. The bile contains no enzymes but it aids the action of the enzymes that are found in the pancreatic juice.

**Bile.**—The analysis of bile shows marked differences in composition. If a fistula is made so that the bile drains away and is lost the bile becomes very dilute, hence we find that fistula bile is more dilute than bile obtained from the gall-bladder by operation.

**Table XXV**

*Composition of Human Bile† in parts per 1,000 by weight.*

<table>
<thead>
<tr>
<th>Component</th>
<th>Bladder bile</th>
<th>Fistula bile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile salts</td>
<td>97.0</td>
<td>10.1</td>
</tr>
<tr>
<td>Mucin and pigments</td>
<td>41.9</td>
<td>4.86</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>9.86</td>
<td>2.61</td>
</tr>
<tr>
<td>Fat</td>
<td>1.9</td>
<td>6.85</td>
</tr>
<tr>
<td>Soaps</td>
<td>11.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Lecithin</td>
<td>2.23</td>
<td>6.42</td>
</tr>
<tr>
<td>Total solids</td>
<td>170.3</td>
<td>29.8</td>
</tr>
<tr>
<td>Inorganic</td>
<td>—</td>
<td>9.2</td>
</tr>
<tr>
<td>Water</td>
<td>829.7</td>
<td>970.2</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>—</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The bile pigments are waste products from the decomposition of hæmoglobin. They give coloured reactions, due to oxidation, with nitric acid,‡ iodine, etc. Thus if some bile is

placed in contact with strong nitric acid a series of colours are seen ranging through green, blue, violet, red, yellow, from the bile towards the acid. The colour of bile from different animals varies. Herbivora have a bile which is predominantly greenish due to the green pigment biliverdin and carnivora have an orange bile due to the red pigment, bilirubin. The chemical relation of these pigments to other substances will be described later (p. r89).

The bile salts are the sodium salts of two acids, glycocholic and taurocholic acids. These are formed by the union of cholalic acid \((C_{24}H_{40}O_{5})^*\) with glycine \((CH_2NH_2COOH)\) and with taurin \((CH_2(NH_2)CH_2SO_2OH)\) respectively. They have the important property of decreasing the surface tension at an air-water surface, the importance of which will be considered later in this chapter.

The tests for bile acids are first of all a purple colour, produced by acting on the solution with strong sulphuric acid in the presence of cane-sugar.† The strong acid produces furfurol by acting on the cholalic acid and the furfurol condenses with the cane-sugar to produce the purple colour. The bile acids can also be detected by their action on the surface tension. Flowers of sulphur float on the surface of pure water but the presence of a little bile salt allows the sulphur to break through the surface and sink.‡ A quantitative measure of the amount of bile salt can be obtained by the stalagmometric method, which consists in comparing the number of drops given by a known weight of liquid with the same weight of liquid without bile salt. The drops must be obtained under standard conditions through a standard orifice.§

The bile acids are absorbed from the intestine into the blood, which passing directly back to the liver by the portal circulation gives up these substances to be passed into the bile once more. This cycle of changes is termed the circulation of the bile acids. Thus:—

* R. H. A. Plimmer, *Practical Organic and Biochemistry* (Longmans), 1915, p. 322, gives the formula as \(C_{26}H_{31}^*\)


The bile is formed continuously with an increase in rate of formation during digestion. The increase in rate is associated with an increased blood flow from the intestine containing products of digestion and the bile acids. These conditions may account for the increased secretion of bile or the increase may be due to secretion (see later). During the periods between digestion the bile is stored in the gall-bladder and during digestion it is forced into the intestine by the contraction of the muscular walls of the gall-bladder. The outpouring is regulated by a local nervous connection between the stomach and the gall-bladder.

Pancreatic Juice.—Digestion in the duodenum is brought about by three enzymes furnished by the pancreatic juice, trypsin, amylopectin (pancreatic amylase) and lipase.

The first of these is a proteoclastic enzyme which presents certain differences from pepsin. These differences are that it acts in an alkaline instead of an acid medium and that the hydrolysis proceeds further, giving rise more rapidly than does pepsin, to polypeptides and amino acids. The second of these differences may be due to the presence of a second enzyme, erepsin. The scheme given on p. 120 shows the steps of the hydrolysis, the metaprotein being the form that exists in alkaline solution and the two last stages are more abundant than with pepsin. The relation of the alkali to the activity of trypsin is described on p. 63.

Trypsin is not found as such in the pancreatic juice. It exists in an inactive form called trypsinogen. Whenever the pancreatic juice comes into contact with the mucous membrane of the duodenum the trypsinogen is converted into trypsin. The activation of trypsinogen is brought about by enterokinase which is an enzyme* formed by the mucous membrane of the duodenum.

Trypsinogen can be converted into trypsin by calcium salts.† This second method of activation is stated to be due to traces

of enterokinase already present in the pancreatic juice. The alkalinity of the solution makes a great difference to the rate of activation by enterokinase and neutralisation increases the rate of activation. Thus if the pancreatic juice is neutralised an amount of enterokinase, insufficient to cause rapid activation, can cause activation. The neutralisation is brought about by the interaction of the calcium salts with the sodium carbonate in the pancreatic juice, whereby calcium carbonate is precipitated.

\[ \text{CaCl}_2 + \text{Na}_2\text{CO}_3 = 2\text{NaCl} + \text{CaCO}_3 \]

The curve of activation is peculiar in that the rate increases as the reaction proceeds. Vernon believes that this is due to an unstable form of trypsin produced by the action of enterokinase and that this unstable form activates the trypsinogen.* Mellanby and Woolley, on the other hand, consider that the increase in rate of activation is not due to an unstable form of trypsin.† It seems possible that if trypsin is produced it will act upon proteins, converting them into amino acids. The amino acids can neutralise the alkali of the pancreatic juice, and as mentioned above neutralisation increases the rate of activation. Hence the increase in rate of activation may be due to a neutralising of the pancreatic juice by the products of protein digestion produced by the trypsin formed during the earlier stages of activation.

The methods for estimating the rate of digestion of protein have been mentioned in connection with pepsin, but one method applies to trypsin which does not give good results with pepsin. Formaldehyde condenses with ammonia compounds to form hexamethylene tetramine and with amino acids to neutralise the amine group. This forms the basis of a method to estimate the amount of amine groups set free by digestion, as each splitting of a CO NH group gives rise to a fresh NH\textsubscript{2} group. The solution is neutralised to phenolphthalein and neutral formaldehyde is added. The combination of formaldehyde with the NH\textsubscript{2} groups causes the amine group to lose its alkaline character, hence the carboxyl group becomes predominant and the amino acid becomes a stronger acid; the amount of alkali required to neutralise these acids gives an indication of the amount of amino acids in the solution. As the amino acids increase in amount during

tryptic digestion the amount of alkali gives a measure of the amount of protein digestion by trypsin.*

Digestion of caseinogen is another method for showing the digestive action of trypsin.†

Pancreatic amylase is very similar to salivary amylase, but is more energetic in its action. It can attack uncooked starch and it produces more glucose than does the latter. The production of glucose is probably due to the presence of some maltase, an enzyme that hydrolyses maltose.

Lipase hydrolyses fats with the production of glycerine and fatty acids. The fatty acids combine with alkali to form soluble soaps. Although the pancreatic juice is alkaline, the production of fatty acids and amino acids neutralises the alkali so that the intestinal contents are not sufficiently alkaline to produce a pink colour with phenolphthalein. Owing to the fat being insoluble the rate of hydrolysis depends on the extent of surface, or in other words on the degree of subdivision, hence emulsification aids the process of digestion of fat.

The manner in which bile aids the action of the pancreatic enzymes can be best dealt with in connection with the digestion of fat by lipase. Bile can dissolve fat, hence the concentration of fat in solution is increased and the rate of hydrolysis is also increased. The solvent power of bile for fat is due to the presence of the bile salts. These salts have the property of decreasing the surface tension at an air-water surface, and if the interfacial tension at an oil-water surface be decreased the emulsification of the oil will be facilitated. If the subdivision of the oil proceeds far enough a true solution is produced. Thus the bile salts aid the digestion of fats by increasing the surface owing to emulsification and by increasing the concentration of fat in solution. The fatty acids formed by hydrolysis of fats are also held in solution by the bile salts.‡

Pancreatic juice is stated to contain a milk-coagulating enzyme, but this may be associated with trypsin in the same way that rennin is said to be associated with pepsin.

The action of Lipase can be shown by the amount of fatty acid set free in the solution. Milk or any other fat emulsion can be sterilised and rendered faintly alkaline to phenolphthalein. By the action of lipase the fats are hydrolysed and

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the fatty acids set free. By measuring the amount of standard alkali necessary to neutralise the fatty acids set free and to once more render the solution faintly alkaline to phenolphthalein the rate of hydrolysis of fats can be estimated.

The secretion of pancreatic juice can be brought about by a nervous reflex just like the secretion of saliva or gastric juice, but the predominant influence is a chemical stimulus. Acid in the duodenum causes a secretion of pancreatic juice even after all the nervous connections of the pancreas have been severed. Bayliss and Starling showed that this was due to a substance which they called secretin.* In the mucous membrane of the duodenum is a substance called prosecretin, which on treatment with acid is turned into secretin. If the mucous membrane of the duodenum is boiled with dilute acid and the extract carefully neutralised, injection of the solution into the circulation causes a secretion of pancreatic juice.

The regulation of pancreatic secretion by secretin is adapted to secure complete neutralisation of the acid gastric contents. The passage of acid into the duodenum causes the formation of secretin. This causes a secretion of pancreatic juice which neutralises the acid. So long as there is any unneutralised acid, secretin is formed, and when all the acid is neutralised the formation of secretin ends. The secretin formed by the last amount of acid must be absorbed and pancreatic secretion continues until this secretin is used up. Hence there is always a slight excess of pancreatic juice over that necessary to neutralise the acid.

In the absence of acid it has been found that other substances can cause the formation of secretin. Thus as is usual in biological processes there is provision against the failure of the usual mechanism. Soaps seem able to produce secretin from the mucous membrane of the duodenum (Babkin).

Secretin is said to have a slight effect in causing secretion by the cells of the intestinal glands and of the liver.

There is an interesting transition from the secretion of saliva which is due to purely nervous influences, through the gastric secretion which is mainly nervous and followed by some chemical stimulus, to the pancreatic secretion which is mainly chemical. These differences correspond to the many sensations associated with food in the mouth and just before it passes into the stomach and the almost complete absence of sensation associated with food in the duodenum.

The relative amounts of the various enzymes in the pancreatic juice have been stated to be adapted to the different kinds of foods,* but this statement has been contradicted.

**Table XXVI**

*Composition of Pancreatic Juice obtained by the injection of Secretin.*†

<table>
<thead>
<tr>
<th>Component</th>
<th>Measurement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity, 10 cc.</td>
<td>= 12.7 cc</td>
<td>12.7 cc</td>
</tr>
<tr>
<td>Total solids</td>
<td>= 12.7 cc</td>
<td>1.6%</td>
</tr>
<tr>
<td>Total protein</td>
<td>= 12.7 cc</td>
<td>0.5%</td>
</tr>
<tr>
<td>Ash</td>
<td>= 12.7 cc</td>
<td>1.0%</td>
</tr>
<tr>
<td>Chlorides</td>
<td>= 12.7 cc</td>
<td>0.28%</td>
</tr>
</tbody>
</table>

During secretion changes take place in the granules like those described in connection with the salivary glands.‡

**Digestion in the Small Intestine**

The final stages of digestion are brought about by enzymes which seem to be associated with the cells lining the small intestine. The carbohydrates have been so altered that the polysaccharides have been mostly converted into maltose; some of the maltose has been converted into glucose. Cane-sugar may have been partially hydrolysed by the acid of the gastric juice and lactose is unaltered. The enzymes of the small intestine complete the hydrolysis of the carbohydrates to monosaccharides. Maltose is acted on by maltase, lactose by lactase and cane-sugar by invertase, producing respectively two molecules of glucose, one molecule of glucose and one of galactose, and one molecule of glucose and one of fructose. Thus the carbohydrates are converted into the simplest, most soluble and most easily diffusible products.

The presence of amylases as mentioned previously can be shown by the production from starch of some substance capable of reducing alkaline copper solution. Invertase can likewise be shown by the production from cane-sugar of some substance capable of reducing alkaline copper solution. Maltose and lactose are reducing sugars, so in order to show the presence of maltase and lactase we must either make a careful quantitative estimation of the reducing power of the solution before and after incubation with the supposed enzyme, or

Barfoed's Reagent (copper acetate in dilute acetic acid) may be used with proper precautions.*

The proteins have been hydrolysed to the stage of peptone with some polypeptides and amino acids. In the intestine there is an enzyme, erepsin, which converts peptone into polypeptides and amino acids.† This enzyme will not act upon natural proteins (except fibrin, vitellin and caseinogen) but it acts readily upon the products of partial digestion. We can show the presence of erepsin by incubating it with some peptone solution. As the peptone is hydrolysed to polypeptides and amino acids, tests with copper sulphate and alkali show that the pink colour given by peptones with these reagents gives place to the blue colour given by polypeptides and amino acids with the same reagents. We can instead precipitate the peptones with some reagent that precipitates peptones and estimate the amount of nitrogen in the solution by Kjeldahl's method. As the peptone is hydrolysed the amount of nitrogen not precipitated by the peptone precipitant increases.

The fats have been hydrolysed to glycerine and fatty acids, so like the other two great classes of food substances they have been reduced to their simplest form.

GENERAL REFERENCE

CHAPTER IX

TRANSFERENCE OF FOOD MATERIALS: ABSORPTION

THE first stage in the transference of food materials is the conversion of non-diffusible into diffusible substances. This change takes place by hydrolysis in the cells of the leaf or of storage depôts in plants and in the alimentary canal of animals.

The second stage is the building up of these diffusible substances into the non-diffusible constituents of other cells. In plants this is brought about by the sap conveying the materials to the place where they are to be used, but in multicellular animals we have to deal with the absorption of materials from the intestine and their transport in the blood.

ABSORPTION FROM THE INTESTINE

The various products formed during digestion are absorbed from the intestine into the blood and lymph streams. The conditions are such as to favour diffusion and osmosis. The diffusible substances are produced in the intestine, thus as digestion proceeds their concentration has a tendency to rise inside the intestine. The blood stream flows continuously in the wall of the intestine, hence any absorbed material is carried away and the concentration of the diffusible substance is kept low. Nevertheless, the absorption is not merely a physical process, as Waymouth Reid has shown that removal of the intestinal epithelium decreases the rate of absorption* and does not increase it as one would expect if the process were purely physical and dependent on the distance between the intestinal contents and the blood stream. Brodie and others have shown, moreover, that there is an increased oxygen consumption during absorption†; this again indicates that the cells perform work during the process of absorption.

* E. W. Reid, Phil. Trans., 1900, B. vol. 192, p. 211.
Hence we conclude that absorption is probably a biological process requiring a transformation of energy.*

Absorption of Fats.—As the fats are the easiest substances to trace their absorption will be described first. We have followed them to the stage of glycerine and fatty acids. The fatty acids are held in solution as soaps and by the bile salts. The contact of the fatty acids with the intestinal wall is promoted by the decrease in surface tension produced by the bile salts. If during digestion of fat the intestine is taken

* One ought to expect that where a purely physical process is predominant the separating wall shall be thin; where chemical changes are concerned the cells will be thicker to furnish the protoplasm for the chemical processes. The cells of the intestine are columnar and not squamous, hence we expect to find chemical processes taking place.
from an animal and is stained with osmic acid or other fat stain it is found that the portion of the cells next to the intestinal contents is unstained, but that fat drops are found in the deeper parts of the cells. The drops are largest in the central portion of the cell, and they become smaller towards the base of the cell. The fat globules can be traced beyond the cell through the connective tissue to the lymphatic vessels which lie close to the mucous membrane. In the lymphatic vessels the fat forms a milk-like emulsion (chyle), and during absorption of fat the lymphatics can be seen as white lines stretching away from the intestine. The fat passes with the lymph to the receptaculum chyli and thence up the thoracic duct to enter the blood stream at the root of the neck. In the blood stream the fat is carried away and soon disappears, although after a meal rich in fat there may be a slight opalescence of the blood plasma indicating the presence of minute particles of suspended fat. Within twelve hours after a meal containing fat at least sixty per cent. of the absorbed fat can be recovered from the thoracic duct, so there is a remainder which may be carried away from the intestine in the blood stream.*

By chemical analysis it is possible to show that during absorption the fatty acid is combined with glycerine to form fats. The contents of the intestine consist mainly of saponified fat. This can be shown by analysis of the material scraped from the surface of the mucous membrane after a meal rich in fat. The fat emulsion collected from the lymphatics (lacteals) consists mainly of neutral fat and analysis of the mucous membrane shows less neutral fat and more free fatty acid.†

These chemical findings with the histological appearances indicate that the fats pass into the cells as fatty acids (or soaps) and glycerine. These unite to form neutral fat, hence the appearance of fat globules about the middle of the cell, although the absorbing border is homogeneous. Even if fatty acids are fed to an animal they are united with glycerine to form neutral fats. The glycerine must be formed by the cells of the body, probably from carbohydrate.*

The digestibility and absorbability of fats depend upon their melting point: the lower the melting point the more complete is their digestion and absorption. A low melting point is due either to fatty acids of low molecular weight or to the presence of unsaturated linkages in the fatty acid molecules. The soaps of the fatty acids and the acids themselves are more soluble the smaller the molecular weight and the more unsaturated the acid. Hence the ease of absorption seems to be related to the solubility and diffusibility of the products formed from them.† The formation of insoluble soaps such as the calcium soaps may be associated with pathological conditions.‡

Butter and cod-liver oil may owe part of their value to the solubility of their digested products. This solubility is due in the former to the presence of fatty acids with low molecular weight and in the latter to the presence of unsaturated acids. The more soluble acids tend to hold the less soluble in solution with them, a circumstance which offers great difficulty when one attempts to separate mixtures of the fatty acids.

Bloor has recently emphasised the fact that fats may be altered during absorption. Fats of high melting point have their melting point decreased and the converse by the time that they reach the thoracic duct. He believes that this alteration occurs in the wall of the intestine. Fats with very low molecular weight pass mainly into the blood stream and not into the lymphatics.§

Absorption of Carbohydrates and of Proteins.—The absorption of carbohydrates and of proteins is not so easy to follow. By placing a canula in the thoracic duct and by collecting the lymph it can be shown that comparatively little of these substances pass into the lymph, hence the blood stream must

be the channel of absorption. The great experimental difficulty is that during absorption the difference in concentration in the blood stream is small. The products absorbed are rapidly removed from the blood stream, hence we can consider that the blood coming to the intestine is always of practically the same composition. During one hour ten litres of blood pass through the blood vessels of the intestine of a dog,* and assuming that as much as 10 grams of sugar are absorbed during one hour, the increase in concentration of sugar in the blood would be 0.1 per cent.

Absorption of Carbohydrates.—During absorption of sugar the portal blood shows an increase in glucose above that in the blood passing to the intestine. This excess of glucose is removed by the tissue cells (mainly of the liver) and formed into glycogen.† Glycogen is the form in which carbohydrate is stored in most animals, just as starch is stored in most plants. When carbohydrate is required the glycogen is hydrolysed and the sugar transferred to situations where it is required.

The experimental data are that following a meal rich in carbohydrate the portal blood going to the liver contains more glucose than the blood coming to the intestine or the hepatic blood coming from the liver. At the same time the amount of glycogen in the liver increases. During the interval, when glucose is not being absorbed the portal blood contains less sugar than the hepatic blood and the amount of glycogen in the liver decreases.

The mechanism that controls the storage of glycogen can be explained by the law of mass action. There is an equilibrium between glucose and glycogen which tends to keep the concentration of glucose constant. When glucose is increased in concentration by the arrival of larger quantities of glucose by the portal blood an increased formation of glycogen occurs, but the concentration of glycogen in solution is not increased as it is precipitated in the cells. When the concentration of glucose in the blood is decreased the reverse reaction occurs, and the concentration of glucose in the hepatic blood is increased above that in the portal blood.

Such a simple chemical explanation requires, however, some modification because the glycogen store in the liver is affected by the splanchnic nerves; stimulation of the splanchnic nerves causes an increased output of sugar from the liver but

† Claude Bernard, Leçons de Physiologie expérimentale appliquée à la Médecine, Paris, 1855.
the presence of adrenaline is necessary to the normal action of the nerve endings.*

Glycogen is stored in other parts than in the liver. The muscles, owing to their large bulk, contain a total amount of glycogen about the same as that in the liver (150 grams in man). The placenta is another part where glycogen is stored as a reserve for the growing embryo.

Absorption of Proteins.—Some years ago the final products of digestion of proteins were believed to be peptones. As no peptone could be found in the blood during digestion of proteins it was assumed that like the fats the proteins were resynthesised in the cells of the intestinal mucous membrane. Now that it is known that most of the protein is decomposed into amino acids these substances have been sought in the blood. The bulk of the evidence is in favour of the absorption of amino acids.

The method of Abel, which consists in passing blood through a series of collodium tubes so that a great surface is exposed for diffusion into a solution outside the tubes, shows that amino acids are present in blood and the same method ought to settle whether the bulk of digested protein finds its way into the blood as amino acids.†

The amino acids are taken from the blood by the individual cells according to their needs and the excess of any amino acids are deamidised and removed from the circulating medium.

Digestion of Cellulose.—Bacterial decomposition in the intestine leads to the formation of various substances not present in the food. These processes are more conveniently described in relation to other bacterial processes, but the bacterial decomposition of cellulose is important in relation to digestion. No enzyme capable of hydrolysing cellulose has been found in mammals but in the large intestine of the horse and the stomach of ruminants cellulose is acted on by bacteria so that soluble substances (organic acids) are formed which can be absorbed and used in metabolism.‡

Some invertebrates possess the power of digesting cellulose, for instance an enzyme can be obtained from the liver of the snail which dissolves cellulose.§

Digestion in Plants and Animals other than Mammals.—The mechanisms described are very instructive as they probably represent elaborations of mechanisms that occur even in unicellular organisms. The regulation of enzymes cannot be so satisfactorily demonstrated in simpler organisms as in the cases where the gland cells are collected into definite groups with special nerve supplies, and often a duct from which the secretion can be obtained. Yet in seeds, plants, and the simpler animals, there are evidences of the regulation of enzyme action. Protozoa, for instance, secrete acid into their digestive vacuoles.

In addition to bacteria other plants have specialised digestive secretions. The scutellum or other portions of the seed furnish enzymes which dissolve the stored food. Enzymes that act upon cellulose; glucosides, such as salicin, etc., are known in addition to those already described.

Fungi and other saprophytic or parasitic plants digest and absorb the organic matter upon which they feed. Insectivorous plants show all stages from those which drown insects and allow their bodies to undergo bacterial decomposition to those which possess specialised digestive secretions with some form of regulation so that the secretion is formed only when required.*

An interesting adaptation is that of some intestinal worms. They do not need digestive secretions of their own as digested products are formed in the intestine surrounding them, so they merely absorb the food products furnished by the digestive activity of their host. The worms are not themselves digested, possibly because they contain an antitrypsin which prevents the action of trypsin.† The alimentary canal may be furnished with antienzymes to prevent it digesting itself.

There is no doubt that in the plant the materials formed in the leaves are transferred by movements of liquid in the stem in the form of simple soluble substances. Thus there must be hydrolysis of the starch, etc., in the leaf. The soluble substances are transferred to food depôts in the seeds, etc., and turned into insoluble substances. When the seed commences to grow the food materials are once more hydrolysed and used as simple soluble substances.

The same processes occur in each individual cell of plants and animals. The simple soluble substances are dehydrated to form insoluble colloidal substances which are the food

* C. Darwin, Insectivorous Plants. Murray, 1875.
reserves. When required the reserves are hydrolysed and used as simple substances. These changes are due to the reversible action of enzymes as shown in the following diagram:

Food reserves by enzyme For transference or oxidation
Polysaccharides ←→ Monosaccharides
Fats ←→ Glycerine and fatty acids
Proteins ←→ Amino acids.

The importance of the simpler substances in cells has been emphasised by Hopkins, who pointed out that chemical changes in cells are mainly concerned with the simpler substances, whilst the larger complex molecules are of more importance from the physical aspects of cell activities.*

Therefore the digestive processes elaborated in this and the preceding chapter are of much wider significance than the mere process of digestion in mammals. Transfer of food materials in plants and the chemical changes in individual cells all depend on reversible hydrolysis of the stored colloidal and insoluble food reserves.

GENERAL REFERENCE

CHAPTER X
NUTRITION

Between the absorption of food and the removal of the waste products of its oxidation are certain chemical changes which form part of the subject of metabolism. In order to understand how cells perform their various processes we must know what they receive and what becomes of their incomings; that is we must examine the material and energy exchanges of cells.

In the present chapter we shall discuss the conservation of mass and of energy in the body and in the next chapter we shall deal with the processes by which oxygen is obtained from the atmosphere and transported to the tissues and by which carbon dioxide is removed from the tissues and given off to the atmosphere.

Let us commence by an examination of the income and expenditure of the whole body. We do this by what is termed the balance-sheet method, in which the income and expenditure are balanced against each other, the gain or loss being included as one of the items in the statement.

We need a balance for materials and a balance for energy. Numerous experiments have shown that the living organism obeys the laws of conservation of mass and of energy, so that any surplus output of energy over intake is derived from a loss in body substance and the converse.

In making out a balance-sheet we need to know the income. This is subdivided into gross income and nett income. Therefore we must know not only the amount of food taken into the body but also the amount of such food that is unabsorbed and passes out by the faeces.

The expenditure is measured by the materials excreted in the urine and the carbon dioxide which escapes by the breath; the small amount of loss by the skin must also be included.

Nitrogenous Equilibrium

Nitrogen is taken in mainly in the form of protein, which has an average content of 16 per cent. of nitrogen.
fore the amount of nitrogen multiplied by $6.25 (16 \times 6.25 = 100)$ is used as the measure of the amount of protein. By deducting the amount of nitrogen in the faeces from that in the food the amount of nitrogen which has been absorbed is obtained. The amount of nitrogen in the urine gives the amount of nitrogen excreted. As the animal does not store appreciable quantities of nitrogen* the normal condition is that of nitrogenous equilibrium in which the nitrogen excreted equals the nitrogen absorbed.

From the nitrogen figures it is possible to calculate the amount of carbon and hydrogen which has been used in the form of protein; by deducting these from the total figures for the carbon and hydrogen of the food, faeces and urine respectively the amount of carbon and hydrogen used in the form of carbohydrate and fat is obtained.

**Respiratory Quotient**

We can gain further information from the measurement of the respiratory gas exchange.

When carbohydrate is oxidised, owing to there being sufficient oxygen in the molecule to combine with the hydrogen to form water, the volume of carbon dioxide formed is exactly equal to the volume of oxygen used up. Thus $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$.

In the oxidation of fat and protein some of the oxygen is required to combine with hydrogen to form water, therefore the volume of oxygen taken in is greater than the volume of carbon dioxide given off. We express this as a ratio of $\frac{\text{volume of carbon dioxide given off}}{\text{volume of oxygen absorbed}}$ called the respiratory quotient (R.Q.).

As pointed out above the respiratory quotient for carbohydrate is 1, but for fat and protein it is less than 1, namely 0.71 and 0.80 respectively.

The amount of nitrogen excreted being known the amount of carbon dioxide and oxygen corresponding to the oxidation of the equivalent amount of protein can be calculated. On subtracting these amounts from the total values for carbon dioxide and oxygen the corrected values give the amount of carbon dioxide and oxygen corresponding to the carbohydrate and fat metabolised.

The corrected respiratory quotient having been thus obtained, a simple sum in proportion gives the relative amount

* Plants store nitrogen as aleurone grains.
of carbohydrate and fat oxidised. Knowing this proportion and the total corrected amounts of carbon dioxide and of oxygen the absolute amounts of carbohydrate and fat are easily calculated.

Each gram of nitrogen in the urine requires 8.471 grams or 5.923 litres of oxygen and gives rise to 9.347 grams or 4.754 litres of carbon dioxide, whilst the values for the oxidation of carbohydrate and fat are given in the following table:

**TABLE XXVII**

*Showing relative amounts and heat value of glycogen and fat for different respiratory quotients.*

<table>
<thead>
<tr>
<th>R.Q.</th>
<th>Glycogen Catabolised grams</th>
<th>Fat Catabolised grams</th>
<th>Heat Produced Calories (large)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.71</td>
<td>0.0000</td>
<td>0.5027</td>
<td>4.795</td>
</tr>
<tr>
<td>0.75</td>
<td>0.1543</td>
<td>0.4384</td>
<td>4.829</td>
</tr>
<tr>
<td>0.80</td>
<td>0.3650</td>
<td>0.3507</td>
<td>4.875</td>
</tr>
<tr>
<td>0.85</td>
<td>0.5756</td>
<td>0.2630</td>
<td>4.921</td>
</tr>
<tr>
<td>0.90</td>
<td>0.7861</td>
<td>0.1753</td>
<td>4.967</td>
</tr>
<tr>
<td>0.95</td>
<td>0.9966</td>
<td>0.0877</td>
<td>5.012</td>
</tr>
<tr>
<td>1.00</td>
<td>1.2071</td>
<td>0.0000</td>
<td>5.058</td>
</tr>
</tbody>
</table>

A respiratory quotient above one may be caused by the conversion of carbohydrate into fat when the volume of carbon dioxide given off is actually greater than the amount of oxygen taken in.

The energy balance-sheet is constructed on similar principles. The energy value of the food can be calculated by combustion of the same substances outside the body. The value for carbohydrates (4.1 C.) and for fats (9.3 C.), is the value for complete combustion, but for proteins a lower value is taken because some of the carbon and hydrogen of the proteins is excreted combined with the nitrogen. Each gram of protein gives rise to one-third of a gram of urea. Therefore the heat value for one gram of protein in the body (4.1 C.) is the heat value for complete combustion of one gram of protein less the heat value of one-third of a gram of urea.

The heat loss is composed of a series of items. There is the amount of heat required to raise the food and drink to body temperature, the amount of heat required to warm the air during respiration, the amount of heat required to evaporate water from the lungs and skin, the amount of heat lost by radiation and convection, and the heat equivalent of the external work performed.

The amounts of protein, carbohydrate and fat oxidised are calculated, as previously described, from the excretion of nitrogen, the respiratory quotient and the volume of oxygen absorbed.

In order to carry out a complete experiment of this nature a respiration calorimeter is used. It consists of a closed chamber in which the animal can be placed and the respiratory gases measured and analysed. The food can be passed through a trap door and the excreta obtained and analysed.

**Fig. 26.—Diagram of Respiration Calorimeter.**

Surrounding the two casings shown in the diagram are several layers of wood, etc., which are intended to protect the apparatus from being affected by variations in the surrounding temperature. They have been omitted from the diagram so as not to confuse it by too much detail. (Modified from Halliburton.)

The energy output is not measured as the separate items, but loss of heat from the chamber is prevented and the heat set free is absorbed by a water circulating apparatus. Any moisture condensed on the heat absorbing apparatus gives up its latent heat and any uncondensed moisture is collected in the gas analysis apparatus and its heat equivalent calculated. The air is passed in at the same temperature at which it comes out, hence this source of heat loss is eliminated. The external work performed must be measured and its heat equivalent calculated.

The loss of heat from the calorimeter is prevented by having two metal casings one inside the other. The temperature of the outer casing is kept the same as the inner, thus there cannot be
To show the construction of a balance

From W. O. Atwater, *Ergebnisse der Physiologie*, 1904

### INCOME.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>100.4</td>
<td>3715.0</td>
<td>366.3</td>
<td>54.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>61.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>69.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water in food and drink</td>
<td>2752.0</td>
<td></td>
<td></td>
<td>305.8</td>
<td></td>
</tr>
</tbody>
</table>

| Balance                         |        | 1308.1                    | 109.7   | 54.6      | 1.7      |
| Total                           |        | 5023.1                    | 476.0   | 414.4     | 18.5     |

In this experiment there was a loss to the body of—

### Material.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>10.8</td>
<td>61</td>
<td>5.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Fat</td>
<td>136.7</td>
<td>1304</td>
<td>104.0</td>
<td>16.1</td>
</tr>
<tr>
<td>Water</td>
<td>339.8</td>
<td></td>
<td>37.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>487.3</td>
<td>1365</td>
<td>109.7</td>
<td>54.6</td>
</tr>
</tbody>
</table>

The material loss as calculated from the experimental data accounts for the energy expenditure with an excess of 57 Calories or just over 1 per cent. on the total energy exchange.

any loss of heat by radiation, convection or conduction. The inner chamber is made of copper and the outer of zinc. Stretching between the two are a series of thermocouples (E), so that if one casing is at a different temperature from the other an electrical current is produced and this current is shown by a galvanometer. If the outer casing is cooler than the inner it can be warmed by an electrical current passing through wires wound round it, and if it is warmer than the inner casing it can be cooled by passing water through lead tubes wound round it. The outer casing is surrounded by wood, air and packing in several layers so as to prevent fluctuations due to changes in external temperature.*

For very small animals a Dewar flask can be used as a heat insulator.*

The heat absorbing apparatus consists of a copper tube with copper discs brazed on to it. Cool water flows through the copper tube and absorbs the heat from the interior of the inner chamber. The temperature of the water entering and leaving the chamber is measured and also the total amount of water passing through. The amount of heat is obtained by multiplying the difference of temperature by the amount of water and by the specific heat of water at the temperature of the experiment.

The gas analysis is carried out by causing the air to pass

through absorbers. The air coming out of the chamber is dried by passing it over pumice soaked in strong sulphuric acid. The increase in weight of the absorber gives the amount of water vapour absorbed by the acid and the amount of heat required to vaporise that amount of water can be calculated.

The carbon dioxide is absorbed by means of soda lime and the increase in weight of the soda lime absorbers gives the weight of carbon dioxide formed. As the reaction between soda lime and carbon dioxide sets free water this moisture must be retained by concentrated sulphuric acid in an absorber and weighed with the soda lime absorber,

$$2\text{NaOH} + \text{CO}_2 = \text{Na}_2\text{CO}_3 + \text{H}_2\text{O}$$

The air, free from moisture and carbon dioxide and deficient in oxygen, is now supplied with oxygen. The oxygen is run in until the pressure of the gas in the closed space is equal to that of the atmosphere. This volume of gas passed in must be equivalent to the volume of oxygen absorbed by the animal. The amount of oxygen run in can be measured by a meter or run in from a cylinder and the loss in weight of the cylinder used to calculate the amount of oxygen used.

The external work can be measured in various ways, such as by converting it into heat by friction or by converting it into electricity and measuring this. If it is ultimately converted into heat the heat may be retained in the respiration calorimeter and measured by the water absorbing system.

The results obtained by complete or partial metabolism experiments cannot all be reviewed here. In connection with nitrogenous metabolism we find that the greater portion of the nitrogen is excreted as urea (see p. 181).

If an animal is kept without nitrogenous food the excretion of nitrogen soon falls to a low level. In complete starvation this low level is maintained until all the carbohydrate and fat stores are exhausted and the body proteins are required for the energy processes of the body. When all the non-nitrogenous food stores are exhausted protein is used and the nitrogen excretion rises shortly before death occurs.

In order to obtain nitrogenous equilibrium (i.e. the intake of nitrogen exactly balancing its excretion) it does not suffice to add just enough nitrogen to correspond to the excretion of nitrogen during starvation. Several times as much nitrogen are required, hence we see that an increased intake of nitrogen is followed by an increased output. The amount of nitrogen necessary for the attainment of nitrogenous equilibrium
depends, moreover, upon other circumstances, such as the amount of carbohydrate and fat present in the diet.

Increased excretion of urea occurs soon after the ingestion of nitrogenous food, and this circumstance gave rise to the hypothesis of Voit that there are two varieties of protein, circulating and tissue. The former is not built up into the tissues and is rapidly destroyed, but the latter is built up into the tissues and is slowly removed.* Further, all proteins are not equally efficient in maintaining nitrogenous equilibrium.

The explanation of these processes is best seen by applying the law of mass action to our modern knowledge of the constitution of the proteins. The proteins are transported in the form of amino acids, and built up into the tissue proteins by the various cells. If one or more of the necessary amino acids are absent the protein cannot be synthesised, hence a diet deficient in one or more amino acids is inadequate to maintain nitrogenous equilibrium.† Thus gelatine, which is deficient in aromatic amino acids, cannot maintain nitrogenous equilibrium.

The amino acids present in excess must be removed, and we find that this is done by removal of the amine group and by utilisation of the non-nitrogenous portion for energy requirements. Owing to bacterial changes in the intestine some of the nitrogen is removed before absorption, and the same arguments would apply to that process, but we shall confine our attention to the fate of the amino acids after absorption.

Some amino acids can be synthesised in the animal body, whilst others must be furnished in the food. Osborne and Mendel have amplified the work of Willcock and Hopkins on zein, a protein deficient in tryptophane and lysine, showing that it does not suffice to maintain the weight of rats, but that if tryptophane is added the animals maintain their weight but will not grow. If, however, lysine is added to zein the animals will not maintain their weight, but if lysine and tryptophane are added to the zein the rats not only maintain their weight but also grow. These authors point out that certain amino acids are necessary for special processes (tryptophane for maintenance, lysine for growth), and that

if these amino acids are absent from the diet the body proteins are broken down to furnish the required amino acid. If that amino acid can be resynthesised it need not be present in the diet, but if it cannot be resynthesised it must be present in the diet, otherwise the proteins of the tissues are destroyed. We must therefore aim at a supply of amino acids that are necessary to the body but cannot be synthesised in the body.*

Applying the law of mass action to these processes the equation for the union of two molecules of amino acid to form a molecule of dipeptid, is $K = \frac{C_x C_y}{C_z}$ where $K$ is the equilibrium constant and $C_x$, $C_y$ and $C_z$ the concentrations of the reacting substances $x$, $y$ and $z$ respectively. As the formation of protein is due to a series of such unions we see that deficiency of one substance will bring the whole process of synthesis to a standstill, and as these represent reversible reactions the destruction of one substance will cause the breakdown of protein until equilibrium is re-established.

In the process of deamidisation it is recognised that deamidisation mainly affects the amino acids present in excess. This likewise follows from the law of mass action. The rate of reaction depends upon the concentration, or $\frac{dx}{dt} = kC_x$, where $k$ is the velocity constant and $C_x$, the concentration of the substance $x$ which is being deamidised. We therefore see that the greater the concentration of any amino acid the more rapidly it must be deamidised. The amino acids which are present in minimal amounts will be only slowly deamidised and they will be rapidly taken up by the synthesis of protein, hence they are used most economically.

The hypothesis of Voit is thus shown to mean that the amino acids present in excess are rapidly deamidised, but that those which are present in minimal amounts are formed into tissue proteins. The requirements of the body for certain amino acids will cause a breakdown of protein in the intervals between absorption and the excess amino acids thus set free will be deamidised. Thus there is a greatly increased excretion of nitrogen after a meal rich in protein, followed by a low nitrogen excretion when tissue proteins must be hydrolysed to furnish special amino acids as they are required.

This discussion assumes that there are not specialised enzymes for deamidisation of special amino acids. As all the

α-amino acids contain the group — CH(NH₂)COOH, one enzyme ought to be able to act upon them all with equal ease.

The relative value of proteins for maintaining nitrogenous equilibrium in man are shown in the following table.* The Biological value is probably related to the kinds of amino acids in the protein.

**Table XXIX**

*Biological Value of Various Proteins.*

<table>
<thead>
<tr>
<th>Source of protein</th>
<th>Relative value to meat proteins (Biological Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>104.74</td>
</tr>
<tr>
<td>Milk</td>
<td>99.71</td>
</tr>
<tr>
<td>Rice</td>
<td>88.32</td>
</tr>
<tr>
<td>Potatoes</td>
<td>78.89</td>
</tr>
<tr>
<td>Beans</td>
<td>55.73</td>
</tr>
<tr>
<td>Wheat Meal</td>
<td>39.56</td>
</tr>
<tr>
<td>Maize</td>
<td>29.52</td>
</tr>
</tbody>
</table>

The main nitrogenous end product, urea, is formed from ammonium carbonate. If, however, an excessive amount of acid is produced or administered the ammonia neutralises the acid and is excreted as ammonium salts. This is one of the ways in which neutrality is maintained in the body, and the amount of ammonia in the urine is an indication of the amount of acid to be neutralised.

Another example of neutrality regulation is mentioned by Dakin, that in the presence of acid the formation of lactic acid from pyruvic aldehyde by glyoxalase is inhibited. As mentioned on p. 112, both these processes can be explained by Le Chatelier's Theorem.†

The metabolism of carbohydrates and fats gives rise to carbon dioxide and water. The intermediate stages are discussed in Chapter VII.

**Sulphur Metabolism**

Sulphur metabolism presents certain features of interest. Sulphur is excreted mainly as sulphates. These are produced either from sulphates in the food or by oxidation of sulphur-containing substances such as the amino acid cystine. The taurine of the bile acid, taurocholic acid, is formed from cystein by oxidation of the sulphur and removal of the carboxyl group.

Some of the sulphates are combined with organic groups to form ethereal sulphates, so we have to distinguish between the inorganic and ethereal sulphates. The ethereal sulphates are formed as a means of protection against certain poisonous substances. The formation of phenol, skatol, indol, etc., in the intestine may lead to absorption of these substances, which will then exert poisonous action on the cells of the body. These substances are rendered non-toxic by combining them with sulphuric acid to form ethereal sulphates. The ethereal sulphates are not precipitated by barium salts, or by benzidin, hence the inorganic sulphates can be estimated without the ethereal sulphates. By boiling with acid the ethereal sulphates are hydrolysed, setting free the organic substance and sulphuric acid. The latter will then give a precipitate with barium salts. The ethereal sulphates are usually estimated by taking the difference between the inorganic sulphates and the sulphates after hydrolysing with acid.*

Neutral sulphur is also found in the excreta. It consists of sulphur not in the form of sulphates. In certain individuals the sulphur metabolism is defective and hexagonal crystals of cystin are deposited in the urine.†

For further observations on sulphur, see sulphur bacteria, p. 211.

**Phosphorus Metabolism**

Phosphorus is excreted in the form of phosphoric acid. The phosphorus is derived from phosphates in the food, nucleo-proteins, phospho-proteins and phospho-lipins.

The discussion of metabolism refers specifically to mammals, but there is no doubt that similar relations hold for all living

cells. We must bear in mind that there are certain differences in the end products of metabolism. For instance, birds and reptiles form uric acid instead of urea as the main end product of metabolism. In plants many special substances such as alkaloids are formed, but their relation to metabolism is still far from clear.

**Dietetics**

The bearing of the description of metabolism given above on dietetics is obvious. We see that it is necessary to have sufficient energy value in the food to maintain the energy exchange of the resting body, and also to provide the energy required for any additional energy expenditure necessary for the performance of external work.

The energy can be obtained from protein, carbohydrate or fat, but it has been found advisable to have certain proportions of these various food substances.

*Minimum Protein Requirement.*—To maintain a healthy condition the body requires a certain daily amount of protein. The least amount that will suffice depends upon the constituent amino acids in the protein of the diet. Many observations have been made on this subject. Chittenden aroused renewed interest in this subject by the statement that nitrogenous equilibrium could be maintained on about 60 grams of protein per day, instead of the higher figures given by previous observers.* Chittenden's conclusions have been established, and it is now recognised that 80 grams of protein of good biological value allows sufficient margin for a normal adult man.

Having determined the amount of protein in the diet, the energy value of this is deducted from the total energy requirement, and the remaining energy is obtained from carbohydrate and fat. The relative amounts of carbohydrate and fat depend on the ease of their digestion and on the fact that where large energy expenditures are concerned the fatty foods are much less bulky. It is possible that the minimum amount of fat ought to be sufficient to furnish 20 per cent. of the total energy value.†

The water and salts of the diet are also to be remembered. The daily loss of water by urine, respiration, etc., must be made good, and the proportions of salts for the normal function of the cells (Chapter V) must be maintained.

Accessory Food Substances.—When, however, purified food substances are fed to animals they do not thrive, and it has been proved that there are unknown substances which are required in minimal amounts. These substances are independent of the energy value of the food, but they are found in various fresh natural food substances.*

The absence of these accessory substances is associated with certain diseases, and at least three accessory substances are required. They are known only by their properties, *i.e.* by the effect of their absence from the diet.

The classification is:

- Vitamin A found in certain fats and green leaves.
- Vitamin B found in the pericarp and germ of cereals, in yeast, etc.
- Vitamin C found in varying quantity in fresh uncooked foods.

The investigation of these substances is still in progress, and the distribution of them in various kinds of food, the effect of cooking and methods of preservation are being examined.

The fat-soluble A substance is probably destroyed by heating and oxidation, † whilst the water-soluble B substance is thermo-stable. The water-soluble substance C is known to be thermolabile.

Absence of fat-soluble A substance is probably a factor in the production of rickets.

Absence of water-soluble B substance causes paralysis, or the disease beri-beri.

And absence of water-soluble C substance causes scurvy.

A relative deficiency of these substances may cause ill health, even if the deficiency is not enough to cause the characteristic symptoms of the diseases associated with their absence from the diet. ‡

* It may be that greater expenditure of energy may require a larger supply of these substances.


‡ Medical Research Committee Report on the Present State of Knowledge Concerning Accessory Food Factors.
FIG. 27.—Deficiency of Vitamin A

Rickets following a diet of 175 c.c. whole milk, white bread *ad lib.*, and 10 c.c. linseed oil per diem. Time of experiment, 5½ months. Increase in weight during period of experiment, 2670 grm.

*From Special Report Series No. 38. By permission of the Medical Research Council.*
FIG. 28.

This photograph represents the condition shown by a young rat which is suffering from a deficiency of water-soluble B. There is no co-ordination of the movements of the hind quarters, and the animal is unable to use the hind legs, which may be seen stretched out in a helpless manner. This animal showed a rapid recovery from this condition and was able to walk about with ease twenty-four hours after a dose of yeast extract had been given by the mouth.

From Special Report Series No. 38. By permission of the Medical Research Council.
### TABLE XXX. Percentage Composition.

<table>
<thead>
<tr>
<th>Food substance</th>
<th>Water</th>
<th>Protein</th>
<th>Biological value of Protein</th>
<th>Carbohydrate</th>
<th>Fat</th>
<th>Ash</th>
<th>Heat of combustion in calories per pound =453:6 g.</th>
<th>Accessory Food Values.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fat Soluble A</td>
<td>Water Soluble B</td>
</tr>
<tr>
<td>Beef</td>
<td>55.5</td>
<td>17.0</td>
<td>17.7</td>
<td></td>
<td>25.3</td>
<td>0.7</td>
<td>1430</td>
<td>++</td>
</tr>
<tr>
<td>Eggs in shell</td>
<td>73.7</td>
<td>13.0</td>
<td>(13.0)</td>
<td></td>
<td>10.0</td>
<td>0.8</td>
<td>695</td>
<td>+++</td>
</tr>
<tr>
<td>Beans, white, dried</td>
<td>12.6</td>
<td>15.8</td>
<td>8.8</td>
<td>59.9</td>
<td>1.6</td>
<td>2.6</td>
<td>1530</td>
<td>++</td>
</tr>
<tr>
<td>Beans, germinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proportion much the same as dried</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>beans only less concentrated as they absorb</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>water during germination</td>
<td></td>
</tr>
<tr>
<td>Bread, white</td>
<td>35.3</td>
<td>7.1</td>
<td>2.8</td>
<td>52.3</td>
<td>1.2</td>
<td>0.8</td>
<td>1195</td>
<td>0</td>
</tr>
<tr>
<td>Bread, wholemeal</td>
<td>38.4</td>
<td>7.5</td>
<td>3.0</td>
<td>49.1</td>
<td>0.8</td>
<td>1.0</td>
<td>1125</td>
<td>+</td>
</tr>
<tr>
<td>Rice, polished</td>
<td>12.3</td>
<td>6.5</td>
<td>5.7</td>
<td>76.9</td>
<td>0.3</td>
<td>0.3</td>
<td>1610</td>
<td>0</td>
</tr>
<tr>
<td>Rice, unpolished</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Probably much the same as polished rice</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize flour, wholemeal</td>
<td>12.6</td>
<td>5.8</td>
<td>1.7</td>
<td>76.3</td>
<td>1.2</td>
<td>0.5</td>
<td>1625</td>
<td>+</td>
</tr>
<tr>
<td>Potato, raw</td>
<td>78.3</td>
<td>1.7</td>
<td>1.3</td>
<td>17.7</td>
<td>0.1</td>
<td>0.8</td>
<td>370</td>
<td>+</td>
</tr>
<tr>
<td>Butter</td>
<td>11.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td>8.0</td>
<td>2.3</td>
<td>3410</td>
<td>++</td>
</tr>
<tr>
<td>Margarine</td>
<td>9.5</td>
<td>1.2</td>
<td>?</td>
<td></td>
<td>79.8</td>
<td>4.7</td>
<td>3335</td>
<td>Varies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>with source of fat</td>
<td></td>
</tr>
<tr>
<td>Olive oil</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4218</td>
<td>0</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4218</td>
<td>+++</td>
</tr>
<tr>
<td>Cabbage</td>
<td>91.5</td>
<td>1.2</td>
<td>1.0</td>
<td>5.5</td>
<td>0.3</td>
<td>0.8</td>
<td>140</td>
<td>++</td>
</tr>
<tr>
<td>Lemon juice</td>
<td>89.3</td>
<td>0.8</td>
<td>?</td>
<td>7.7</td>
<td>0.6</td>
<td>0.4</td>
<td>180</td>
<td>+++</td>
</tr>
</tbody>
</table>

We can summarise the food requirements in tabular form.

**TABLE XXXI**

*Showing daily food requirements for an individual doing moderate work.*

The gross values are given and the nett values are obtained from these after deducting the loss by non-absorption.

<table>
<thead>
<tr>
<th>Food substance</th>
<th>Amount.</th>
<th>Required.</th>
<th>Energy value.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>grams 8.0</td>
<td>To maintain nitrogenous equilibrium</td>
<td>328.0</td>
</tr>
<tr>
<td>Fat to furnish at least 20% of total energy</td>
<td>65</td>
<td>Variable in proportion but they furnish the remainder of the energy required</td>
<td>604.5</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>504</td>
<td></td>
<td>2066.4</td>
</tr>
<tr>
<td>Water</td>
<td>Variable</td>
<td>For solvent action and to promote heat loss by evaporation</td>
<td>2998.9 Nil</td>
</tr>
<tr>
<td>Salts</td>
<td>Variable</td>
<td>To maintain physico-chemical integrity of cells</td>
<td>Nil</td>
</tr>
<tr>
<td>Accessory food substances</td>
<td>?</td>
<td>To maintain healthy conditions</td>
<td>Nil</td>
</tr>
</tbody>
</table>
CHAPTER XI

RESPIRATION

The great importance of gaseous exchange lies in the liberation of energy by oxidation. All organic compounds can take up oxygen, and are finally converted into carbon dioxide and water. During the process of oxidation in living organisms a certain amount of energy is rendered available for biological processes. The maximum amount of energy is shown by the amount of heat liberated when the substance is completely oxidised, but only a certain proportion of this is available energy, the remainder being lost. It is usual to designate the factor energy used for external work total energy as the efficiency of the process. As mentioned in the preceding chapter the carbohydrates and fats yield the same amount of energy on oxidation in the body as when oxidised outside of it, but the proteins yield less owing to a certain amount of the energy being carried away in incompletely oxidised substances such as urea.

Unicellular organisms can obtain a supply of oxygen and get rid of carbon dioxide by diffusion between themselves and their surroundings, but multicellular organisms require some means of transport whereby the gases can be transferred to and from the cells.

In most animals the gases are transferred in solution by some form of circulatory mechanism, the simplest of which consists of an exchange of water either by rhythmical movements or by ciliary action. When a closed circulatory mechanism has been developed the gases are exchanged in one organ (gill or lung), they are transferred by the circulatory mechanism and used by the cells. Thus there are three processes to consider, external respiration, transport of gases and internal respiration corresponding respectively to the division mentioned above.

EXTERNAL RESPIRATION

In plants the gas exchanges take place through minute openings. On the under surface of the leaves are stomata...
guarded by cells which open or close according to the wetness or dryness of the atmosphere. These openings regulate the escape of moisture by evaporation, and they also influence the gas exchanges. Lenticels are minute openings on stems.

In the leaf are air spaces into which the stomata open. Gas exchange takes place between the cells of the leaf and the air spaces. The air spaces exchange gases with the external air through the stomata.

**Fig. 29.—Drawing of under surface of leaf.**

A, opening leading into air spaces of leaf between, B, the guard cells of the stoma.

_Slightly modified from "Vegetable Physiology," J. R. Green (Churchill)._  

**Fig. 30.—Drawing of lenticel showing the manner in which gaseous exchange can take place between stems and the surrounding atmospheres._

_Copied from "Vegetable Physiology," J.R. Green (Churchill)._  

Diffusion through the openings in septa has been studied by Brown and Escombe,* who have found that in plants the stomata are more than sufficient for the exchange of gases by diffusion. Under natural conditions leaves and branches are always moving in air currents. These movements cause deformation of the air spaces in them so that the air in the spaces is kept moving, thus the mixing of gases in the spaces and movements of gases through the stomata and lenticels are promoted.

Water plants sway in the water, thus their surfaces are brought into contact with fresh portions of water. The exchange of gases is facilitated by these movements. Some water plants contain air sacs so that gas exchange can take place into the air sacs as well as into the surrounding water.

Aquatic animals are furnished with gills and by movements of the animal water currents are forced over the gill fringes

* H. T. Brown and F. Escombe, Phil. Trans., 1900, B. 193, p. 223.
so that fresh portions of water are being continually brought into contact with the gills, just as the swaying movements of water plants bring them into contact with fresh areas of water.

Some fishes contain air sacs which serve two purposes: (1) as a reserve for gas exchanges; (2) as a float for keeping the animal almost of the same specific gravity as the water.

The composition of the contained gas varies according to whether rapid changes of volume are required. Those fishes that remain at about the same depth have a gas in their swim bladders which is mainly nitrogen, but those that change rapidly from one depth to another have mainly oxygen instead of nitrogen. The reason for this difference is that nitrogen is soluble to only a small extent in blood, whilst oxygen can be carried in larger quantity.

The swim bladders of fish that contain large quantities of oxygen are furnished with an oxygen-secreting gland. The problem of secretion of oxygen is of the same nature as other secretions. The increase in concentration of oxygen can be accomplished only as the result of expenditure of energy. Absorption of oxygen takes place from another portion of the swim bladder.*

Secretion of oxygen in the swim bladder of fish is important because it shows the possibility of secretion of a gas. During normal quiet respiration gas exchange in the lungs of mammals is easily accounted for by the physical process of diffusion. When living at a high altitude where the oxygen tension in

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the air is low, or when the oxygen requirements of the body are increased by strenuous exercise, it may be that the oxygen pressure in the blood may rise above that of the alveolar air, or that the amount of oxygen taken up by the blood is greater than can be accounted for by the process of diffusion.

In either of these cases we must assume that the lung secretes oxygen from the alveolar air into the blood, and it is important to have such a definite case as the swim bladder of oxygen-secreting fish to show the possibility of oxygen secretion.

The process of diffusion can be calculated by the difference in oxygen tension between the alveolar air and the pulmonary blood, after taking into consideration the area of the lung surface through which diffusion can take place and the length of time during which it is taking place.

The recent experiment by Barcroft and his co-workers* shows that at a low oxygen pressure and during the performance of work, the oxygen tension in the blood, as calculated from the percentage saturation of haemoglobin, is less by about 7 mm. than the oxygen tension of the alveolar air. In the absence of direct information as to the rate at which oxygen will diffuse through the lung at this difference of pressure we cannot say whether this disproves any secretory activity: as the pressure is less in the blood it certainly indicates that

the passage of oxygen into the blood may be a process of diffusion.

Land animals have an exchange between air and tissue fluids. Insects possess tubes (tracheæ) which pass from the exterior to reach all parts of their bodies, but the usual arrangement is an air sac or lung through which exchange takes place between the contained gases and the circulating fluid.

Birds have lungs, and in addition they have air sacs. The function of these air sacs is probably two-fold. First of all they penetrate into the bones and in that way are useful mechanically in that they permit a greater degree of strength with less increase in weight, just as hollow bicycle tubing is stronger for the same weight than solid steel rods. The other function is respiratory. The thorax of flying birds must be rigid for the attachment of muscles, therefore the lungs cannot expand so freely as in non-flying animals. The air sacs pump air in and out through the bronchi, thus mixing fresh air with the air in the lung alveoli.

Lungs are bellows which by expansion and contraction draw in and expel air. The structure of lungs is really that of a number of minute bellows. The larger air conducting tubes (trachea and bronchi) divide and subdivide until a multitude of extremely minute tubes are formed. Each of these ends in a dilated extremity with a number of pouches in its walls.

The expansion of the lungs is really the integrated expansion of the large number of these small air sacs (alveoli).

Gas exchange takes place between the air in the air sacs and the blood in the capillaries covering the wall of the air sac. The air in the conducting tubes is not of any use for respiratory exchange. We distinguish between the air in the tubes and in the alveoli by calling the former the air in the dead space and the latter the alveolar air. If we wish to measure the actual amount of air used in the alveoli we must subtract the volume of the dead space from the total volume breathed.

From the paragraph above it is clear that the expired air
is a mixture of air from the dead space and alveolar air. If we wish to obtain a sample of the alveolar air we must take a sample of the expired air at the end of expiration after the dead space has been flushed out with the air from the alveoli.

From "Human Physiology" A.D. Waller (Longmans).

**TABLE XXXII**

*Volume of Air Spaces in Man.*

<table>
<thead>
<tr>
<th>Description</th>
<th>C.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead space</td>
<td>170</td>
</tr>
<tr>
<td>Quiet respiration (tidal air)</td>
<td>500</td>
</tr>
<tr>
<td>Amount that can be inspired at the end of a</td>
<td></td>
</tr>
<tr>
<td>quiet inspiration</td>
<td></td>
</tr>
<tr>
<td>Amount that can be expired at the end of a</td>
<td></td>
</tr>
<tr>
<td>quiet expiration</td>
<td></td>
</tr>
<tr>
<td>Total possible respiratory volume (vital capacity)</td>
<td>4000</td>
</tr>
<tr>
<td>Amount left after greatest possible expiration</td>
<td></td>
</tr>
<tr>
<td>(Residual air)</td>
<td>1500</td>
</tr>
</tbody>
</table>

From these figures we see that a quiet respiration is the mixing of about 330 c.c. (500 — 170) fresh air, with about 3500 c.c. of air and the removal of 330 c.c. of the mixture after exchange has taken place between it and the blood. Deeper respirations such as occur during performance of work increase the amount of exchange.
In this case expired air is approximately one part of inspired air (from dead space) and two parts of alveolar air (170 c.c. from dead space and 330 c.c. from alveoli = 500 c.c.).

To study the chemical changes of respiration we need to bear the above facts in mind. If we wish to study the total oxidation in the body we must measure the total volume of air respired and the changes in its composition, but if we wish to study the exchange of gases between the blood and the alveolar air it is the composition of alveolar air that is required.

**Transport of Gases**

*Respiratory Pigments.*—When the activity of cells becomes greater than a certain value a current of water cannot supply enough oxygen. In order to overcome this difficulty respiratory pigments are used. These pigments can serve two purposes: first, as local deposits, which can accumulate a supply of oxygen to be used up during periods of activity; and secondly, as part of the circulatory mechanism to convey oxygen from the gills or lungs to the tissues. The colours of anemones, etc., have been stated to be storehouses of oxygen.

There are two main respiratory pigments. One, hæmocyanin, a copper-containing compound which is colourless when deprived of oxygen and becomes slightly bluish when brought into contact with the oxygen of the atmosphere. This pigment is confined to some invertebrates and is much less efficient than the other pigment.

The second pigment, hæmogoblin, is one containing iron, and it is found in the circulating blood of vertebrates; it also occurs in isolated situations in the invertebrate phyla. We shall proceed to a consideration of the behaviour of this pigment.

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**Hæmoglobin**

The amount of gas dissolved in a liquid is proportional to the partial pressure of the gas above the liquid. The presence of respiratory pigments upsets this relation as we no longer deal with a solution of gas in the same molecular condition as in the vapour space above the liquid. We have a small amount of gas in solution and a larger amount associated in some way with the respiratory pigment. The result is that at low pressures of oxygen relatively more is contained in the solution than at high pressures. This result is due to oxygen uniting with the hæmoglobin at low pressures.
pressures, but at higher pressures the hæmoglobin is nearly saturated, so that a further rise of pressure causes only the small increase due to the increase of solubility associated with higher pressure. The relation instead of being linear is parabolic.

From the diagram it can be seen that at 70 mm. pressure of oxygen the blood contains almost as much (90 per cent.) oxygen as at 100 mm. pressure (pressure of oxygen in lung alveoli). A fall to 10 mm. pressure liberates 76 per cent. of the combined oxygen, and would therefore allow the escape of 66 \((76 - 10)\) per cent., or 13.2 volumes of oxygen from every hundred cubic centimetres of blood containing 15 per cent. of hæmoglobin.

This process accounts for the transport of oxygen from lungs or gills to the tissues. In the respiratory organs the hæmoglobin is exposed to comparatively high pressures of oxygen and the hæmoglobin takes up oxygen until almost saturated. In the tissues there is a low oxygen pressure: the oxygen is given off from the hæmoglobin and the hæmoglobin passes back to the respiratory organs to obtain a fresh supply of oxygen.

In the blood the hæmoglobin is not dissolved in the plasma but it is contained in the red blood corpuscles. It is due to the hæmoglobin in these corpuscles that the blood has a red colour. If the hæmoglobin were not contained in the corpuscles it would escape by the kidneys and be lost to the body.

The curve relating oxygen pressure to the amount of oxygen united with hæmoglobin is not always the same, as it varies from animal to animal. Bohr believed that this difference indicated that the hæmoglobin from different animals was of different composition. The presence of different proteins united to the iron containing group (hæmatin) was held to be responsible for the different oxyhæmoglobin curves and the difference in shape of the oxyhæmoglobin crystals. The experiments of Barcroft show that the differences in the oxyhæmoglobin curves can be reproduced with the same hæmoglobin.

Barcroft found that the hæmoglobin curve varies according to whether the hæmoglobin is dissolved in distilled water or solutions of different salts. If the same hæmoglobin is dissolved in solutions containing salts in the proportions in which they occur in the corpuscles of different animals, the oxyhæmoglobin curves agree with those obtained from the
corresponding animals. Thus the differences in the oxy-
haemoglobin curves may be due to the saline constituents
associated with the haemoglobin in the red blood corpuscles.

In Barcroft's experiments one factor was of extreme
importance, namely the pressure of carbon dioxide. Thus a
series of curves showing the dissociation of oxyhaemoglobin
with increasing concentration of carbon dioxide shows that
for the same pressure of oxygen the oxygen is given off more
easily the higher the pressure of carbon dioxide.

![Dissociation curve of Barcroft's blood](image)

**Fig. 38.** Dissociation curve of Barcroft's blood.

Exposed to 0, 3, 20, 40 and 90 mm. CO₂.
Ordinates = percentage saturation.
Abscissae = oxygen pressure.

(From "Respiratory Function of the Blood," J. Barcroft, by permission of the Editor of the "Journal of Physiology.")

The physiological value of this relation is that it aids the
gaseous exchanges. In the capillaries of the tissue the pressure
of carbon dioxide is increased as the result of tissue activity.
There is also a low oxygen pressure owing to the use of oxygen
by the tissues. Both these factors favour the giving up of
oxygen from the oxyhaemoglobin.

In the capillaries of the respiratory organs the oxygen
pressure is raised and the carbon dioxide escapes from the
blood. These two changes favour the absorption of oxygen
because the higher the oxygen pressure the more oxygen com-
bines with haemoglobin, and the lower the carbon dioxide
pressure the more oxygen combines with haemoglobin for the same pressure of oxygen.

We therefore see that the oxygen and carbon dioxide pressures aid each other in connection with the absorption and liberation of oxygen by haemoglobin.

The action of carbon dioxide is believed to be due to its acidity. Thus all solutions containing alkaline salts show a curve which is shifted in the opposite direction from those with increasing concentrations of carbon dioxide. There is one pair of salts which require special mention. These are potassium and sodium chlorides. Barcroft found that haemoglobin dissolved in equimolecular solutions of these salts gave different curves. The potassium chloride solution behaved as if it were more alkaline than the sodium chloride solution.

The explanation of these results seems to be that the haemoglobin is an amphoteric substance with more marked acidic than basic properties. The addition of oxygen to the haemoglobin makes it a stronger acid, just as sulphuric acid is stronger than sulphurous and nitric than nitrous. In support of this hypothesis the experiments of Christiansen, Douglas and Haldane can be quoted, who find that increase in oxygen tension of blood causes an increase in carbon dioxide tension. The increase in oxygen tension makes the haemoglobin into a stronger acid. More alkali combines with haemoglobin, and there is less alkali left to combine with carbon dioxide. Therefore carbon dioxide is set free and the carbon dioxide tension rises.*

The effect of increase in acidity in favouring the reduction of oxyhaemoglobin can be explained on the Law of Le Chatelier (see pp. 59, 112), because the increase in acidity will prevent the change which causes an increase in acidity, i.e. the combination of oxygen with haemoglobin to form oxyhaemoglobin.

Barcroft and Hill have suggested that the effect of salts on haemoglobin is due to an effect on the degree of aggregation of the haemoglobin. At the isoelectric point haemoglobin, like other amphoteric colloids, has a greater tendency to run together to form larger particles containing a greater number of molecules than when the solution is more acid or alkaline. The normal haemoglobin is alkaline, hence the addition of acid causes the system to approach the isoelectric point of haemoglobin. By the running together of haemoglobin the

number of molecules of hæmoglobin is reduced and the mass
law relations are altered.

A. V. Hill suggests the following equation:  \[ \frac{y}{100} = \frac{Kx^n}{1+Kx^n} \]
where \( y \) = percentage saturation of the hæmoglobin with oxygen, \( x \) = oxygen pressure, \( K \) is the equilibrium constant of the curve, and \( n \) = average number of hæmoglobin molecules in the aggregations. This equation agrees with the experimental results.

**The Nature of the Combination of Oxygen with Hæmoglobin**

One of the criteria of a chemical combination is that there must be a definite fixed relation between the number of atoms united in the molecule, hence we must find out the molecular weight of hæmoglobin and the number of atoms of oxygen united with the hæmoglobin.

The minimum molecular weight of hæmoglobin has been calculated from the percentage of iron contained in it. Hæmoglobin contains about 0.4 per cent. of iron. The minimum molecular weight is therefore about 14,000.

Direct measurements of the molecular weight of hæmoglobin are difficult to obtain, but Hüfner and Gansser found that under the conditions of their experiments the osmotic pressure of hæmoglobin solutions indicate a molecular weight of about 16,000. (Horse = 15,115. Cow = 16,321.)

The values obtained depend upon the condition of the hæmoglobin in the solution as acid, alkali and salts markedly affect the condition of the hæmoglobin.

The amount of hæmoglobin containing 1 gram of iron when completely saturated with oxygen absorbs 401 c.c. of oxygen at 0° and 760 mm. pressure. Therefore for each atom of iron hæmoglobin absorbs two atoms of oxygen, thus showing a definite atomic relationship between the iron and oxygen in the hæmoglobin.

Barcroft and Hill have made measurements of the rate of reduction of hæmoglobin at different temperatures. From these rates they calculated the molecular heat of combination of one molecule of hæmoglobin with oxygen. They measured the heat produced when one gram of hæmoglobin unites

with oxygen. By dividing the molecular heat of combination by the heat produced by one gram of haemoglobin uniting with oxygen, the molecular weight was found to be 15,200.* Thus a result based on the assumption that oxygen unites chemically with haemoglobin gives practically the same molecular weight that is given by other methods of measurement, thus indicating that the union is probably chemical.

Another criterion of chemical combination is that the properties of the compound must be different from a mixture of the original substances. In some cases the difference may be comparatively slight, e.g. sulphurous acid uniting with oxygen to form sulphuric acid. In the case of haemoglobin we find that there is a marked difference between the spectral appearances of oxyhaemoglobin and haemoglobin, such a spectral difference being greatly in favour of a chemical change when haemoglobin combines with oxygen.

The various compounds and derivations of haemoglobin show absorption in different portions of the visible spectrum, and we must also remember that lines and bands occur in the ultra violet and infra red portions of the spectrum.

The various compounds and derivatives of haemoglobin and some of the corresponding spectra are given in diagrammatic form. The diagram shows that haemoglobin can unite with oxygen, carbon monoxide or nitric oxide. The compound of oxygen with haemoglobin is least stable and that with nitric oxide most stable, the compound with carbon monoxide being intermediate. Oxygen is easily removed from oxyhaemoglobin by reducing agents or by the tissues, but carbon monoxide is so firmly united that the haemoglobin is rendered useless as an oxygen carrier. The relative combining powers of oxygen and carbon monoxide with haemoglobin are as 1: 224 † and 1: 290 for two samples of human blood when the carbon dioxide pressure and salts are the same.

Methaemoglobin is formed from oxyhaemoglobin in a variety of ways, amongst which are the action of ferricyanides, nitrates, iodine, excess of neutral salts. It contains the same amount of oxygen as oxyhaemoglobin, but united in a different way so that the oxygen is not given off to the tissues. By rendering methaemoglobin alkaline it passes into alkaline methaemoglobin, and on treating this solution with ammonium sulphide oxyhaemoglobin is formed which is then reduced to haemoglobin.

The decomposition of haemoglobin proceeds by the formation of Hæmatin, which contains the iron, and a protein (globin) belonging to the histone class. By removing iron from hæmatin hæmatoporphyrin is formed. Both hæmatin and hæmatoporphyrin can exist in acid and alkaline forms. Hæmatoporphyrin is closely allied to such substances as the bile pigments.

Hæmatoporphyrin when decomposed is found to contain pyrrole groups which are identical with the decomposition products of chlorophyll, so that we see that there is a structural relationship between the two important animal and vegetable pigments.

Haemoglobin and many of its derivatives can be easily crystallised. This can be brought about by lowering the solubility (by alcohol, salts, etc.) and at the same time cooling the solution.

One of the crystalline decomposition products is Hæmin. Blood pigment is heated with glacial acetic acid, and if it is an old sample from which the chlorides have been removed by washing a crystal of sodium chloride is added. On cooling dark brown crystals appear. This is the well-known Teichmann's Test for blood* and the crystals are composed of the chloride of hæmatin. Other solvents, such as acetone, can be used instead of glacial acetic acid.

The crystals of haemoglobin from different animals are different in shape; in most cases they are rhombic (horse, dog, man, etc.), but in other cases they are hexagonal plates (squirrel) or tetrahedra (guinea-pig).

![Blood Spectra Diagram]

**Fig. 39.**—Blood Spectra.

1. Oxyhaemoglobin.
2. Haemoglobin (reduced).
3. Carboxyhaemoglobin.
4. Acid haematin.
5. Alkaline haematin.
6. Reduced Haematin.
7. Methaemoglobin.
8. Haematoporphyrin.
9. Wave lengths in $10^{-3}$ of a mm.

(From "Human Physiology," A. D. Waller. Longmans.)

**TRANSPORT OF CARBON DIOXIDE**

The transport of carbon dioxide is a much simpler problem than the transport of oxygen. Carbon dioxide is more soluble than oxygen in water, hence more of it can be carried in solution. It combines with alkalies to form carbonates and bicarbonates, hence we find that some of the carbon dioxide
is united with alkali. Finally, carbon dioxide may unite with protein and some of it may be carried in this form. The blood coming away from the tissues contains more bicarbonate than the blood going to the tissues. In the lungs carbon dioxide is removed and some of the bicarbonate is turned into carbonate. The influence of oxyhaemoglobin on the removal of carbon dioxide from carbonates has been mentioned previously (p. 165). It is also possible that carbon dioxide can unite directly with alkaline haemoglobin.*

Owing to the increase in carbon dioxide the venous blood is slightly more acid than the arterial blood.

That serum proteins can act in the same way as haemoglobin in competing with carbonic acid for the alkali of the blood is suggested by Moore. As carbon dioxide is removed from the bicarbonate the solution does not become markedly alkaline because the base set free unites with the protein. Thus the presence of protein keeps down the alkalinity and allows the carbon dioxide to come off, but in the absence of protein the rise of alkalinity would prevent the removal of more than a minimal quantity of carbon dioxide.†

Recent experiments show that the red blood corpuscles carry most of the extra carbon dioxide from the tissues to the lungs. This is probably dependent on the haemoglobin in the corpuscles.‡

**INTERNAL RESPIRATION**

Oxygen is carried to the tissues by the blood and carbon dioxide is removed on the return journey of the blood from the tissues.

During rest there is a continuous oxidation occurring in the tissues, and whenever a tissue becomes active it absorbs more oxygen and gives up more carbon dioxide. The energy changes during oxidation and the possible course of the oxidation have already been indicated, here we are mainly concerned with the exchange of gases between the blood and the tissues.

Between the blood and tissue cells there is usually a layer of lymph, but that can be regarded merely as an indifferent medium through which diffusion can occur.

Increased activity causes an increased absorption of oxygen and there are various ways in which that can be brought about.

First of all, it may be due to a secretion of oxygen by the capillary wall. We shall put that aside because there is no evidence in favour of it and because there are several purely physico-chemical possibilities.

Secondly, it may be due to an increase in the difference of oxygen tension between the blood and the tissue the result either of a rise in oxygen tension in the blood or a fall in oxygen tension in the tissues.

The blood coming by the arterioles is always fully saturated with oxygen, so that apart from breathing a gas mixture containing more oxygen than does atmospheric air the tension of oxygen cannot be increased in arterial blood.

In the capillaries, however, the blood becomes partially reduced so that the oxygen tension is less in the capillaries. By increasing the blood flow the venous blood coming from the tissues is less reduced than usual, so that the mean capillary oxygen tension is higher than before the increased blood flow. During activity the blood flow is increased and the venous blood less reduced, but this is not the sole cause of the increased oxygen intake because mere dilation of blood vessels does not cause an increased intake of oxygen.

During activity there is an increased output of carbon dioxide and an increased carbon dioxide tension causes a rise in oxygen tension of a solution of oxyhemoglobin. However, this does not initiate the increased intake of oxygen because oxygen intake occurs before the increased output of carbon dioxide.

The rise of oxygen tension by vaso-dilation and by increase of carbon dioxide tension both help to increase the oxygen intake, but as pointed out above they cannot be considered to be the cause of the increased oxygen intake.

Before we can consider whether a fall in oxygen tension in the tissues is accountable for the increased oxygen intake we must know that there is an oxygen pressure in the tissues.

It is stated that if a tissue is placed in a vacuum no oxygen is given off, therefore there can be no oxygen tension in the tissue. It may be that any free oxygen is used before the tissue can be placed in the vacuum.

Methylene blue when injected into the circulation is reduced by many tissues to a colourless compound. This is believed to be possible only when there is complete absence of oxygen. The reduction of methylene blue is due to addition of two hydrogen atoms, thus the reaction is that water is split up, oxygen being taken by the tissues and hydrogen by methylene blue. This reaction requires further investigation before it
can be taken as absolute proof that there is no oxygen tension in the tissues.

On the other hand there is some evidence that an oxygen tension does exist in the tissues. Verzar* carried out experiments in which the rate of oxidation in the tissues was followed whilst the animal breathed gas mixtures with less and less oxygen in them. If there is no oxygen tension in the tissues the rate of absorption of oxygen by the tissues should fall as soon as the oxygen tension in the blood falls below its usual value. He found that with some tissues the oxygen consumption fell with a small decrease in oxygen tension in the lungs but that with others the oxygen consumption remained at the same level until there is a marked decrease in the alveolar oxygen tension.

The results indicate that skeletal muscle has a low oxygen tension, not more than 19 mm. mercury at most, whilst the submaxillary gland has a considerable oxygen tension which can be only a little below the oxygen tension in the vein from the gland.

From these figures it is legitimate to conclude that during resting metabolism the tissues have an oxygen tension at least equal to the decrease of tension necessary to cause a decrease in their rate of oxygen absorption.

If it can be shown that there is an oxygen tension in the tissue it is obvious that increased use of oxygen during activity will cause a fall in oxygen tension and an increased absorption of oxygen from the blood.

An increased rate of oxidation without an increase in the oxygen tension can be produced in three ways:

(1) By an increase in oxidisable substance.
(2) By a quicker removal of the products of oxidation.
(3) By the liberation of an increased amount of oxidising enzyme.

In discussing these three we must remember that complete oxidation is a series of changes and that an increase in rate of one reaction may allow the whole series to proceed more quickly.

(1) We must distinguish between the initial changes and the later changes during activity. Increase in activity is associated with increase in oxygen intake and in carbon dioxide output.

If we examine a quick reaction such as the contraction of voluntary muscle we are faced by the difficulty that ordinary analytical methods are not quick enough to follow the rapid changes, but by electro-chemical methods it can be demonstrated that chemical changes such as an increase

in acidity precede the mechanical shortening of muscle.*

(2) Blood analyses indicate that the carbon dioxide output lags behind the oxygen intake, therefore escape of carbon dioxide cannot be the cause of the increased rate of oxidation. We must remember, however, that the escape of carbon dioxide from the cells and diffusion through the lymph to the blood stream takes an appreciable interval of time. Lillie,† from studies on isolated cells, believes that an increase in permeability allows carbon dioxide to escape, thus permitting the oxidation to proceed more quickly because of removal of the products of oxidation. The difficulty here is to be sure that the escape of carbon dioxide is not the result of a rise in carbon dioxide pressure due to activity.

(3) Oxidation is brought about by oxidising enzymes, and it is possible that more enzyme is set free during activity. Change in hydrogen ion concentration always affects the rate of reaction brought about by enzymes, and as mentioned above increase in acidity precedes muscular contraction; thus the action of an oxidising enzyme may be accelerated without an actual increase in amount of enzyme.

In plants various toxic substances start reactions, possibly due to the liberation of an enzyme.‡

At present the only evidence that we have about the early stage of activity is that there is a chemical change preceding activity. This change is shown by an increase in acidity probably by the formation of lactic acid.

The process of muscular contraction is possibly analogous to other kinds of activity, but in any activity lasting a longer time the products of reaction appear and cloud the picture.

Heat production in muscle takes place in two stages: (1) an early stage corresponding to the mechanical shortening; (2) a later stage of repair, during which the tissue is restored to its resting state.

The amount of heat in each of these stages is the same, and this is what one would expect from the mechanics of the reaction. A. V. Hill agrees with Macdonald in ascribing stage 1 to the liberation of some chemical reaction without oxidation, and stage 2 to an oxidation to restore the system to its former state. A reversible isothermal reaction would require the same amount of energy to restore the products to their original condition as to produce the reaction. §

The sequence of events is most probably some sort of chemical alteration (such as production of lactic acid from glucose) which causes an increase in oxidation by furnishing an increase in amount of oxidisable substance. Increased oxidation causes a fall in oxygen tension, hence increased absorption of oxygen. Increased oxidation is followed by liberation of the products of oxidation such as carbon dioxide and water.

It has been stated that oxidation may furnish a larger supply of energy than can be accounted for by the heat of combustion of the substance oxidised. In some chemical reactions this has been realised because the chemical affinity is sufficient to cause combination with a fall in temperature, the extra heat value being absorbed from the surroundings.

In living cells this is not possible as the temperature of the cell is above that of its surroundings, and to say that one molecule of glucose gives more energy than its heat of combustion is to demand that something else, for instance another molecule of glucose, must be oxidised at the same time. It is more logical to regard the energy produced as the result of the combined oxidation because both are necessary for the normal activity of the tissue.

We thus see that respiration requires a respiratory organ in which gas exchange takes place between the external medium and the circulating blood. This exchange is due to diffusion, but active oxygen secretion may occur in some instances.

The transport of gases in the blood is aided by chemical combination of oxygen with haemoglobin and of carbon dioxide with sodium and possibly also with proteins.

The oxidation in the tissues liberates energy for various processes, and the amount of oxygen taken in can be used to calculate the energy production. The output of carbon dioxide also furnishes an indication of the energy production, but one must bear in mind certain fallacies in the latter mode of estimation. These fallacies depend on the nature of the substance oxidised and on the possibility of removing excess of carbon dioxide by forced respirations. Assuming an average respiratory quotient of 0.85 the estimation of carbon dioxide output is a method which can be used in circumstances under which the more elaborate respiratory measurements are not possible.*

GENERAL REFERENCE


EXCRETION OF WASTE PRODUCTS

URING metabolism various waste products are formed. Plants do not possess any special excretory mechanism such as that found in animals. Carbon dioxide can be excreted from the plant by the leaves or used for photosynthesis. In the latter case oxygen is excreted in its place. Some nitrogenous substances may be resynthesised into proteins.

In plants the waste products are frequently formed into insoluble substances which are deposited either inside the cell or in the cell wall. Excretion of waste products by the roots has been said to occur (De Candolle), but the evidence of such excretion is not conclusive.* Grass grown round trees has, however, an injurious effect on fruit trees.† The cell walls are required for support, so although they may contain substances from the cells we cannot always be certain that they are waste products.

Thus it may be that waste substances may still be used for supporting the structure of the plant. We cannot attempt any discussion of excretion in plants as there are not any general points to build upon, the processes being mainly local and dependent on the activity of individual cells, diffusion and precipitation being sufficient in most cases for the removal of the waste products.

Shedding of dead leaves and loss of dead branches represent excretory processes.

In multicellular animals excretion may occur through the lungs (or gills), alimentary canal, skin, or special excretory organs.

From the lungs volatile or gaseous substances are removed. The excretion of carbon dioxide from the blood is a process of diffusion described in Chapter XI. Volatile substances can pass into the lungs until the vapour pressure in the lung

* C. Daubeney, Phil. Trans., 1845, p. 179.
alveoli is equal to the vapour pressure of the same substances in the blood. Usually the volatile substances are so small in amount that they are negligible. Volatile anaesthetics such as chloroform, acetone and possibly traces of ammonia may be found at times in the expired air. The diffusion of soluble substances from gills is also a method by which a certain amount of easily diffusible substance can escape from the blood.

The alimentary canal has well-marked excretory functions. Bile pigments and cholesterol are excreted from the liver. Calcium and iron are two substances excreted by the alimentary canal. Iron is known to have a beneficial effect in restoring haemoglobin in some forms of anaemia. This is easily understood, as iron is necessary for the formation of haemoglobin, it being a constituent portion of the haematin. When iron salts are given by the mouth it is found that the iron apparently passes through the alimentary canal without being absorbed. In order to explain the beneficial effect of iron it was assumed that only organic forms of iron can be absorbed and that inorganic iron was beneficial by preventing the destruction of the organic compounds containing iron. The real explanation is that iron is excreted by the alimentary canal and that the beneficial effect of iron is due to its absorption, but that owing to simultaneous excretion of iron there is apparently no iron absorbed.*

Calcium behaves like iron in being absorbed and excreted by the alimentary canal. It has an additional importance as it may form insoluble soaps, with fatty acids, which cannot be absorbed, and these may give rise to concretions in the alimentary canal.†

The skin in some animals is of importance both for excretion and respiration (frog), but in mammals the skin is mainly protective. Loss of heat by evaporation is important in some animals and the sweat may sometimes contain waste products such as urea.

**SPECIAL EXCRETORY ORGANS**

In most animals the excretory mechanism is built on the plan of the nephridia found in worms. These consist of a tube with a bulbous extremity opening to the coelom by the bulbous extremity and to the exterior by the opposite end of the tube. The mammalian kidneys consist of a series of long tubes

uniting together to form excretory ducts which all open into the two ureters. The ureters open into the bladder and this communicates with the exterior by a single tube, the urethra. Each tube in the kidney consists of a bulbous extremity, Malpighian corpuscle, and a tube with various convolutions in it. The bulbous extremity consists of an epithelium formed by a single layer of flat cells. The wall has been invaginated so that the Malpighian corpuscle consists of a tuft of blood vessels covered by a layer of pavement epithelium. Surrounding this is another layer of pavement epithelium, so that any liquid excreted from the blood is contained between the two layers of flattened epithelium.

At the side of the Malpighian corpuscle, away from the part where blood vessels enter, the neck is continued into the convoluted tubules. These tubules are lined by cubical epithelial cells except in one portion, the descending loop of Henle, where flat cells are found.

In those sea animals in which the internal fluids are of the same composition as the surrounding water there is no need of special excretory mechanism, but in the others in which the internal fluids are of different composition the difference in composition may be maintained by selective absorption or selective excretion.

We have previously considered the equilibrium conditions between cells and their surroundings, and similar relations hold in regard to the maintenance of the composition of internal fluids of multicellular animals. The kidneys possess a marked regulatory function as it is due to their activity that the blood serum of animals is kept of uniform composition. The salts contained in the food vary widely, but the kidney excretes the excess of salts present so that the blood remains of uniform composition.

**FIG. 40.**—Diagram of kidney tubule.
A, Malpighian corpuscle containing glomerulus.
B, Convoluted tubules.
C, Loop of Henle.
D, Collecting tubule.
E, Duct of Bartolimi.
(Copied from "Elementary Physiology," Huxley. Macmillan.)
The composition of the blood must either be the result of an equilibrium or work must be done in maintaining a uniform composition. It must be clearly understood that we have here to deal with the difference in composition between blood and urine. For instance, in the human being the blood plasma contains 0.03 per cent. of urea and the urine approximately 2.0 per cent. In certain fish, however, the osmotic pressure of the blood is maintained by urea and the blood contains 2.61 per cent. urea, whilst the urine contains less.* These differences must depend upon the activity of the kidneys in removing urea.

Two processes are known to occur in the kidney, one a filtration and the other a process requiring the transformation of energy. The former occurs through the flat epithelial cells of the Malpighian corpuscle. The energy for this process is derived from the blood pressure and is thus due to the activity of the heart muscle. The urine thus formed is almost the same composition as the blood plasma, but without the proteins. It is of equal osmotic concentration (isosmotic) with the blood and its production is not accompanied by increased oxidation in the kidneys.† If the cells allow some substances to filter through more easily than others there will be a partial separation of the constituents.

The second or selective function is believed to be a function of the cubical cells lining the kidney tubules. The composition of this secretion differs markedly from the composition of the blood plasma and the energy for its separation is obtained by oxidation in the kidney cells.‡

I shall not attempt any explanation of the phenomenon as there is no satisfactory one yet. We can look upon it like secretion in being a pumping action of the cell which requires a transformation of energy and of which we can calculate the efficiency or relative amount of useful work compared to the total energy used.

Cushny suggests that the glomerulus filters the crystalloids from blood and that the tubule absorbs the ideal composition of the crystalloids of serum, but this amounts to the same thing so far as the energy requirements are concerned. Brodie and Cullis stated that both glomerulus and tubule have a selective secretory power.§

Nussbaurn tied the renal arteries in frogs, and by micro-

scopic examination after injecting the blood vessels he showed that the blood supply of all the glomeruli was cut off. With such preparations he showed that diuresis resulted after the injection of urea, therefore the tubules which are supplied by the renal-portal system must be able to form urine; that is, the tubules excrete even when the glomeruli are deprived of their circulation.* This has been confirmed by later experiments and the only possible fallacy is that some glomerular filtration can occur by back pressure from the anastomosis with the renal-portal system.

**FIG. 41.—Diagram of circulation in frog's kidney.**

The arterial blood supply (A) enters the glomerulus (G), from which it passes to the tubule (T), where it mixes with the venous blood of the renal-portal vein (P), and finally leaves by the renal vein (V).

In the mammalian kidney the circulation is the same, with the exception that there is no renal-portal vein to join with the capillaries surrounding the tubule.

(Copied from "The Secretion of Urine," Cushny. Longmans.)

**COMPOSITION OF URINE**

The composition of the excretion of the kidney varies according to the preponderance of the processes of filtration and secretion. It varies in different species and at different times in the same animal. The substances found in the urine indicate the end products of metabolism.

Birds and reptiles furnish a solid excretion. This may possibly be due to economy of water. Reptiles frequently live in dry places where there is not a large supply of water. Less water means less weight for birds to carry when flying. We also find that the substances excreted by birds and reptiles are relatively insoluble in water, whilst in mammals where a fluid excretion is the rule water-soluble substances predominate. The presence of insoluble substances is a great advantage

in birds and reptiles as the osmotic pressure cannot rise above that of a saturated solution. The greater the osmotic pressure the greater the work done by the kidney in separating the excretion.

On the other hand, insoluble substances in mammalian urine would lead to precipitates and concretions which would irritate the urinary passages.

**Table XXXIV**

*Showing average composition of urine from different types of animals in grams per litre.*

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen</td>
<td>11.2</td>
<td>53.8</td>
<td>30.02</td>
<td>7.5</td>
<td>7.62</td>
<td>36.38</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.6</td>
<td>2.4</td>
<td>0.176</td>
<td>0.06-0.1</td>
<td>0.08</td>
<td>70.14</td>
</tr>
<tr>
<td>Urea</td>
<td>20.9</td>
<td>114.3</td>
<td>Not estimated</td>
<td>Not estimated</td>
<td>15.43</td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.27</td>
<td>Absent</td>
<td></td>
<td>0.266</td>
<td>0.35</td>
<td>0.018</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>0.5</td>
<td></td>
<td>7.59</td>
<td>11.85</td>
<td>Not estimated</td>
<td></td>
</tr>
<tr>
<td>Phenols</td>
<td>0.02</td>
<td></td>
<td>1.19</td>
<td>0.153</td>
<td>Not estimated</td>
<td>(Allantoino, 0.264) (Kynurenlic acid, 0.422)</td>
</tr>
<tr>
<td>Diet</td>
<td>Eggs, Milk</td>
<td>Meat</td>
<td>Hay, Oats, etc.</td>
<td>Dry feed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In what follows we shall consider chiefly the relations of the various substances in human urine. The same principles apply to other animals, but slight differences occur owing to differences in the end products. The excretion of inorganic salts requires no further notice as their amounts are dependent on the amount of the salts in the food, except in disease, when retention of salts may occur. The specific gravity varies according to the relative amounts of liquid and solid to be removed from the body. In normal human urine the specific gravity varies between 1.015 and 1.025.

**Reaction of Urine**

The reaction of the urine depends upon various conditions. In carnivora the reaction is predominantly acid and in herbivora alkaline. This difference is due to the excess of alkaline salts in the food of herbivora. The reaction of the urine reflects the reaction of the blood, as the kidney is the

regulating mechanism for the composition of the blood. Increase in acidity of the blood will cause a greater increase in the acidity of the urine and the reverse. We must refer once more to the difference between the true acidity or hydrogen ion concentration and the titratable acidity. The body possesses regulating mechanisms, hence we find that weak acids or bases are often formed to neutralise excess of alkali or acid respectively.

Sometimes the urine has the composition of a stabilising solution containing phosphates. Thus if a drop of such a urine is placed on red litmus paper it neutralises the acid, so that a purple spot is produced which appears blue against the red of the paper. If a drop of the same urine is placed on blue litmus paper the alkali is neutralised and the purple colour of the neutral litmus appears red in contrast to the blue background. Such urine is called amphoteric, as it apparently turns blue litmus red and red litmus blue.

**THE NITROGENOUS CONSTITUENTS OF URINE**

The total nitrogen of urine is important as it is used as an indication of the total amount of protein destroyed. Protein contains on the average sixteen per cent. of nitrogen, hence the total nitrogen multiplied by 6.25 gives the weight of protein destroyed. It is customary to subdivide the total nitrogen into various fractions and to estimate the percentage of the various nitrogenous constituents to the total nitrogen.

**Table XXXV**

*Showing the distribution of Nitrogen and Sulphur on different Diets.*

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity.</td>
<td>Per cent.</td>
</tr>
<tr>
<td>Volume</td>
<td>1170 c.c.</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>16.8 g.</td>
</tr>
<tr>
<td>Urea Nitrogen</td>
<td>14.70 g. = 87.5</td>
</tr>
<tr>
<td>Ammonia Nitrogen</td>
<td>0.49 g. = 30</td>
</tr>
<tr>
<td>Uric Acid Nitrogen</td>
<td>0.18 g. = 11</td>
</tr>
<tr>
<td>Creatinine Nitrogen</td>
<td>0.58 g. = 36</td>
</tr>
<tr>
<td>Undetermined Nitrogen</td>
<td>0.85 g. = 49</td>
</tr>
<tr>
<td>Total Sulphur as SO₃</td>
<td>3.64 g.</td>
</tr>
<tr>
<td>Inorganic Sulphur as SO₃</td>
<td>3.27 g. = 90.0</td>
</tr>
<tr>
<td>Ethereal Sulphur as SO₃</td>
<td>0.19 g. = 5.2</td>
</tr>
<tr>
<td>Neutral Sulphur as SO₃</td>
<td>0.18 g. = 4.8</td>
</tr>
</tbody>
</table>

*Urea* forms the major portion of the nitrogenous excretion. It is mainly an indication of the amount of protein in the diet. The protein has been traced, during digestion, into the

individual amino acids, and a certain amount of these are deamidised with the formation of ammonia. Ammonia is formed into urea mainly by the cells of the liver. If the liver is removed from the circulation ammonium salts accumulate in the blood and increase in the urine.*

Urea is formed from ammonium carbonate by a process of dehydration, as shown by the following formulæ:

\[
\begin{align*}
\text{ONH}_4^+ - H_2O & \rightarrow \text{ONH}_4^- C=O & \text{C}=O \\
\text{NH}_2 & \text{ONH}_4^- C=O & \text{NH}_2 \\
\text{Ammonium Carbonate} & \text{Ammonium Carbamate} & \text{Urea}
\end{align*}
\]

These changes can be shown by perfusing the excised liver with solutions of either ammonium carbonate or ammonium carbamate, urea being formed in each instance.† The same synthesis can be accomplished but in lesser degree by other tissue cells.

By the formation of urea the alkaline salt ammonium carbonate is turned into the neutral substance urea, thus there is a decrease in alkalinity, or in other words an increase in acidity. According to previous discussions (pp. 59, 112) an increase in acidity will therefore interfere with the formation of urea. It is found that administration of inorganic acids causes a decrease in urea and an increase of ammonium salts in the urine.‡ In conditions when large quantities of organic acids are formed owing to incomplete combustion of fatty acids, there is an increase in the ammonia of the urine.

Urea is often estimated by measuring the amount of nitrogen liberated when it is decomposed by sodium hypobromite. This method is not very accurate, as other substances are also decomposed. By hydrolysis urea can be converted into ammonia and by estimating the increase in the ammonia of the urine the amount of urea can be calculated. The best method of hydrolysis is by the enzyme, urease, of the soy bean.§

Ammonia.—There is always a small amount of ammonia in the blood, therefore, also in the urine. Increase in acids causes an increase in ammonia, as mentioned above. In

spite of the neutralisation of the acids by ammonia certain amounts of the fixed bases, sodium and potassium are removed by combination with the acids. There is thus a danger of coma and death from acid poisoning. Administration of alkaline salts of sodium and potassium is useful in these conditions.

Ammonia can be estimated in two ways. In the first way the solution is neutralised to phenol phthalein and formaldehyde is added. This condenses with ammonia to form hexamethylene tetramine, which is neutral, hence an amount of acid equivalent to the ammonia is liberated. By measuring the amount of alkali necessary to neutralise the solution once more the amount of ammonia is given.* This method gives slightly too high results as other substances besides ammonia, such as amino acids (see p. 127) react with formaldehyde. The second method consists in distilling the ammonia by a current of air at a low temperature after the addition of a weak base such as magnesium carbonate.†

Uric Acid.—This substance is mainly the end product of the decomposition of nucleo proteins. Nucleo proteins are split into nuclein and protein. Nuclein is hydrolysed into nucleic acid and protein. Nucleic acid is decomposed into phosphoric acid, carbohydrate and purine bases and pyrimidine bases. All these changes are brought about by hydrolysing enzymes. The purine bases are deamidised by enzymes and the resulting hypoxanthine and xanthine are oxidised to uric acid.

The excretion of uric acid depends partly on amount of purine substances in the diet and on its formation in the body by the decomposition of nucleo protein according to the following schema: ‡

---


Plant (yeast) nucleic acid differs from animal nucleic acid in containing pentose instead of hexose and uracil instead of thymine.
In the schema the various enzymes responsible for the
various steps are inserted. The absence of any one of these enzymes will prevent that stage of the reaction and the appearance of some intermediate product in the urine. Thus the absence of adenase will prevent the conversion of adenine to hypoxanthine, and adenine will appear in the urine.

The uricolytic enzyme seems to be deficient in man and the higher apes, hence these species excrete a fair amount of uric acid in their urine. Uric acid administered to an animal is partially destroyed and partially excreted unchanged.

Adenase is absent from the human body, hence adenine in the food passes into the urine, but in the destruction of nucleic acid the adenine is diamidised in a combined state and not set free as adenine.

In birds and reptiles the main end product of nitrogenous metabolism is uric acid. In these cases the uric acid seems to be synthesised from ammonia and lactic acid, mainly in the liver.*

In addition to uric acid and other oxy-purines, methyl purines are found in the urine. They are probably derived from the methyl purines caffeine, theophylline and theobromine of coffee, tea and cocoa respectively.

We therefore see that uric acid metabolism is rather complicated. The amount of uric acid excreted depends upon the amount of purine in the food, the amount of nucleic protein destroyed and the activity of the uricolytic enzyme. Pathological conditions upset these various activities and disorders of uric acid metabolism are frequently encountered.

Uric acid is estimated either by precipitating it as ammonium urate in the presence of excess of ammonium salts: the precipitate being titrated with potassium permanganate in the presence of sulphuric acid † or colorimetrically.

Creatinine.—The amount of this substance is fairly constant, and therefore it depends upon some metabolic factor. If protein food is withheld the amount of urea in the urine is decreased, and as the creatinine remains constant in amount it becomes a larger proportion of the total nitrogenous excretion.

The importance of creatinine has been shown since Folin introduced a convenient colorimetric method of estimating it. The method consists in comparing the colour produced under standard conditions by the well known Joffe test with picric acid and alkali against a standard colour.‡

The source of the creatinine is not definitely known. Muscle contains creatine, and it has been suggested that the constancy of the creatinine output is dependent on the amount of muscle in the body.

Creatinine is formed from creatine by dehydration, usually carried out in vitro by heating with acid.

The liver has been suggested as the seat of transformation of creatine into creatinine.* If the creatinine is an indication of muscular metabolism it is related to the metabolism independent of contraction, as muscular movements do not increase the amount of creatine in muscle nor creatinine in urine.

Creatine is not usually found in the urine, but it occurs in the urine of children,† in the urine of women under certain conditions, such as menstruation and pregnancy,‡ and in the urine during starvation. Cathcart has found that the administration of sugar abolishes the excretion of creatine in fasting individuals.§

Creatine is a normal constituent of the urine of birds.||

Cathcart suggests that the presence of sugar allows the creatine formed by tissue breakdown to be reformed into the structures of the tissues. In the absence of sugar the tissues are decomposed and the non-nitrogenous portion used for energy transformations. The creatine thus set free appears in the urine. If sugar is present the creatine is reformed into tissue material. This result would follow from the law of mass action and can be expressed by the equation on p. 57. The reversible process can be understood to be as follows:—

\[
\begin{align*}
\text{protein} & \leftrightarrow \text{Sugar} \\
\text{Muscle tissue} & \leftrightarrow \text{and} \leftrightarrow \text{amino acids} \leftrightarrow \text{and} \leftrightarrow \text{urea.} \\
\text{creatinine} & \downarrow \text{excreted.} \\
\text{creatine} & \downarrow \text{excreted.}
\end{align*}
\]

**Hippuric Acid** is a compound formed by the union of benzoic acid and glycine.

\[
\text{COOH} + \text{CONH} \cdot \text{CH}_2\text{COOH} = \text{COOH} \cdot \text{CONH} \cdot \text{CH}_2\text{COOH}
\]

Benzoic Acid. \hspace{1cm} Glycine. \hspace{1cm} Hippuric acid.

In the excretion of all the previous substances the function of the kidney has been passive, in that it has merely excreted the substances brought to it in the blood, but in the case of hippuric acid the kidney causes its synthesis from benzoic acid and glycine. A mixture of benzoic acid and glycine perfused through the blood vessels of the kidney in the presence of oxygen gives rise to the formation of hippuric acid.* The synthesis occurs even with chopped up kidney tissue.

Hippuric acid is especially predominant in the urine of herbivora, as they ingest a fair amount of benzoic acid. The synthesis of hippuric acid depends on the amount of benzoic acid supplied, glycine being always present in sufficient amount.

The interest of this synthesis is that it is a type of reaction which is employed to render toxic substances non-toxic. Salicylic acid and similar substances are combined with glycine: camphor and other substances are combined with glycuronic acid to form glycuronates. The union of phenolic substances with sulphuric acid to form ethereal sulphate has been already described.

Kynurenic acid is found in the urine of dogs. Its formula is—

\[
\text{C-OH} \uparrow
\]

Allantoine is the form in which the purine derivates appear in the urine of animals which can decompose uric acid.

**Sulphur Compounds.**—Sulphur is found in the urine in three forms. The inorganic sulphates and ethereal sulphates have been described under sulphur metabolism. The third form,

neutral sulphur, consists of compounds which can yield sulphuric acid only after complete combustion, but not after mere hydrolysis.

The neutral sulphur is derived from the cystin of the protein, and is related to the taurine of the bile and thiocyanate of the saliva.

Phosphorus.—The phosphorus of urine is mainly in the form of inorganic phosphates derived from the phosphates of food, nucleoproteins, phosphoproteins and other phosphorus-containing substances.

**Pigments of Urine**

Normal urine is yellow in colour, but it becomes darker after being passed or on heating with acid; thus it contains chromogens in addition to pigments.

Urochrome.—The chief pigment of the urine is a yellow pigment called urochrome. The yellow colour of the blood and tissues of fowls has been shown to be derived from xanthophyll of the food,* and it may be that the yellow colour of urine may be traced to the same source.

Urobilin is present in small quantities as its chromogen urobilinogen. Under pathological conditions it is greatly increased in amount.

Hæmatoporphyrin is present in small quantities, and its amount may be greatly increased in diseased conditions.

These two pigments are derived from hæmoglobin. Hæmatoporphyrin is the iron free derivative of hæmatin. Hæmatoporphyrin is the source of the bile pigments which become reduced in the intestine to stercobilin and stercobilin is identical with urobilin.

Uroerythrin is another pigment. It frequently colours uric acid deposits a brick-dust colour.

The excretion of waste products is concerned with the physico-chemical means by which waste products are removed and with the chemical nature of the substances excreted.

The nature of the waste products is related to the problems of metabolism and they vary in different species of animals. As this subject has such important medical applications a vast literature exists. In this chapter only an outline of the subject has been possible.

**General References**

See books on urinalysis and allied subjects.


CHAPTER XIII
CHEMICAL REGULATION OF CELL ACTIVITIES

In previous chapters we have dealt with various aspects of the chemical activities of living organisms. We have treated these activities from the chemical aspect, but it is well known that the activities of cells vary from time to time and that they are influenced by outside agencies.

In the animal kingdom a specialised system has been built up by which the various tissues are regulated and controlled so that they all work together for the good of the organism. How the nervous system performs this function is unknown, but it is a physico-chemical problem which must be solved in the future. We know that by means of nerves various activities can be augmented or diminished, and that when such a result is produced an electrical change occurs in the conducting nerve; it is probably related in some way to a setting free of electrolytes.

Stimulation of sensory nerve endings is brought about by photochemical changes, or by some shift in equilibrium produced by pressure, temperature, or other physical change, similar to the changes shown by the dilatometer, where volume changes are registered during allotropic transformations, or to the changes in allotropic form which occur at certain transition temperatures.

The development of a conducting system of nerves gives rise to a special form of tissue. The conducting portions of this tissue differ somewhat from the cellular parts where the nerve cells are situated, and from the chemical point of view the difference in composition between executive structure, such as muscle or glands, and controlling and conducting tissues, is of interest.

In addition to the regulation by nerve impulses there is a regulation by means of chemical substances which in the higher vertebrates are transported by the circulating blood. This is the modern counterpart of the early "humoral" conceptions.

We have seen several examples of this regulation in preceding chapters. The accessory food substances, secretin and gastrin, are examples of these regulators.

Waste products from the tissues act on other organs. Carbon dioxide, which is produced in increased amount by
any cellular activity, acts on the respiratory centre in the medulla, causing increased ventilation of the lungs, thus favouring the removal of carbon dioxide from the blood.* The carbon dioxide acts because it is an acid and raises the hydrogen on concentration of the blood.

**Table XXXVI**

<table>
<thead>
<tr>
<th>Composition of Nervous Tissue and of Muscle.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey Matter †</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Solids</td>
</tr>
<tr>
<td>Solid of which</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Extractives</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Lecithins and Kephalins</td>
</tr>
<tr>
<td>Cerebrins</td>
</tr>
<tr>
<td>Lipoid S. as SO₃</td>
</tr>
<tr>
<td>Cholesterin (by diff.)</td>
</tr>
<tr>
<td>Gelatin</td>
</tr>
<tr>
<td>Muscle †</td>
</tr>
<tr>
<td>Water</td>
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<tr>
<td>Solids</td>
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<td>Solid of which</td>
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</tr>
<tr>
<td>Gelatin</td>
</tr>
<tr>
<td>Muscle</td>
</tr>
</tbody>
</table>

The result is that the tension of carbon dioxide in the alveolar air is kept constant, and as the production of carbon dioxide is almost proportional to the use of oxygen the larger amount of air required to remove more carbon dioxide during tissue activity will bring enough oxygen into the lung alveoli.

Urea, the main nitrogenous end product of the metabolism of amino acids, acts on the kidney as diuretic, thus aiding the removal of itself from the blood.

Drugs administered to animals also act on the chemical processes in the body.

Over and above such instances of chemical regulation, which is a common attribute of all cells, there are a number of regulating substances produced by special organs. It is these special organs that must be more especially studied in this chapter. The organs which produce these internal secretions are usually glandular in structure, but without ducts to the exterior, hence they are called ductless glands, and any secretion that they form is carried away by the blood or lymph streams.

**Thyroid Gland**

Swelling of this gland with symptoms of mental dullness

and overgrowth of mucoid tissue under the skin were compared with similar symptoms resulting from removal of the thyroid gland. Experiments showed that removal of the thyroid glands causes mental dullness and a tendency to deposit mucin in the subcutaneous tissues,* that these symptoms can be prevented by grafting the gland into other portions of the body or by feeding with the fresh or dried gland.†

Various conditions, such as cretinism (congenital) or myxœdema (acquired), are associated with deficient activity of the thyroid gland, whilst exophthalmic goitre, which is characterised by excessive nervous activity, is associated with increased activity of the thyroid. The former conditions are relieved by feeding with thyroid glands, and the symptoms of the latter can be produced by excessive doses of the same.

The effect of removal of the thyroid varies with the species‡ and age§ of the animal. In some cases death occurs, whilst in others the results are very slight.

Associated with the thyroid are structures termed parathyroids. It is claimed that removal of the thyroid causes death when the parathyroids are also removed, but not when the parathyroids are uninjured.

The thyroid glands contain spaces filled with a material which stains uniformly with acid dyes, and it has been given the non-committal name of “colloid.” The thyroid gland, unlike other tissues, contains appreciable amounts of iodine in organic combination, and this iodine is said to be connected with the active substance of the gland.

Thyreoglobulin is a protein which can be isolated from the thyroid gland: it contains iodine and has the curative effect of thyroid tissue.||

The thyroid gland is thus seen to contain a hormone which stimulates metabolism, causing increased mental and physical activity. The active substance is associated with the presence of a compound containing iodine.

Quite recently Kendall has isolated a crystalline iodine containing a compound which he calls thyroxin,¶ and to which he ascribes the tautomeric formulae: ---

* Schiff, Arch. f. exper. Path. u. Pharm., 1884, vol. 18, p. 25.
Fig. 42.—Photograph showing a thyroidectomised cretin lamb (left) about fourteen months old, and a normal sheep (right) of the same age.

The thyroids (with the parathyroids) had been removed from the cretin about twelve months previously (Sutherland Simpson).

(From "The Endocrine Organs," E. S. Schafer. Longmans.)
FIG. 45.—Exophthalmic Goitre
Note protrusion of eyeballs and anxious expression of face
(Photolent by Dr. R. Hutchison.)
This substance contains as much as 58–60 per cent. of iodine.

**ADRENAL GLANDS**

These are another interesting example of internally secreting glands. In 1855 Addison showed that a disease characterised by muscular weakness and bronzing of the skin was associated with tuberculous disease of the adrenal glands.*

Oliver and Schafer showed that injections of extracts of the adrenal glands caused a marked rise in blood pressure, which is due mainly to constriction of the blood vessels.†

Takamine isolated the active substance,* and as the result of the work of Abel and others the chemical formula was identified and the substance was synthetised by Friedmann.†

\[
\text{CHOH} \cdot \text{CH}_2(\text{NHCH}_3)\]

This formula contains an asymmetric carbon atom, thus there are two optical isomers, and the physiological activity is due to the lævo form.

There are two types of structure in the adrenal glands, named cortex and medulla respectively. The substance that produces a rise in blood pressure is obtained from the medulla.

The medulla of the adrenal glands stains brown when placed in solutions of potassium bichromate. Other tissues which stain brown with bichromate give extracts which cause a rise of blood pressure. We speak of such tissues as chromaffine, and the extracts as pressor. Such substances are found throughout the vertebrate kingdom‡ and also in invertebrates.§

The cortex of the adrenal glands contains a large amount of lipoid tissue, and it is probably related to the sexual glands. It is of interest that the cortex is derived from the germinal epithelium and is related to the sexual glands, whilst the medulla is derived from the sympathetic nervous system and its extract acts on the terminations of the sympathetic nerve fibres.

**Chemical Constitution and Physiological Action**

The active substance obtained from the adrenal gland (adrenaline) is interesting from the pharmacological point of view. The action of drugs depends upon their physical and chemical properties. The physical properties predominate in those substances which possess a general action, whilst the chemical characteristics are associated with specific or localised activity. A detailed examination of active substances shows that their activity depends upon the presence of special groups and their relation to each other in the molecule. In

other words, the physiological action of drugs depends, like their chemical reactions, on the stereochemical configuration of their molecules. We thus see why a good working knowledge of organic chemistry is necessary for the biological chemist.

One of the compounds which has been thoroughly investigated is the substance adrenaline. Its specific action can be shown in more or less degree by many substances of allied chemical nature. Its activity resembles the effect of stimulation of the sympathetic nervous system, so Barger and Dale have suggested the term "sympathomimetic" for drugs that behave like adrenaline.*

The main factor in producing the sympathomimetic action is an NH₂ group or substituted NH₂ group (NHCH₂ in adrenaline). Thus many amines produce a rise of blood pressure on injection into an animal. The presence of a benzene ring intensifies the activity, if the NH₂ group is separated from the aromatic nucleus by at least one carbon atom. The addition of hydroxyl groups to the benzene ring makes the activity greater.

The secondary alcohol group intensifies the activity, but it is found that the levorotatory substance accounts for all the activity and the dextrorotatory compound is inactive.†

The ketone—\[\text{CO} \cdot \text{CH}_2(\text{NHCH}_3)\]

is much less active than the secondary alcohol—\[\text{CHOH} \cdot \text{CH}_2(\text{NHCH}_3)\]

Adrenaline.

In the above synopsis we see that each portion of the molecule has some effect on the physiological activity, and that comparatively trifling changes caused marked alterations in the activity of the compound. Alkaloids show a similar relation: slight differences in chemical constitution make a great difference in their physiological activity.

**PITUITARY GLAND.**

This organ, which is composed of three distinct portions,

is another internally secreting organ. The anterior portion is derived from the epithelium of the nasopharynx, the posterior portion is derived from the central nervous system, and the intermediate portion is like the anterior portion an outgrowth from the naso-pharynx.

The anterior portion regulates the growth of the body. Enlargement with hyperactivity leads to an overgrowth of the skeleton, which in young people leads to gigantism and in older people to acromegaly. The extract from the whole gland was found by Oliver and Schafer to cause a rise of blood pressure.* Howell has shown this to be due to the extract of the posterior lobe.† This extract from the posterior portion has an action which greatly resembles that of the adrenal medulla, but differs from the latter in causing dilation of the renal vessels and a flow of urine. It probably acts directly on involuntary muscle and not on the sympathetic nerve endings as does adrenaline.

By histological methods Herring has traced a colloid material from the intermediate portion through the posterior portion to the third ventricle. This substance is probably the material which is responsible for the rise of blood pressure, in which case it is formed by the intermediate portion, and the activity of the extract of the posterior portion is due to this substance passing through it.‡

Robertson has isolated a substance which he claims is the growth-promoting substance of the anterior lobe. This substance he calls tethelin: it contains phosphorus, nitrogen and inosite. A portion of the nitrogen is in the form of an iminazolyl group. §

The Pancreas.

In addition to its external secretion the pancreas furnishes an internal secretion. The evidence for this belief is that if the pancreas is removed the metabolism of carbohydrates is upset and sugar is excreted in the urine. || Cohnheim has claimed that neither muscle nor pancreas extract has any glycolytic action, but that mixing extracts of the two produces some substance capable of destroying sugar.¶||

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* G. Oliver and E. S. Schafer, Journ. Physiol., 1895, vol. 18, p. 277.
Fig. 46.—Twelve-months-old hypophysectomised dog (left) and control of same litter (right) (Ashner). The operation was performed at eight weeks.

(From "The Endocrine Organs," E. S. Schafer. Longmans.)
Fig. 47.—Four photographs of the same person, showing the gradual development of the facial appearance characteristic of the acromegalic (Cushing).

A at 24 years of age (prior to the commencement of the disease); B at 29 (onset of disease); C at 37; D at 42 years of age.

(From "The Endocrine Organs," E. S. Schafer, Longmans.)
Levene and Meyer have pointed out that the disappearance of sugar is not necessarily due to destruction of sugar, but that the sugar may have been condensed into a disaccharide or polysaccharide.*

**GLYocosuria**

Removal of the pancreas produces an excretion of sugar in the urine which has been shown to be due to the absence of some chemical substance, as it is not due to nervous action. There is always a small amount of sugar in the blood (0.1–0.15 per cent.) and a trace of sugar in the urine, but under certain conditions the amount of sugar in the urine becomes increased. The excretion of sugar seems to be accompanied by an inability of the body to utilise sugar, and sugar seems to be formed from many other substances. Glycosuria has two important bearings. First, the disease diabetes is characterised by glycosuria, in which large quantities of sugar are lost in the urine. Even if large quantities of food are taken the body becomes wasted owing to the conversion of fat and proteins to sugar, hence these substances are lost from the body. In advanced stages the excessive decomposition of fats leads to the excretion of "acetone" bodies in the urine. The aceto-acetic acid and β-hydroxybutyric acid cause an increased excretion of ammonia and fixed bases in the urine. Coma and death may result from removal of the bases potassium and sodium. The second important point about glycosuria is that various forms of experimental glycosuria are used to show the conversion of other substances into carbohydrate (p. 107).

Removal of the pancreas does not produce glycosuria owing to the absence of its external secretion because blocking the duct or diverting the external secretion from the intestine does not produce glycosuria.

In addition to removal of the pancreas, after which there is an increase in the amount of sugar in the blood, there are several other ways in which glycosuria can be produced.

**Alimentary Glycosuria.**—If the amount of sugar is increased in the blood the kidney excretes a greatly increased amount of sugar in the urine. This increase of sugar in the blood has been mentioned above as the cause of glycosuria after removal of the pancreas. If large quantities of carbohydrate are given by the mouth there is an increased absorption of sugar into the blood. If more sugar is absorbed than can be removed by the liver the excess of sugar passes into the general circula-

tion and causes glycosuria. This form of glycosuria depends upon the rapidity of absorption, hence it is most easily produced when the carbohydrate is given in the form of glucose. If given in the form of starch the carbohydrate must be hydrolysed before absorption, hence the concentration of sugar in the intestine depends on the rate of hydrolysis and the sugar is absorbed as it is formed. The absorption of sugar is therefore slower and the liver can deal with it better, thus a larger quantity of carbohydrate can be given in the form of starch than of glucose, without producing glycosuria. Maltose, lactose and cane-sugar, if injected into the blood, are excreted unchanged in the urine, but if given by the mouth they must be hydrolysed. In normal individuals it is found that alimentary glycosuria can be produced by more than about 150-180 g. of glucose.

120-150 g. of fructose.
20 g. of galactose.
120 g. of lactose.
150-200 g. of cane-sugar.*

If glycosuria is produced by less amounts than these the sugar "tolerance" is said to be decreased. The measurement of sugar tolerance is frequently performed as an indication of an individual’s liability to diabetes.

The limits for alimentary glycosuria are not absolute, but they serve as a general indication of the ability of the individual to absorb and store carbohydrate.

**Phloridzin Glycosuria.**—Administration of the glucoside phloridzin produces glycosuria. This is not due to the sugar in the glucoside as the decomposition product, phloretin, produces a similar but weaker result. It is stated that the amount of sugar in the blood is not increased, and that this form of glycosuria differs from other forms in that the permeability of the kidney for sugar is increased.†

This form of glycosuria is largely used for experimental purposes to show what substances can be converted into glucose by the animal body (p. 107). Phloridzin, suspended in olive oil, is injected and the dose repeated until the excretion of sugar is uniform. This sugar is mainly formed from protein, as the ratio of glucose to nitrogen in the urine \( \frac{D}{N} \) ratio is the same as the ratio of the total amount of carbon when converted into sugar to the nitrogen in protein. Some

substance is then given, and it is seen if there is any formation of extra sugar. If more sugar is formed the $\frac{D}{N}$ ratio may alter. If the $\frac{D}{N}$ ratio does not alter the extra sugar may be due to an extra breakdown of protein, so it is important to compare the $\frac{D}{N}$ ratio with the ratio of the amount of sugar which can be formed from the carbon to the nitrogen of the substance administered.

Sugar has been shown to be formed from glycerine, glyceric acid, lactic acid, propyl alcohol, glycocoll, alanine, aspartic acid and glutaminic acid.

**Table XXXVII**

*Formation of Glucose from Amino Acids in Animal Metabolism.*

<table>
<thead>
<tr>
<th>Substance</th>
<th>Relative amount of sugar formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocoll</td>
<td>All the carbon is formed into glucose.</td>
</tr>
<tr>
<td>Alanine</td>
<td>All the carbon is formed into glucose.</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>Three out of the four carbon atoms are formed into glucose.</td>
</tr>
<tr>
<td>Glutaminic acid</td>
<td>Three out of the five carbon atoms are formed into glucose.</td>
</tr>
</tbody>
</table>

From Graham Lusk, pp. 381 and 382.

**Nervous Glycosuria.**—Claude Bernard, about 1850, showed that injury to the medulla causes glycosuria.* He ascribed this to the presence of a diabetic centre in the medulla. It seems that this form of glycosuria is brought about through the splanchnic nerves which act upon the liver, upsetting the normal glycogenic function. Administration of adrenaline produces glycosuria, probably by stimulation of the splanchnic nerve endings.

Glycosuria can be brought about by anaesthetics, asphyxia and other agents. Some animals are more easily affected than others.

The internal secretions affect the metabolism and numerous observations on nitrogenous and carbohydrate metabolism have been made. Presence or absence of some of these hormones produces glycosuria or increase the sugar tolerance, the details of which must be found elsewhere.

Other organs furnish internal secretions, but in many cases the observations have not reached the stage in which they can be considered from the chemical point of view, but a few examples will be of interest.

The development of the mammary glands has been shown

to be due to extracts from the foetus* or corpus luteum.†

The reproductive organs furnish internal secretions which regulate the development of the secondary sexual characteristics, but the reader must be referred to other books for further details. The interstitial cells of the testis correspond in origin to the cells of the suprarenal cortex.

From these observations we see that the chemical processes in cells can be affected by chemical substances. When these chemical substances are obtained from some source outside the body they are called drugs, but substances formed inside the body are known as internal secretions. The organs that furnish internal secretions seem to be related to each other, so that interference with one may upset the action of all.

All cells add waste products to the circulating blood; some of these substances stimulate other cells to activity. In the case of the ductless glands the production of substances to stimulate other cells has become specialised, so that there are aggregations of tissue with apparently no other function than to produce hormones. Various intermediate stages such as the pancreas, ovary and testis are known where the function of producing an internal secretion is combined with some other kind of activity.

Chemical regulation is predominant where slow reactions such as growth are concerned, whilst the quicker reactions are brought about by nervous influences.

In plants chemical regulation must occur as is evidenced by experiments on the dominance of the apical buds of Picea excelsa. So long as these buds remain the geotropism of other buds is inhibited, but after the apex is removed another bud exhibits geotropism and inhibits the other ones.§

The term hormone is used for the special substances that affect activity without having any other obvious function, but the substances which are excretory products and affect activity as a subsidiary effect, such as urea and carbon dioxide, are called parahormones.

GENERAL REFERENCES


CHAPTER XIV
ACTION OF MICRO-ORGANISMS

We have traced the process of synthesis by green plants and the using of the products of synthesis by animals. The waste products of animals and the dead parts of plants are converted into the simple substances from which our cycle started. The achlorophyll plants behave like animals in decomposing the complex substances formed by green plants.

The final stages of this decomposition are brought about by animal and vegetable micro-organisms. Some of these organisms are saprophytic, i.e., they act on dead organic matter, whilst others are parasitic, i.e., they act injuriously upon living plants and animals. The micro-organisms are present everywhere, in the alimentary canal, soil, etc., so they are ready to act upon the substrate whenever favourable conditions arise. The subject of bacteriology owes its origin to the researches of Pasteur, a chemist who studied the decompositions brought about by micro-organisms.

Carbohydrates are decomposed in various ways. As in animal metabolism the chief end products are carbon dioxide and water, but other substances are also found, e.g., hydrogen, marsh gas, acids, etc. The formation of alcohol from sugar by yeast has been the subject of much study.

Alcoholic fermentation does not depend upon the intact yeast cells, as Buchner showed that a cell-free extract, prepared by grinding the yeast at a low temperature and by subjecting the mass so obtained to hydraulic pressure, is capable of producing alcohol from sugar. He called the active substance Zymase.*

The various factors in the conversion of glucose into carbon dioxide and alcohol have been carefully studied. The initial rate of reaction depends upon the concentration of sugar.

* Symbiosis is a partnership of organisms for their mutual benefit; see, for instance, Plant-Animals, by F. Keeble (Cambridge University Press, 1912).
† E. Buchner, Ber., 1897, vol. 30, p. 117.
The reaction is very rapid at first but it soon falls to a slower rate. Addition of phosphates increases the rate once more until the phosphate is used up, and even then the final slow rate may be greater than it was before the addition of phosphate.

One of the stages of the reaction is the union of sugar with phosphoric acid to form a hexosephosphate. The hexosephosphate is hydrolysed into sugar and phosphate by an enzyme called hexosephosphatase. The rate of hydrolysis of hexosephosphate depends upon its concentration. Free phosphate seems to be necessary for the action of zymase upon glucose, hence addition of phosphate increases the rate of conversion of sugar into alcohol, but the phosphate is soon removed by conversion into hexosephosphate. The rate now falls off, but owing to the hydrolysis of hexosephosphate free phosphate is being formed continuously and the rate of alcohol formation becomes uniform. The initial rise in the rate of fermentation depends upon the amount of phosphate added and it is found that each molecule of phosphoric acid added gives rise to one molecule of carbonic acid.

The final rate of fermentation depends upon the rate at which phosphoric acid is liberated from hexosephosphate, and this, like other enzyme actions, depends upon the concentration of hexosephosphate and hexosephosphatase. If the latter is present in excess the addition of fresh phosphate, leading to a higher continuous supply of free phosphate, and the final rate of fermentation will be greater than the final rate before the addition of fresh phosphate. If, on the other hand, the hexosephosphate is already in excess the addition of more phosphate will cause an initial rise in rate of fermentation, but the final uniform rate will not be increased.

There are therefore two stages, an early stage in which the rate of reaction depends upon the concentration of free phosphate and a later slower stage the rate of which depends upon the rate of formation of free phosphate from hexosephosphate by hexosephosphatase. The fermenting complex is composed of two parts, the enzyme and co-enzyme. The latter of these is not destroyed, whilst the former is destroyed by heating. The phosphate is believed to play the part of co-enzyme. The enzyme, zymase, cannot ferment the di-saccharides cane sugar and maltose, but the intact yeast contains invertase and maltase, whereby these sugars are hydrolysed into the monosaccharides glucose and fructose, which can be fermented by zymase.
Other forms of organisms produce different end products; thus instead of alcoholic fermentation we may have the formation of acetic, butyric or lactic acids, acetone, etc. The different kinds of carbohydrates are acted upon to different degrees by different forms of bacteria, hence the behaviour of an organism towards the various sugars is used as a means of identifying bacteria.

Most carbohydrates are fairly well dealt with by animals, but one form of carbohydrate is apparently not utilised by higher animals. Cellulose is resistant to the enzymes of the mammalian alimentary canal but it is attacked by micro-organisms. Certain bacteria can dissolve cellulose and use it as a source of energy. By the action of bacteria in the alimentary canal of herbivora cellulose is formed into substances which can be absorbed and used by the animal.*

The bacterial decomposition of proteins is spoken of as putrefaction. The hydrolysis of proteins into amino acids has been considered under digestion, so we must now consider the action of bacteria upon amino acids.

The amino acids may be reduced with the formation of ammonia,

\[ \text{R·CH}_2\text{CH·NH}_2\text{COOH} + H_2 \rightarrow \text{RCH}_2\text{CH}_2\text{COOH} + \text{NH}_3, \]

or they may be oxidised with the formation of ammonia and a ketone acid

\[ \text{RCH}_2\text{CH·NH}_2\text{COOH} + \frac{1}{2}O_2 \rightarrow \text{RCH}_2\text{COCOOH} + \text{NH}_3. \]

A process of oxidation and reduction leading as a net result to an apparent hydrolysis may occur,

\[ \text{RCH}_2\text{CHNH}_2\text{COOH} + H_2O \rightarrow \text{RC H}_2\text{CH}_2\text{CH} + \text{NH}_3 + \text{CO}_2. \]

Carbon dioxide may be removed, leading to the formation of an amine,

\[ \text{RCH}_2\text{CHNH}_2\text{COOH} \rightarrow \text{RCH}_2\text{CH}_2\text{NH}_2 + \text{CO}_2. \]

The further behaviour of these substances is important. The fatty acids and alcohols are oxidised in the manner described in Chapter X. The amines may affect the blood pressure (see p. 195) and ultimately produce degenerative changes in the blood vessels. The aromatic amino acids give rise to phenolic compounds such as phenol, and to such substances as indole and skatole. The latter are the typical evil-smelling products of putrefaction. By the overgrowth of putrefactive organisms in the intestine such substances are produced and they produce toxic symptoms unless there is

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sufficient sulphuric acid to combine with them and render them harmless (p. 150).

Metchnikoff suggests that chronic toxæmia by bacterial putrefaction may have an influence in hastening the degenerative changes in the body characteristic of old age.* He further suggests that the activity of putrefactive organisms can be inhibited by weak acids such as lactic acid and that administration of bacteria that can produce lactic acid kills the putrefactive bacteria.†

The ripening of cheese and other processes due to micro-organisms must not be forgotten.

In addition to the micro-organisms which grow outside the body there are those which grow inside the living organism and produce a series of chemical changes. The mere presence of micro-organisms is usually harmless, but they produce their bad effects by poisons or toxins. The toxins may produce a rise of temperature and other symptoms depending on the nature of the toxin produced. The toxin is usually peculiar to the species of micro-organism that produces it and the specific action is frequently very marked. In some cases special groups of cells are affected before others; for instance, the toxin of tetanus bacteria tends to attack the cells of the facial nerve in the medulla, and the toxin of diphtheria bacteria frequently acts upon the nerves to the soft palate.

The defence of the body against bacterial invaders consists of two methods, the first a biological process and the second a series of chemical reactions. The biological method must be considered here, as there are certain chemical points connected with it. Certain of the white corpuscles of the blood can undergo what are termed amœboid movements, that is flowing movements whereby they can surround and engulf particles. The presence of bacteria in the tissues causes the white corpuscles to escape from the blood vessels and move towards the invaders. The stimulus to these movements is the diffusion of substances from the bacteria and is thus similar to taste or smell: it is called chemotaxis.

If the corpuscles engulf the bacteria we speak of it as phagocytosis, and this is an important method of destroying bacteria. The phagocytic corpuscles have been called the policemen of the body.‡ If, on the other hand, the corpuscles

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become killed they form small round white corpuscles called pus corpuscles. Suppuration is the result of the killing of the white corpuscles, probably brought about by lack of oxygen when the corpuscles are thickly massed in one place.

The power of engulfing the bacteria depends on something else than the mere presence of bacteria and white corpuscles, because if the white corpuscles are thoroughly washed so as to remove all traces of blood serum, they will not engulf bacteria. The addition of a little blood serum to the mixture of bacteria and white blood corpuscles causes the corpuscles to engulf the bacteria. Wright and Douglas have ascribed this to the presence of something in the serum which renders the bacteria palatable to the corpuscles; this they term an opsonin."

Chemical protection can be best illustrated by the case of diphtheria. The horse is relatively resistant to diphtheria, so that if a small dose of diphtheria toxin is injected into it there is practically no effect on the horse except a slight rise in temperature. It is found that afterwards the horse can withstand a larger dose of toxin. Increasing doses of diphtheria toxin are given until the animal is said to be immunised. By mixing some of the blood serum from such an immunised animal with diphtheria toxin it is found that a certain amount of toxin is neutralised. This is shown by the absence of symptoms when a small amount of the mixture is injected into a susceptible animal. An antitoxin is said to be produced in the immunised animal. The relation between toxin and antitoxin is probably some sort of chemical union or adsorption.†

Not only can antitoxins be produced by the injection of toxins but there are a whole series of substances, of a protective nature, formed by injection of various substances. The stimulating action of serum on the phagocytosis of bacteria by white blood corpuscles, can be increased by inoculation of bacteria, even if the bacteria have been killed beforehand. That is the opsonins are increased in amount. Injection of bacteria also leads to the production of substances which cause even motile bacteria to fall together in clumps, thus interfering with their activity. We say that an agglutinin has been produced. Injection of foreign proteins leads to the production of some substance that precipitates these proteins (precipitins) and injection of foreign red blood corpuscles leads to the production of something which causes hæmolysis or destruction of these corpuscles (hæmolysins).

These various substances are extremely specific. The agglutinins are used for diagnostic purposes. Suppose an individual is suspected to be infected with typhoid bacteria. A sample of his blood serum is diluted and mixed with a suspension of a culture of typhoid bacillus. If the individual has or has had typhoid fever the bacteria are caused to clump, but if not they remain separated and in active movement.

The precipitin test is used for medico-legal purposes and for the determination of the relationships of species. Blood serum of the human species is injected on several different occasions into a rabbit. After this has been done it is found that the blood serum of this rabbit will cause a precipitate with human blood serum even if the former is diluted. The serum from the same rabbit will not give a precipitate with the serum of the sheep, ox, etc. In a case of suspected murder blood stains can be examined to see if they are from human blood or that of domestic animals.

The production of antitoxins, agglutinins and opsonins is the underlying object in the production of immunity by bacterial vaccines. An emulsion of dead bacteria injected into the body tissues causes a reaction whereby the power is increased of resisting the same species of bacteria that were used for the inoculation.

The serum of a rabbit immunised to human serum may show a slight precipitate with the serum of higher apes. The degree of dilution at which the rabbit's serum can still give this precipitate shows the nearness of kin in the different species, and this method has been used to show the relationship of various species to each other.*

The striking point about these reactions is the high degree of specificity. We cannot distinguish, by ordinary chemical means, between the blood serum of different animals, but in the precipitin test we have a means of distinguishing the various sera with extremely small amounts of material.

Some time after the injection of a foreign serum a stage results in which a second injection of even a small amount of serum from the same foreign species is followed by collapse, and even death. This is called anaphylactic shock and it is extremely undesirable when using serum treatment.† The anaphylactic condition can be prevented by injecting an extremely small dose of serum; this removes the anaphylactic condition without bad results, after which larger doses can be

used. The anaphylactic condition is due to the reaction of all the cells of the body.†

All the above serum reactions have certain features in common. They all require the presence of two substances: one to produce the action (complement) and the other to unite the complement to the substance which is acted upon (alexin). The second substance is called the amboceptor. This is like the chemistry of dyeing, in which one molecular group confers the colouring property and another group the power of uniting with the fabric. Sometimes a mordant is required before the dye will unite with the fabric.

The complement is not specific, but it is rapidly destroyed by heating the serum to 55°C.; that is, it is thermolabile. Such inactivated serum is used in studying the phenomena of immunity as the amboceptor remains undamaged.

The amboceptor is specific and is thermostable; as stated above an activated serum is used to study the amboceptor. By adding fresh complement (in non-immune serum) to inactivated immune serum the activity is restored. By varying the proportions of fresh serum and inactivated immune serum the dosage of amboceptor and complement may be determined.

The following experiment illustrates these points:

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Fresh guinea-pig serum</td>
<td>o</td>
<td>Sheep's red blood corpuscles 5% suspension in saline do.</td>
<td>No hæmolysis</td>
</tr>
<tr>
<td></td>
<td>Serum from rabbit immunised against sheep's red blood corpuscles</td>
<td>do.</td>
<td>No hæmolysis</td>
</tr>
<tr>
<td></td>
<td>Inactivated by heating to 55°C.</td>
<td>do.</td>
<td>Hæmolysis</td>
</tr>
<tr>
<td>Fresh guinea-pig serum</td>
<td>do.</td>
<td>do.</td>
<td></td>
</tr>
</tbody>
</table>

We must here refer to zymoids (p. 73) which we saw were substances derived from enzymes that cannot digest their proper substrate but prevent the action of other enzymes. This behaviour is explained by assuming that enzymes are composed of amboceptor and complement. If the comple-

ment is altered so that it will not cause hydrolysis and the altered complement is united to the substrate by an amboceptor a portion of fresh enzyme cannot unite with the substrate owing to the substrate being saturated with amboceptor and altered complement.

There are certain practical applications of the above relations between complement and amboceptor.

**Wassermann Reaction**

In the Wassermann reaction for diagnosis of syphilis the complement is used up by a special reaction and then the absence of complement is demonstrated by the use of red blood corpuscles and an inactivated hæmolytic serum. The mixtures are made as follows:

A Inactivated blood serum + Antigen consisting of lipoid from healthy person extract
B Inactivated blood serum + Antigen consisting of lipoid from suspected case of syphilis extract
C Inactivated blood serum + Antigen consisting of lipoid from known case of syphilis extract
to each of which is added fresh serum containing complement.

In A the serum does not unite the complement to the antigen, but in C the complement is fixed. In B the complement is or is not fixed, depending on whether the case is or is not syphilitic.

To each of these is added an emulsion of red blood corpuscles which have been "sensitised" by inactivated hæmolytic serum; so that they are hæmolysed by the addition of complement.

In A complement is present, but it is absent in C. Thus hæmolysis occurs in A but not in C. If hæmolysis occurs in B the unknown serum is not syphilitic and the converse.

This test is not infallible, but it is a very useful guide in diagnosis. Using specific agglutinating serum it is possible to show complement "deviation" by bacteria, and this method is used for the differentiation of bacterial species.

**Serum Test for Pregnancy**

By digesting an emulsion of placental tissue (autolysis) with blood serum it is claimed that the digestion occurs more rapidly if the serum is from a case of pregnancy than from a non-pregnant case. This is shown by allowing the digestion to occur in dialysing tubes of parchment paper so that the amino
acids formed during digestion escape into the surrounding liquid and by testing this dialysate for amino acids.

The underlying principle is that the placenta in pregnancy causes an immunity reaction so that the serum of the pregnant female acquires the power of digesting placental tissue.*

The above description refers to vegetable parasites, but within recent years chemical reactions in relation to animal parasites have acquired considerable importance. That malaria parasites at certain stages of their existence can be easily destroyed by quinine has been known for a long time; but we must refer to some points in connection with trypanosomes, the parasites that cause sleeping sickness.

Arsenic is toxic to all animal tissues, but by combining arsenic with an organic carbon group it can be shown to be toxic to animal parasites, but not markedly toxic to higher animals.

Various organic compounds containing arsenic have been used successfully in the treatment of trypanosomiasis† (sleeping sickness), and in syphilis (infection with spirochaeta pallida).

Another element which kills animal parasites in the human body is antimony.

**BIO-CHEMICAL CHANGES IN THE SOIL**

Returning now to our main theme we find that the final stages in the decomposition of organic matter occur in the soil; the aim of cultivation is to aid the proper transformation of the materials in the soil.

The soil consists of a framework of particles of different sizes with water and air filling up the spaces between. In the soil water are dissolved various substances, and it is these substances which are taken up by the roots of plants.

The inorganic salts are of importance. As water drains through the soil it removes some of the soluble material in solution and some constituents wash away more rapidly than others. The supply of inorganic material is maintained by the disintegration of the mineral substances contained in the soil. In the growth of a plant deficiency or excess of a substance is injurious. Mineral fertilisers are used to keep the inorganic constituents up to the optimum concentration.

As in the law of mass action, if one constituent is deficient

its concentration exercises a predominant influence on the reaction, similar deficiency of one constituent in the soil causes the amount of this substance to exert a predominant influence on the rate of growth of the plant. This is expressed as the Law of the Minimum, namely, that no matter how great an excess of food material there is, if one substance is relatively deficient the rate of growth is small and is regulated by the amount of deficient substance in the soil.*

The supply of nitrogenous substances is not so simple as that of the inorganic salts such as phosphates, potassium, calcium, etc. Nitrogenous substances can be supplied in the form of animal or vegetable waste matters. Proteins and amino acids are decomposed, giving rise to ammonia. Urea is rapidly hydrolysed to ammonium carbonate by urease, which is formed by micrococcus ureæ (cf. p. r82). The main supply of nitrogenous material is therefore in the form of ammonia. Ammonia, however, is not used directly by plants but it must be oxidised to nitric acid. This oxidation is accomplished in two stages, namely, the formation of nitrous acid from ammonia and the oxidation of nitrous acid to nitric acid.

The first stage is accomplished by nitrosomas and the second stage by nitrobacter. The energy liberated by the reaction is made use of by these organisms. A supply of oxygen is necessary, and if this is not forthcoming nitric acid is not formed. Under anaerobic conditions or by the overgrowth of organisms that prevent the growth of the normal nitrifying organisms some of the ammonia is decomposed with the liberation of atmospheric nitrogen, which causes a distinct loss to the fertility of the soil.

There are, on the other hand, organisms which "fix" atmospheric nitrogen and furnish a supply for plants. These organisms were first shown to be associated with certain nodules on the roots of leguminosæ; hence the fertilising value of growing such crops as clover.† Later still organisms independent of a symbiotic relationship have been found to oxidise atmospheric nitrogen.

The organisms which cause loss of nitrogen in the form of gas are provisionally believed to be protozoa. These organisms are more easily destroyed than bacteria, hence the value of partial sterilisation of soil consists in killing the protozoa without killing the bacteria. The bacteria afterwards com-

mence to grow and the processes of nitrification go on rapidly before the soil is reinfected by protozoa. Sick soil can be partially sterilised by steam or volatile substances such as toluol; care must be taken not to oversterilise as that kills the bacteria also and prevents the advantages of a fresh growth of bacteria.

In cultivating the soil the main aim is to drain the soil so as to prevent it from becoming waterlogged, to prevent acidity by lime and to break up the soil so that there will be a good supply of oxygen in the spaces of the soil. All these are steps to promote the growth of nitrifying bacteria as well as aid the "weathering" of mineral constituents so that potassium, phosphates, etc., are rendered available for the roots of plants.

The conditions in the soil present a superficial resemblance to fermentation in the large intestine. When there is an overgrowth of putrefactive organisms toxic substances are produced which by absorption cause mental dullness and headache and are said to be instrumental in hastening the onset of old age. These organisms can be removed by infecting the alimentary canal with lactic acid organisms. The acid inhibits the growth of putrefactive organisms and allows the normal bacteria to reassert themselves.

We have seen that when the supply of oxygen is deficient the process of nitrification is stopped and free nitrogen is given off. This leads us to consider anaerobic metabolism, sometimes miscalled anaerobic respiration.

Certain bacteria grow best in the absence of oxygen (anaerobes). Their energy changes must be carried out by some molecular change which does not involve the addition of oxygen. Such a change is exemplified by the explosion of gunpowder or other explosive in which the various atoms are rearranged with the liberation of considerable energy.

A passing reference must also be made to the interesting sulphur organisms which decompose hydrogen sulphide and deposit sulphur in their protoplasm. When there is a deficiency of energy-furnishing food the sulphur is oxidised and the energy set free is utilised for the life processes of the bacteria. These organisms can decompose carbon dioxide and form carbohydrate from the energy of the above oxidation; in fact they grow better if there is no carbohydrate in their surroundings.* This process of chemosynthesis can be compared to the photosynthesis of green plants. In the one case energy obtained from the sunlight and in the other from

a chemical process is used to reduce carbon dioxide and to form energy-yielding food materials.

This completes our survey of the cycle of Biological Chemistry. We have traced our materials from simple substances through their synthesis into complex compounds and the decomposition of these compounds to the simple substances from which we started. During the cycle we have seen the accumulation of energy and the use of this stored energy for muscular movement, growth, secretion, etc.

Various branches of chemistry are included. For some purposes one branch is more important than another, but all are valuable and the particular line of work taken by an investigator depends upon his taste and training.

The main outstanding feature is that life is a continual struggle for a supply of energy and to expend that energy in the most advantageous manner. The former process involves the First Law of Thermodynamics that energy cannot be created or destroyed, therefore it must be obtained from some supply of energy. The latter involves the Second Law of Thermodynamics, that the free or available energy tends to a minimum and that a certain proportion of energy is transformed into a form which is not available for other purposes (entropy). This is due to the fact that all forms of energy can be converted into heat, but that heat by itself cannot always be quantitatively transformed into other forms of energy.

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