THE

CHEMICAL CHANGES AND PRODUCTS

RESULTING FROM

FERMENTATIONS

BY

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INTRODUCTION.

THE majority of the chemical changes which are the result of fermentation occur in two large classes of compounds—the carbohydrates and the albumins. These are the materials used by young plants and animals as food-stuffs; as such, however, they cannot be assimilated, but must first undergo the changes which will be described in the following pages, in order that they may be made assimilable and really serviceable as food-stuffs.

The changes, so far as they are known, generally consist in a splitting up of a complex molecule into simpler ones, but, at present, only in a few cases can the stages in the transformation be followed. The reverse changes, or synthetic processes, no doubt, also take place both in plants and animals, and they consist in the building up of a complex molecule from simpler ones; at the present time, however, we have no knowledge as to how they are produced, but during the last few years experimental *synthetic fermentation processes* with the ferments maltase (Hill, Emmerling), lipase (Kastle and Loevenhart, Hanriot), lactase, and emulsin (Fischer and Armstrong), have been carried out in the laboratory, and it is very possible that processes of this kind take place in nature on similar lines.

All these changes are brought about either directly or indirectly by means of living organisms. In the latter case the change is caused by a soluble ferment, or enzyme, which is secreted by the organism, and which can be employed in the laboratory as a reagent; similar changes to those which occur in nature can thus be produced, and the different products which result at the various stages of the process can be obtained. In the former case, so far as is at present known, these changes are produced by the living organism as the result of its metabolism, though, in all probability, an enzyme is secreted, not excreted, which produces these metabolic changes in the interior of their constituting cells. The researches of recent times upon this subject have made this supposition very probable; for example, a soluble ferment, or enzyme, has been extracted by Buchner from yeast, which can cause the alcoholic fermentation of saccharine liquids—a change which until then had been regarded solely as the result of the life of the yeast-cells.

The chemical changes which occur as results of fermentation are generally brought about by hydrolysis; but oxidation, as well as reduction, also takes place. All enzymes, as pointed out by Schönbein, are capable of decomposing hydrogen peroxide with the evolution of oxygen, and of producing a blue colouration with guaiacum tincture, beer yeasts only being exceptions with regard to the latter test. It has lately been shown that the blue colouration produced in guaiacum tincture is due to the action of a separate enzyme, belonging to the class of oxydases, and Loew has still more recently made experiments showing that the power of decomposing hydrogen peroxide is due to the action of another separate enzyme of general occurrence, termed by him catalase.

The changes which the carbohydrates undergo are by far the most important, on account of the fact that the products obtained from them have been the substances upon which most investigation and research have been expended, and many of them are of commercial importance, *e.g.* the production of alcohol from starch. It will, therefore, be more advantageous to describe firstly the changes which these undergo.

The carbohydrates are generally divided into four classes: (1) the Polysaccharides; (2) the Trisaccharides, or Saccharotrioses; (3) the Disaccharides, or Saccharobioses; (4) the Monosaccharides, or Monoses.

To the monosaccharides, the glucosides, most of which are derivatives of glucose, are very closely allied, and the chemical changes which these undergo as the result of fermentation are best considered before those which take place in the monosaccharides, especially glucose, and which result in the formation of products other than alcohol, *e.g.* the production of lactic acid. Hydrolytic changes also occur in the fats and oils, and in urea, and these are best reviewed before the changes caused by oxidation, the most important of which is the production of acetic acid from alcohol; we shall then come to reduction-changes and the processes of nitrification and denitrification, and finally to the changes occurring in the albumins, which are of a much more complicated nature, but no whit less interesting or important than those occurring in the carbohydrates.

CHAPTER I.

CHANGES IN THE POLYSACCHARIDES.

Of the polysaccharides, starch is by far the most important, not only on account of the great *rôle* which it plays in nature as a reserve material in plants, but also on account of the numerous investigations which have been carried out by various observers, in order to elucidate the chemical changes which it undergoes in plants as the result of the action of the ferment, or enzyme, known as diastase, upon it. These investigations have been made with the ultimate view of determining its constitution, as well as the constitution of the several decomposition products obtained from it during the process.

As is well known, a thin starch paste, containing about one or two per cent. of starch, to which a little diastase has been added, gradually becomes clear and the starch dissolves. This solution now is found to possess the power of reducing Fehling's solution, a property which it did not possess before the addition of the diastase, and, if it be tested at intervals, during the process of this transformation, with a solution of iodine in potassium iodide, a series of colour-changes will be observed: at first a blue colour is formed, showing still the presence of starch, but quickly this colour changes to purple, and then gradually to reddish-brown, until finally no colour-change at all is seen. The purple colour is due to a mixture of starch and dextrin, the reddish-brown to a dextrin, and the cessation of colour-change indicates the presence of another dextrin and maltose. These changes, then, are the result of the hydrolysis of the starch through a series of dextrins to a reducing sugar, now known to be maltose.

Payen and Persoz, who, in 1833, first prepared diastase, were the first to examine the products resulting from this change in starch, and they found that a reducing sugar was not the only product obtained, but that a dextrin, not coloured by iodine and soluble in dilute alcohol, was also formed.

In 1860 Musculus observed that the dextrin and the sugar were formed simultaneously, and that the dextrin was not further altered. Independently of each other, about ten years later, Griessmayer, O'Sullivan, and Brücke ascertained that at least two dextrins were formed in this change, one of which was coloured brown by iodine, whilst the other was not visibly affected. Brücke named these respectively erythrodextrin and achroodextrin. O'Sullivan considered them to be identical, and said that they were convertible into maltose by malt extract.

Further investigations were made by Musculus and Gruber in 1878, who came to the conclusion that at least three achroodextrins were produced, the starch molecule breaking down by a series of hydrations and decompositions, maltose being obtained at each stage, together with a dextrin of decreasing, or lower, molecular weight.

In 1879 Brown and Heron published the results of the work which they had done upon the decomposition of starch by the action of diastase. Their conclusions, which do not differ on the whole from the theories put forward by Musculus and Gruber in the previous year, are to the following effect: Starch has a formula of at least $10C_{12}H_{20}O_{10}$; in the hydrolysis by diastase, groups of $C_{12}H_{20}O_{10}$ are gradually split off, a dextrin of lower molecular weight being left at each stage, and eight of these dextrins are possible; the group of $C_{12}H_{20}O_{10}$, which is split off, is hydrolysed to maltose, $C_{12}H_{22}O_{11}$, the elements of a molecule of water being taken up.

In the same year O'Sullivan stated that four dextrins were formed, one of them being erythrodextrin and the other three achroodextrins.

About the same time Herzfeld brought forward still another view of the changes produced in starch, as the result of his own experiments. He observed that the products obtained were different when the hydrolysis was carried out below or above 65° C.; at the lower temperature maltose and achroodextrin were formed, and the latter was subsequently converted into a body, which he called maltodextrin, and maltose; at the higher temperature, in addition to these products, dextrin and erythrodextrin were formed. He concluded, therefore, that there was consecutively a conversion of the starch into soluble starch, erythrodextrin, and achroodextrin, and that the latter was then transformed into maltodextrin and maltose. For maltodextrin he suggested the composition of two dextrin groups united to a sugar group of the formula $C_6H_{12}O_6$; thus: $\left\{\begin{matrix} C_6H_{10}O_5 \\ C_6H_{10}O_5 \\ C_6H_{12}O_6 \end{matrix}\right\}$.

In 1885 the results of a very thorough investigation by Brown and Morris of the products obtained from starch by the action of diastase were published. These investigators found that 81 per cent. of maltose and 19 per cent. of dextrin were formed as end products, and that the dextrin was further hydrolysed only with great difficulty. They obtained also an intermediate product which they called maltodextrin, and this was found to be produced in varying amounts; this product was different to that of the same name obtained by Herzfeld, which they considered to be impure. The following theory was framed to account for these facts: Since only four-fifths of the starch is readily converted into maltose, the formula of starch is at least $5(C_{12}H_{20}O_{10})_3$, and consists of five amylin or dextrin-like groups, four of which are arranged symmetrically round the fifth, which is changed with greater difficulty, and finally remains as dextrin. At each stage a group of $(C_{12}H_{20}O_{10})_3$ is split off before complete hydration takes place, and a dextrin-like residue is left. The group which is split off is hydrolysed and forms maltodextrin $\left\{\begin{matrix} C_{12}H_{22}O_{11} \\ (C_{12}H_{20}O_{10})_2 \end{matrix}\right\}$, one molecule of water being taken up. It is then further changed into maltose by the assimilation of two molecules of water.

That maltodextrin is a definite chemical compound, and not a mixture of maltose and dextrin, is shown by the following properties: (1) it cannot be separated into maltose and dextrin by dialysis; (2) it is completely converted into maltose by the action of diastase, whereas a mixture would leave a residue of dextrin; (3) it is not fermentable by yeast directly.

In 1874 Naegeli obtained, by the action of dilute acids upon starch, a substance which he called amylodextrin. This substance formed the subject of a paper by Brown and Morris in 1889; they found it to be a definite chemical compound, and not a mixture of maltose and dextrin, although its composition could be expressed in the terms of maltose and dextrin, and that it was analogous to maltodextrin in composition. They represented it by the formula $\left\{\begin{matrix} C_{12}H_{22}O_{11} \\ (C_{12}H_{20}O_{10})_6 \end{matrix}\right\}$, *i.e.* constituted as consisting of six amylin or dextrin groups, and one amylon or maltose group. It was converted, by the action of diastase, immediately and completely into maltose, and could not therefore contain the stable amylin group. It was also quite different from soluble starch, with which it was supposed by Naegeli and others to be identical.

In the same year the same investigators published their further views upon the constitution of starch, and the hydrolytic action of diastase upon it. The starch molecule was still supposed to consist of four complex amylin groups arranged symmetrically round a fifth similar group, which constituted a molecular nucleus; each of the five amylin groups had a formula of $(C_{12}H_{20}O_{10})_{\cancel{12}}^{20}$, and a molecular weight of 6480 (from results obtained by Raoult's method), which was considerably greater than they had originally supposed. The entire starch molecule, or, more correctly speaking, that of soluble starch, was represented by the formula $5(C_{12}H_{20}O_{10})_{20}$, with a molecular weight of 32,400. The first action of hydrolysis by diastase was to break up this molecular complex, thereby liberating all the five amylin groups; four of these, by successive hydrolysations through maltodextrins, were completely converted into maltose, whilst the central amylin nucleus, by a closing up of the molecule, withstood hydrolysis, and constituted the stable dextrin which is so slowly acted upon. The four readily hydrolysable amylin groups were regarded as of equal value, which is in accordance with the view put forward by O'Sullivan in 1879, which stated that the dextrin molecules do not become smaller at each step, and that "the difference in their behaviour must be accounted for by a difference of relation in the arrangement of the molecules to one another, probably in solution alone." This hypothesis, they maintain, provided for intermediate maltodextrins or amylodextrins, whose number was only limited by the size of the original amylin group.

In 1890 Emil Fischer synthesised a new disaccharide from glucose, which he termed isomaltose; and Lintner, in 1891, who had been investigating the diastatic hydrolysis of starch, obtained a sugar of which he made the osazone, which corresponded in its properties with the osazone obtained by Fischer from his sugar: he therefore concluded that isomaltose was a product of the hydrolysis of starch, and not maltose, as had been previously supposed. Two years later, together with Düll, he criticised Brown and Morris's theory, and suggested that the various amyloïns were mixtures of dextrin and isomaltose. At the same time, they brought forward their theory upon the action of diastase on starch, which is as follows: In the hydrolysis of starch five compounds are formed—isomaltose, maltose, and three dextrins, viz.

amylodextrin, erythrodextrin, and achroodextrin, of which they made molecular weight determinations. The first product is amylodextrin $(C_{12}H_{20}O_{10})_{54}$, which is the chief constituent of amidulin, or soluble starch; then erythrodextrin $(C_{12}H_{20}O_{10})_{17}.C_{12}H_{22}O_{11}$ is formed; and subsequently achroodextrin $(C_{12}H_{20}O_{10})_5.C_{12}H_{22}O_{11}$, isomaltose, and maltose. They give the following equations to represent the formation of these substances:—

$$\underset{\text{Amidulin, or amylodextrin.}}{(C_{12}H_{20}O_{10})_{54}} + 3H_2O = \underset{\text{Erythrodextrin.}}{3[(C_{12}H_{20}O_{10})_{17}.C_{12}H_{22}O_{11}]}$$

$$\underset{\text{Erythrodextrin.}}{3[(C_{12}H_{20}O_{10})_{17}.C_{12}H_{22}O_{11}]} + 6H_2O = \underset{\text{Achroodextrin.}}{9[(C_{12}H_{20}O_{10})_5.C_{12}H_{22}O_{11}]}$$

$$\underset{\text{Achroodextrin.}}{9[(C_{12}H_{20}O_{10})_5.C_{12}H_{22}O_{11}]} + 45H_2O = \underset{\text{Isomaltose.}}{54C_{12}H_{22}O_{11}} = \underset{\text{Maltose.}}{54C_{12}H_{22}O_{11}}$$

All these changes are supposed to occur simultaneously.

Schiffer also considered that isomaltose occurs amongst the transformation-products of starch. About the same time (1893), Scheibler and Mittelmeier evolved another theory, as they did not agree with that of Lintner and Düll. Scheibler and Mittelmeier thought that there was no real difference between the constitution of starch, dextrin, and the bioses, etc., except in the difference of the number of glucose residues in the molecule. The glucose molecules are capable of combining in two ways, either one or both of the carbonyl groups taking part; in the former case an aldehyde is formed, but not in the latter; thus there are monocarbonyl and dicarbonyl linkings. In starch the glucose residues are combined by dicarbonyl linkings since starch cannot reduce Fehling's *solution; hydrolysis by acids or by diastase takes place* at the carbonyl linkings, two molecules, each containing an aldehyde group, being formed, and the particular glucose residue is connected with the remainder of the molecule by a monocarbonyl linking. These intermediate substances form dextrins, and as hydrolysis takes place probably simultaneously at several of the dicarbonyl linkings, a number of dextrins are simultaneously formed; by the further action of diastase, dextrins of smaller molecular weight, and finally maltose, are obtained.

Brown and Morris, in 1895, noticed that maltose, when liberated from starch by diastase, was in the state of half-rotation, and that on standing, or on heating, it acquired the normal rotatory power; O'Sullivan and Tompson have observed this same peculiarity with dextrose when obtained by the inversion of cane sugar.

In the same year, Ling and Baker, and Brown and Morris investigated Lintner's isomaltose, and concluded that the sugar obtained by Lintner was not identical with that obtained by E. Fischer, as it could be separated by fractionation with alcohol into maltose and dextrins of the maltodextrin type. Further, that maltose is the only sugar obtained in the hydrolysis of starch which can give a crystalline osazone.

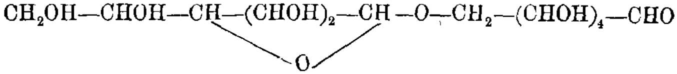
In 1895 Ulrich repeated Lintner's work, and found that maltose and not isomaltose was formed; Jalowetz obtained the same result as Ulrich, and considered that maltose was the only sugar obtained. Ost, in 1896, again confirmed the presence of maltose, and Pottevin, in 1899, also came to the same conclusion. These various observations definitely prove that maltose is the sugar resulting from the hydrolysis of starch by the action of diastase.

Mittelmeier, in 1895, promulgated a still different view upon the diastatic resolution of starch. According to him there are two stages: in the first stage, a small portion of the starch is rapidly converted into amylo-, achroo-, and erythro- dextrins, and sugar; these are not identical with those formed later; and he calls them, according to the stage, primary and secondary dextrins. From the primary dextrins maltose, and from the secondary a new sugar, which he terms metamaltose, are obtained. This view he modified two years later (1897), thus: the first action of diastase on starch results in the formation of equal quantities of two different amylodextrins, one of which is more easily attacked by diastase than the other; thus, while the one is in the erythrodextrin stage, the other has passed from dextrin to sugar; the existence of a sugar molecule and a dextrin molecule in one molecular complex was thus said to have been made intelligible.

In 1898 Pottevin described a number of experiments which tended to show (1) that the conversion of starch into maltose took place in two stages, dextrin being the intermediate product; (2) that the various dextrins differed only in their physical states; (3) that the different parts of the starch granule differed in the ease with which they were converted into dextrin, and the dextrin then into maltose. Thus, the conversion of starch proceeds with unequal rapidity in the different portions of the mass. In the next year he stated that the dextrins could be divided into three groups: (1) achroodextrins not coloured; (2) amylodextrins coloured blue; (3) erythrodextrins coloured red, by iodine. Amylodextrin, he considered, was not a definite substance, as it gave α- and β- amylodextrins; the formation of dextrin was independent and distinct from the formation of maltose from dextrin. Brown and Morris's maltodextrin he considered to be a mixture, as he had separated it into maltose and dextrin.

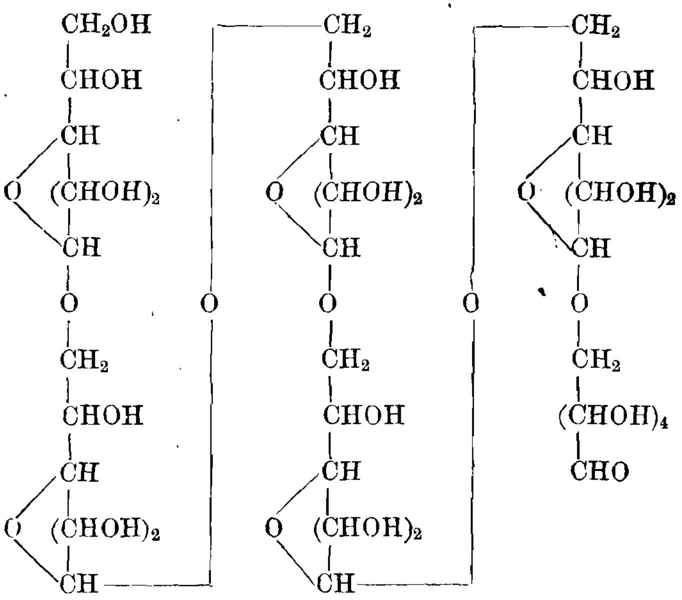
In 1899 Brown and Millar published two very important papers upon maltodextrin and dextrin. To maltodextrin the empirical formula $\left\{ \begin{matrix} C_{12}H_{22}O_{11} \\ (C_{12}H_{20}O_{10})_2 \end{matrix} \right\}$ was assigned by Brown and Morris in 1885.

When this was prepared in a pure state—a very laborious process—and oxidised with mercuric oxide and barium hydroxide until it no longer had any reducing power, a definite and complex carboxylic acid, called maltodextrinic acid A, was obtained. By the hydrolytic action of diastase 40 per cent. of maltose and 60 per cent. of another carboxylic acid—maltodextrinic acid B—were formed; dilute acids gave 85·8 per cent. of d-glucose and a simpler acid containing five carbon atoms, which is a quite different result. The B acid gave the same products, on hydrolysis, as the A acid. The five carbon atom acid appeared to be a normal acid derived from a pentose, and had properties in common with those of xylonic acid. From these results they conceived an idea of the constitution of maltodextrin and the derived acids which is as follows: maltodextrin is made up of a number of C_{12}- or maltose residues, since it is completely hydrolysable to maltose, but still retains a terminal CHO group owing to its possessing reducing properties; oxidation goes further than the conversion of this CHO group into a COOH group, since it splits up the terminal C_{12}-group into a C_5- acid residue, which remains attached to the hydrolysable portion of the polysaccharide. If now Fischer's constitutional formula for maltose—

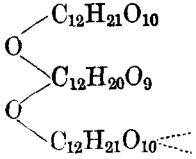


$$\mathrm{CH_2OH{-}CHOH{-}\underbrace{CH{-}(CHOH)_2{-}CH}_{O}{-}O{-}CH_2{-}(CHOH)_4{-}CHO}$$

be accepted, the polysaccharide maltodextrin may be regarded as made up of three such molecules, with the elimination of two molecules of water, and may be represented as follows :—



or abbreviated thus—



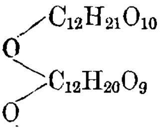
$$\begin{array}{l} \quad C_{12}H_{21}O_{10} \\ O \\ \quad C_{12}H_{20}O_9 \\ O \\ \quad C_{12}H_{21}O_{10} \end{array}$$

the *sign* ⋰ *being used to* denote the open termina carbonyl group, as suggested by Scheibler and Mittel meier.

The C_{12}- groups and the C_6- groups are linked to on another through oxygen atoms, and at these points th molecule breaks down in hydrolysis.

The difference between acid and diastatic hydrolysis in that glucose is only formed by the former, may b accounted for by spatial relations between the adjacen C_{12}- groups, which may determine the differential action o diastase, or by the relative size or mass of the C_6- or C_{12} groups. This resembles the selective action of maltase o glucase, and emulsin, which was shown by E. Fische to exist in their behaviour to glucosides.

The constitution of maltodextrinic acid A can only be explained by assuming that the terminal C_{12}- group o the maltodextrin is attacked in such a way that by simultaneous or successive oxidation $C_7H_{12}O_5$ is removed, and $C_5H_{10}O_6$ is left still in combination with the poly-saccharide residue. The following is thus the only formula which it can possess :—

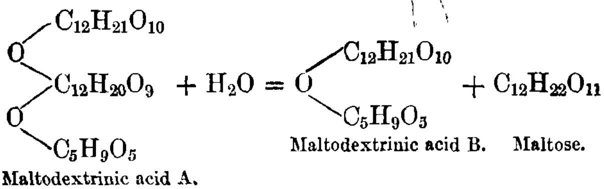


$$\begin{array}{l} \quad C_{12}H_{21}O_{10} \\ O \\ \quad C_{12}H_{20}O_9 \\ O \end{array}$$

the reducing C_{12}- group of maltodextrin having become —O—CH_2—$(CHOH)_3$—COOH—the residue of a normal C_5- carboxylic acid.

The hydrolytic decompositions of maltodextrinic acid A can be expressed by the equations :—

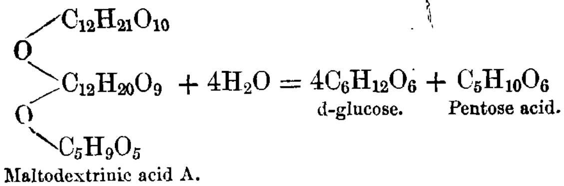
1. *Diastatic*—



$$\begin{array}{l} \quad C_{12}H_{21}O_{10} \\ O \\ \quad C_{12}H_{20}O_9 \\ O \\ \quad C_5H_9O_5 \end{array} + H_2O = \begin{array}{l} \quad C_{12}H_{21}O_{10} \\ O \\ \quad C_5H_9O_5 \end{array} + C_{12}H_{22}O_{11}$$

Maltodextrinic acid A. Maltodextrinic acid B. Maltose.

2. *Acids*—

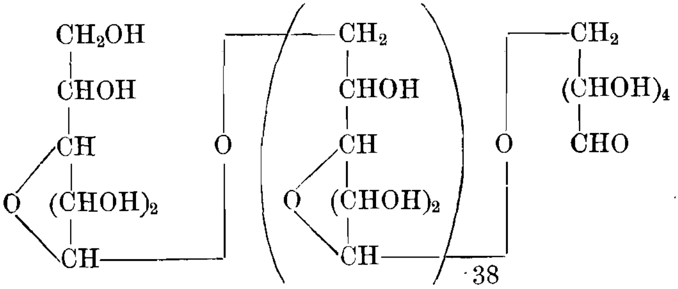


$$\begin{array}{l} \quad C_{12}H_{21}O_{10} \\ O \\ \quad C_{12}H_{20}O_9 \\ O \\ \quad C_5H_9O_5 \end{array} + 4H_2O = 4C_6H_{12}O_6 + C_5H_{10}O_6$$

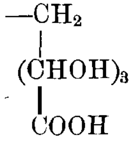
Maltodextrinic acid A. d-glucose. Pentose acid.

The dextrin, which is formed together with maltose in the diastatic hydrolysis of starch, was obtained in the purest possible state by fractionation with alcohol and occasional fermentation, and was found to possess a feeble reducing power. On oxidation with mercuric oxide and baryta, it gave a complex carboxylic acid, called dextrinic acid. By hydrolysis with acids it was resolved into d-glucose and a C_5- acid identical with that obtained from maltodextrin, but diastatic hydrolysis gave maltose and glucose, which are also obtained in almost equal quantities from dextrin itself by the action of diastase. Here there is no selective action of diastase to split off maltose rather than glucose, and the difference in linkings or spatial relations between the C_{12}- groups and their constituent C_6- groups cannot account for these results. The stable dextrin must therefore be made up of C_6- complexes, or as a combination of 39 groups of $C_6H_{10}O_5$ and one $C_6H_{12}O_6$ group, or as $40(C_6H_{10}O_5) + H_2O$.

Its constitution may be regarded as a condensation of 40 glucose molecules with the elimination of 39 molecules of water, expressed by the formula :—



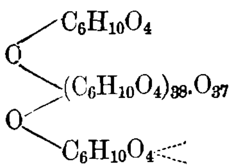
The constitution of dextrinic acid will then be given by converting the right-hand group into—



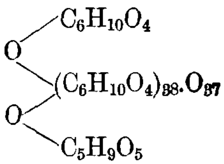
$$\begin{array}{l}—CH_2\\ \quad |\\ (CHOH)_3\\ \quad |\\ COOH\end{array}$$

the residue of a C_5- acid given by complete hydrolysis.

These formulæ can be abbreviated thus :—



Stable dextrin.



Dextrinic acid.

The size and constitution of the starch molecule are suggested from the total results which Brown has obtained with his various co-workers. The stable dextrin obtained with maltose cannot have a simpler formula than 40 $C_6H_{10}O_5 + H_2O$, and the equation representing its formation from starch together with maltose in the proportion of 1 to 4 becomes :—

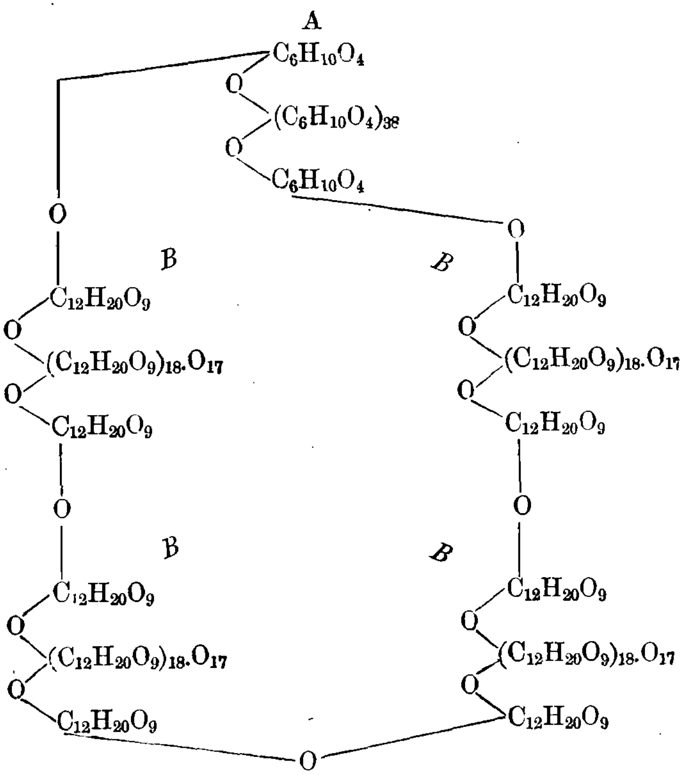
$$100\ C_{12}H_{20}O_{10} + 81\ H_2O = 80\ C_{12}H_{22}O_{11} + \begin{cases} (C_6H_{10}O_5)_{39} \\ C_6H_{12}O_6 \end{cases}$$

The starch molecule cannot be less than five times that of stable dextrin, on account of this result, and dextrin from molecular weight determinations has a formula of 39 $(C_6H_{10}O_5).C_6H_{12}O_6$, giving a molecular weight of 6498.

Its molecular weight cannot be less than 32,400, and its empirical formula can be represented by :—

$$100\ C_{12}H_{20}O_{10},\ \text{or}\ (80\ C_{12}H_{20}O_{10}\ .\ 40\ C_6H_{10}O_5)$$

Starch also does not possess a carbonyl group, as it is a non-reducing polysaccharide, and its constitution can be expressed most simply as made up of 80 maltose groups and 40 dextrin groups, linked in ring form through oxygen atoms, thus :—



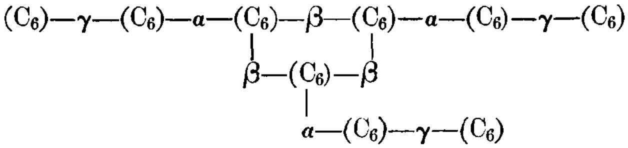
On hydrolysis with diastase, the dextrin complex A is split off, forming stable dextrin, 39 $C_6H_{10}O_5.C_6H_{12}O_6$, whilst the maltose portion B of the ring is attacked at the oxygen linkings of the C_{12}- groups, forming, by successive stages of hydrolysis, maltodextrins or reducing dextrins, and finally maltose.

Also in 1899 W. Syniewski obtained from starch by the action of water, alkalies, and sodium peroxide, a number of substances which he termed amylodextrins, of which the simplest was amylogen. Starch and the amylodextrins are therefore considered to be made up of a number of these amylogen molecules associated together in the form of anhydrides, by loss of water. The amylogen obtained by the action of sodium peroxide has the formula $C_{54}H_{96}O_{48}$, from molecular weight determinations, and consists of three maltose residues combined with a dextrin residue of eighteen carbon atoms; and the dextrin consists of three glucose residues, two of which are combined together in the form of isomaltose.

Diastase hydrolysis was performed with the amylodextrin obtained by the action of boiling water under pressure, and the process is supposed to be a gradual splitting off of maltose groups leaving a maltodextrin, until a dextrin remains which does not contain maltose, and is called "Grenzdextrin," a glucodextrin. This is then converted into simpler glucodextrins and glucose, then into isomaltose and glucose, and finally into glucose. The final result, which is a mixture of dextrin and maltose, is explained by supposing that the maltose is not so easily removed from some of the intermediate dextrins as it is from others.

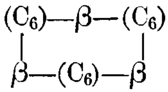
By the action of malt extract, previously heated to 78°, upon amylodextrin, Syniewski has recently obtained a substance, which he has termed Grenzdextrin II.; it differs from Grenzdextrin I., which he formerly obtained by the action of fresh unheated malt extract, and is identical with Brown and Morris's maltodextrin. To account for these results he supposes that the amylogen residue contains three kinds of carbonyl linkings; he denotes them by α, β, γ, and the glucose residue by (C_6);

and the following schematic formula is given to the amylogen residue:—



$$\begin{array}{ccccccccccc}(C_6) & -\gamma- & (C_6) & -\alpha- & (C_6) & -\beta- & (C_6) & -\alpha- & (C_6) & -\gamma- & (C_6)\\ & & & & | & & | & & & & \\ & & & & \beta & -(C_6)- & \beta & & & & \\ & & & & & | & & & & & \\ & & & & & \alpha & -(C_6)- & \gamma & -(C_6) & & \end{array}$$

When fresh malt extract acts upon the amylogen residues of amylodextrin, the α linkings are attacked, maltose $(C_6)-\gamma-(C_6)$ and Grenzdextrin I., made up of the residues—

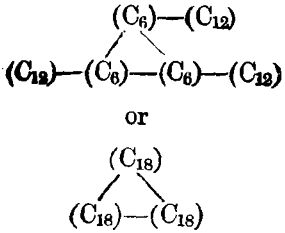


$$\begin{array}{ccc}(C_6) & -\beta- & (C_6)\\ | & & |\\ \beta & -(C_6)- & \beta\end{array}$$

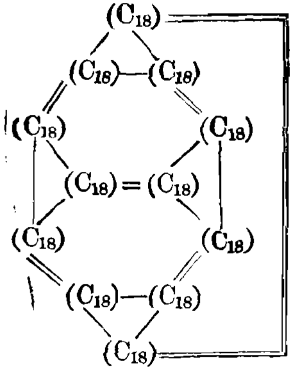
being obtained; when heated malt extract acts upon the amylogen residues, the β linkings are attacked, and Grenzdextrin II., made up of the residues—

$$(C_6)-\gamma-(C_6)-\alpha-(C_6)$$

is obtained. He has previously shown that Grenzdextrin I. has the formula $C_{72}H_{124}O_{62}$, and consequently it contains four of these residues; amylogen, which has the formula $C_{54}H_{96}O_{48}$, can only give one of these residues, so that starch must contain at least four amylogen residues; if (C_6) and (C_{12}) represent glucose and maltose residues respectively, the following formulæ are then obtained:—



Amylogen.



Starch.

where the single lines between the (C_{18})- residues represent β-carbonyl linkings, and the double lines two carbinol linkings.

H. Brown's last publication, with Glendinning, is upon the velocity of diastase hydrolysis, and he finds that it does not follow the law of mass action. A. J. Brown has obtained a similar result in the case of invertin.

Before passing to the next polysaccharide, the other sugars which have been said by different investigators to be products of diastase hydrolysis might be enumerated. Some observers have supposed glucose to be a product, but the conditions of the experiment were probably the cause of this observation, other enzymes being present in the diastase solution. Nasse has obtained a sugar which he calls ptyalose, differing from both glucose and maltose, by the action of ptyalin upon starch.

The action of diastase upon starch is considered by Arthur Meyer as catalytic, the amylose taking up the elements of water and breaking up into two or more molecules of amylodextrin, which subsequently forms dextrin and isomaltose; maltose is formed by intramolecular rearrangement from isomaltose as well as from dextrin.

From the foregoing it will be seen that very many theories have been advanced by many workers to explain the hydrolysis of starch by diastase. Two facts, however, stand out clearly from the mass of complicated results: firstly, the production of maltose; and secondly, the production of a dextrin much more difficult to convert into maltose, but eventually convertible. Intermediate between the starch and maltose many dextrins, no doubt, are formed, and they have been named with the various terms enumerated above. Of these, Brown's maltodextrin only has been prepared in anything like a state of purity; but this may possibly be the simplest of them, as its constitution has been determined: the other dextrins are no doubt mixtures of several of the intermediate ones, and have not yet been prepared in such a state of purity that their properties could be established. Opposite is a table showing schematically the various results which have been obtained.

Scheme of the Results of the Hydrolysis of Starch.

Name.	Date.	Products, according to the various Authors.
Payen and Persoz	1833	Dextrin and reducing sugar.
Musculus	1860	Ditto , but simultaneously.
Griessmayer	1871–72	Two dextrins : one coloured brown, one not coloured, by iodine. Sugar. ‖ ‖
Brücke	Ditto	Ditto : erythrodextrin, achroodextrin. Ditto.
O'Sullivan	Ditto	Ditto ; but identical → maltose.
Musculus and Gruber	1878	Erythrodextrin, and 3 achroodextrins. Maltose at each stage.
Brown and Heron	1879	8 possible dextrins. Maltose at each stage.
O'Sullivan	Ditto	1 erythrodextrin and 3 achroodextrins. Maltose.
Herzfeld	Ditto	Starch → soluble starch → erythrodextrin → achroodextrin → maltodextrin + maltose.
Brown and Morris	1885	Dextrin $\frac{1}{5}$ and maltodextrin → maltose $\frac{4}{5}$.
Ditto	1889	Same results as above, but starch molecule larger than originally supposed.
Lintner and Düll	1893	Starch → amylodextrin → 3 erythrodextrin → 9 achroodextrin → 54 isomaltose → 54 maltose.
Scheibler and Mittelmeier	Ditto	2 dextrins → dextrins of smaller molecular weight → maltose.
Mittelmeier	1895	(1) Primary dextrins (amylo-, erythro-, achroo-) → maltose. (2) Secondary dextrins → metamaltose.
Ditto	1897	2 amylodextrins → one quickly to sugar. → one slowly to sugar.
Pottevin	1898	Starch → dextrins → maltose.
Ditto	1899	Dextrins = α and β amylo-, erythro-, achroo- dextrins.
Brown and Millar	1899	Dextrin and maltodextrin → maltose. ↓ Maltose, with difficulty.
Syniewski	1899	Starch → amylodextrins (simplest amylogen) → maltodextrins + maltose. ↓ Glucose + isomaltose ← simpler glucodextrins ← grenzdextrin + maltose. ↓ glucose.

CHAPTER II.

CHANGES IN THE POLYSACCHARIDES.—Continued.

THE next polysaccharide, inulin, which is also found as a reserve material in certain plants, undergoes hydrolysis by the action of the ferment known as inulase, discovered by Reynolds Green in 1887. The formula $C_{12}H_{20}O_{10}.2H_2O$ is usually given to this polysaccharide, which, according to Bourquelot, is converted by fermentation into lævulose or fructose.

Kiliani, from results of combustion, gave the formula $C_{36}H_{62}O_{31}$ or $6C_6H_{10}O_5 + H_2O$ to this carbohydrate; but Brown and Morris, in 1889, from determinations made by Raoult's method, considered the formula to be $2C_{36}H_{62}O_{31}$. They also stated that inulin resembled amylodextrin in its properties very closely, and they gave the following formula to express its constitution :—

$$\left\{ \begin{matrix} (C_{12}H_{22}O_{11})_2 \\ (C_{12}H_{20}O_{10})_4 \end{matrix} \right\}$$

which is closely analogous to that of amylodextrin. The ultimate products of hydrolysis of inulin are different, and they therefore thought that the amylin and amylon groups in its molecule were different to those in amylodextrin, because lævulose is obtained, whereas in the latter case maltose is obtained. Also, that it is a product of the hydrolysis of an unknown carbohydrate, and stands in the same relation to this body as amylodextrin does to starch.

According to Düll, the molecular weight of inulin is 2950, which agrees with the formula $(C_6H_{10}O_5)_{18}$ or $(C_6H_{10}O_5)_{18} + H_2O$; this observer has found that the lævulins obtained from inulin by acid hydrolysis are really reversion products of lævulose, and therefore they are not hydrolytic decomposition products.

In animals, *carbohydrates are stored up in the form* of glycogen; this reserve material is found in greatest amount in the liver, where it is converted into maltose and glucose when required for use by the animal.

The chemical changes which this body undergoes by the action of diastase have been investigated by Miss Tebb, who has compared this hydrolysis with that produced by acids. The former method gives achroodextrin and another dextrin named dystropodextrin, whilst dilute acids give soluble glycogen, erythrodextrin, and achroodextrin. The sugar formed is supposed to be maltose, from which, by the action of glucase, glucose is produced in the liver. Miss Tebb has identified her dystropodextrin with the product of the same name obtained from glycogen by Seegen.

As regards the molecular weight of glycogen, the most recent determinations have been made by Jackson, who used carefully purified glycogen, and found that the amount of lowering of the freezing-point corresponded to a molecular weight of 9500–10,000.

Caroubin, a polysaccharide of the formula $C_6H_{10}O_5$, has been obtained by Effront from the seeds of the carob-bean

(*Ceratonia siliqua*); it is hydrolysed by the enzyme, caroubinase, obtained from the germinating seeds, to a non-crystallisable reducing sugar, which is a hexose. This hexose is fermentable with yeast, and gives an osazone melting at 183° C; its reducing power is the same as that of glucose, but its specific rotatory power of $[\alpha]_D = +24°$ is different. Effront called this sugar caroubinose, but Marlière considers it to be a mixture of glucose, fructose, and galactose; Von Ekenstein has since identified it as d-mannose.

Bourquelot and Hérissey have found that the so-called horn-albumin, which occurs in the fruit of the carob-tree in the species of *Medicago sativa* and *Trigonella fœnum græcum*, is a manno-galactan; this is hydrolysed by the enzyme seminase, which can be extracted from the germinating seeds, and converted into mannose and galactose.

The albumin of the seeds of *Phœnix canariensis* contains mannans; these are decomposed by a soluble ferment present in the germinating seeds into mannose (Bourquelot and Hérissey). Mannose, according to Hérissey, is also the product of the mannan of orchid tubers when they are acted upon by a soluble ferment present in them.

The cell-walls of plants consist of cellulose and certain bodies closely allied to, and found together with it, termed pectoses. The changes which these undergo when acted upon by the enzyme cytase, are probably very complicated, like those of starch; the ultimate products being a reducing sugar from cellulose and metapectic acid from the pectose; metapectic acid is probably identical with arabic acid, which is found in gum arabic, and which can be hydrolysed to galactose and arabinose.

Cross and Bevan, who have investigated the hydrolytic decomposition products of the various celluloses, state that glucose and mannose are formed from oxycelluloses; in addition to these two sugars, galactose, arabinose, and xylose are formed from pectocelluloses. Tollens and Ganz state that they have obtained a sugar—seminose—from cellulose by acid hydrolysis; and Reiss has also found the same carbohydrate.

According to Omeliansky, cellulose is fermented by *Bacillus fermentationis cellulosæ,* with the result that 70 per cent. fatty acids, chiefly acetic and butyric acids, together with 30 per cent. of gases (carbon dioxide, hydrogen, and in the last phases methane), are formed.

Besides cellulose, lignin, the woody substance, is contained in the cell-walls of plants. Czapek has observed that this is dissolved by an enzyme, which he has termed hadromase. The woody substance is regarded as an ester —a compound of cellulose and hadromal—and it is decomposed by the soluble ferment into its constituents. Hadromal has the properties of a phenol and of an aldehyde, and is probably a para-compound. The cellulose formed in this decomposition is then acted upon by cytase, which is also secreted by the mould fungi from which the hadromase was obtained.

The transformation, which many fruit juices undergo, into a jelly, is, according to Frémy, due to an enzyme called pectase. The substance so changed is the pectose which is found with the cellulose. Frémy states that pectine is formed from pectose by the action of acids, and that the conversion by the enzyme takes place in two stages, resulting in the successive formation of pectosic and pectic acids, which are gelatinous bodies.

The most recent investigations have been carried out by Bertrand and Mallèvre, who have found that calcium is contained in the jelly, which consists of calcium pectate, and not of free pectic acid, and that gelatinisation depends upon the presence of calcium; this can, however, be replaced by barium or strontium. Free mineral acids, and even malic, citric, or oxalic acids, hinder the formation of this coagulum, but the effect of acids is decreased by excess of pectase or calcium salts. Coagulation, therefore, depends on the proportion of pectase, calcium salts, and free acid.

The power of gelatinising possessed by pectine under the influence of pectase, is, according to Bourquelot and Hérissey, destroyed by the action of the soluble ferment pectinase, which converts it into a reducing sugar. This change can take place even when the pectine has been coagulated, but coagulation by pectase cannot occur after the decomposition produced by pectinase.

CHAPTER III.

CHANGES IN THE TRISACCHARIDES AND DISACCHARIDES.

CHEMICAL changes as the result of fermentation are produced in the trisaccharides raffinose, melizitose, and gentianose.

Raffinose, melitose, or melitriose, $C_{18}H_{32}O_{16}$, was discovered in 1876 by Loiseau, and identified with melitose by Tollens. It is hydrolysed by an extract of yeast or of *Aspergillus niger* (Bourquelot) into fructose, glucose, and galactose, thus—

$$\underset{\text{Raffinose.}}{C_{18}H_{32}O_{16}} + 2H_2O = \underset{\text{Fructose.}}{C_6H_{12}O_6} + \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Galactose.}}{C_6H_{12}O_6}$$

This change occurs in reality in two stages, in the first of which fructose is split off, and melibiose, a disaccharide, is left; this substance is then hydrolysed into glucose and galactose, and the following two equations represent these changes:—

$$(1)\quad \underset{\text{Raffinose.}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{Fructose.}}{C_6H_{12}O_6} + \underset{\text{Melibiose.}}{C_{12}H_{22}O_{11}}$$

$$(2)\quad \underset{\text{Melibiose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Galactose.}}{C_6H_{12}O_6}$$

This hydrolysis was formerly attributed to the action of the enzyme invertin, but Fischer and Niebel state that an extract of the small intestines of the horse or dog, which contain invertin, cannot produce this conversion.

The enzyme which produces this change has been termed raffinase, and it occurs in bottom fermentation yeasts. Fischer and Lindner have extracted an enzyme from these, which hydrolyses melibiose, as also has Bau, who attributes the first stage in the conversion to invertin, and the second stage to this enzyme, which he terms melibiase. Scheibler states that these two changes can also be produced by very dilute acids at a low temperature.

Melizitose, discovered by Berthelot in 1859, in Brançon manna, is resolved, according to Bourquelot, by an extract of *Aspergillus niger* into glucose and a disaccharide, touranose :—

$$\underset{\text{Melizitose.}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Touranose.}}{C_{12}H_{22}O_{11}}$$

Touranose is isomeric with maltose; its products of hydrolysis by dilute acids being two molecules of glucose. The enzyme termed melizitase, which produces the change, apparently is unable to produce this latter conversion.

Gentianose, a trisaccharide extracted from the fresh gentian root by Bourquelot and Hérissey, is converted by invertin into fructose, and a new disaccharide, gentiobiose :—

$$\underset{\text{Gentianose.}}{C_{18}H_{32}O_{16}} + H_2O = \underset{\text{Fructose.}}{C_6H_{12}O_6} + \underset{\text{Gentiobiose.}}{C_{12}H_{22}O_{11}}$$

An extract of *Aspergillus niger* is capable of completely hydrolysing this trisaccharide into one molecule of fructose and two molecules of glucose:—

$$\underset{\text{Gentianose.}}{C_{18}H_{32}O_{16}} + 2H_2O = \underset{\text{Fructose.}}{C_6H_{12}O_6} + \underset{\text{Glucose.}}{2C_6H_{12}O_6}$$

The changes which the disaccharides undergo as the result of fermentation have been very thoroughly investigated; they are hydrolytic in their character, one molecule of water being always taken up.

Cane sugar is the oldest known representative of this group of compounds, on account of the fact that the products obtained from it subsequently undergo alcoholic fermentation. The change which it undergoes in the process of fermentation was shown by Berthelot, in 1860, to be due to the action of the enzyme invertin. This ferment converts cane sugar into d-glucose and d-fructose, and the change can be demonstrated in two ways: (1) the solution before the action of the ferment is dextro-rotatory, but it becomes lævo-rotatory when the change is completed; (2) it is originally unable to reduce Fehling's solution, but afterwards it becomes capable of doing so. The products formed, d-glucose and d-fructose, are both capable of reducing cupric oxide in solution; the lævo-rotation of the solution is due to the fact that d-fructose has a stronger rotatory power to the left than d-glucose to the right.

The equation which represents this change is :—

$$\underset{\text{Cane sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{d-glucose.}}{C_6H_{12}O_6} + \underset{\text{d-fructose.}}{C_6H_{12}O_6}$$

The rate of this change is at first very rapid, but as the hydrolysis proceeds, it gradually diminishes; according to O'Sullivan and Tompson, this change follows Harcourt's rule, *i.e.* the conditions remaining the same, the rate of change is proportional to the amount of the substance undergoing the change, and is represented by the equation :—

$$\frac{1}{\theta} \log \frac{1}{1-x} = C$$

where C is a constant, θ the time in minutes, and x the quantity changed in unit time.

The rate of the change is influenced by the reaction of the solution, the temperature (optimum at 55°), and the concentration of the cane sugar, 20 per cent. being the most favourable strength. O'Sullivan and Tompson state that the products of the reaction exert no influence, but according to Müller they are inhibitory. Quite recently A. J. Brown has taken up this subject, and he finds that the change does not follow the law of mass action, and that the products have an inhibitory effect. He considers that time is a factor in this change, on account of molecular combination of the enzyme with the cane sugar, the combination having a resistance for a definite period of time before final disruption and change take place. Molecular combination between the invertin and the cane sugar was shown to be very probable, since a solution of invertin and cane sugar can withstand a higher temperature than a solution of invertin alone (O'Sullivan and Tompson).

Henri has also found that the products of the hydrolysis of cane sugar lessen the rapidity of its inversion by invertin, and that if more cane sugar be added during the process, acceleration is produced.

Brown and Pickering, in 1897, worked upon the thermo-chemistry of carbohydrates ; both in the hydrolysis of cane sugar by invertin, and of starch by diastase, it was found that heat was evolved.

According to Schardinger, cane sugar is decomposed by a mucus-forming bacillus, with the result that hydrogen, alcohol, acetic, succinic, and lactic acids are formed, the last acid being the principal product. A carbohydrate of the composition $C_6H_{10}O_5$ was isolated from the mucus formed; this is a galactan, and yields on oxidation oxalic and mucic acids.

Maltose, which has been seen to be a product of the diastatic hydrolysis of starch, becomes changed, under the influence of the ferment maltase or glucase, into two molecules of glucose:—

$$\underset{\textit{Maltose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\textit{Glucose.}}{2C_6H_{12}O_6}$$

This hydrolysis has been investigated lately by Croft Hill, whose results are very important; he found that when maltase acts on maltose, the hydrolysis proceeds up to a certain point and then ceases, and that this point depends on the temperature, and on the concentration of the maltose. If the glucose be now removed from the solution, further hydrolysis of the remaining maltose takes place until the same point is again reached. If, however, at this point more glucose be added, a reconversion into maltose takes place. It appears, therefore, as if a state of equilibrium were set up during the process, just as in the case of esterification, which can be represented by the equation:—

$$CH_3.COOH + CH_3.OH \underset{\leftarrow}{\overset{\rightarrow}{=}} CH_3.COOCH_3 + H_2O$$

That for maltose would be:—

$$C_{12}H_{22}O_{11} + H_2O \underset{\leftarrow}{\overset{\rightarrow}{=}} C_6H_{12}O_6 + C_6H_{12}O_6$$

From these experiments it appears that the enzyme maltase can exert a synthetical action as well as an analytical.

Emmerling has lately confirmed these results of Croft Hill's, as regards the synthetic action of maltase, but he states that isomaltose, not maltose, is the synthetical product. Hill has denied this, saying that the conditions of the experiments were different in the two cases, and that the osazone of Emmerling's was probably impure, and, as has already been shown, it is very difficult to purify. Emmerling has replied, saying that isomaltose was the sugar obtained in his experiments.

Croft Hill has since notified another case of reversed, or synthetical, ferment action. A 60 per cent. solution of glucose, when treated with taka-diastase containing maltase, was changed into a solution containing 58 per cent. of glucose and 2 per cent. of maltose hydrate; he had observed, before making this experiment, that equilibrium was obtained in a solution of glucose and maltose in the presence of taka-diastase and maltase, when the solution contained 2 per cent. of maltose: he thinks that other polysaccharides are also formed under the same conditions. Using pancreatic ferment and dextrose, he has also found that a reversible reaction occurs. Similar results of synthetic action by ferments have been lately observed in the case of lipase, lactase, and emulsin.

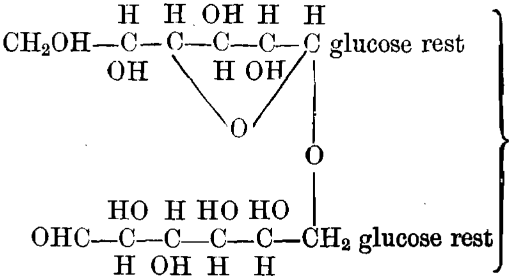
Lactose, another member of this group, has also been known for a long time. It is owing to its presence in milk that the transformation known as lactic acid fermentation is due. This change takes place in two stages; in the first stage a molecule of water is taken up under the action of the enzyme called lactase, and two sugars, glucose and galactose, are the products:—

$$\underset{\text{Lactose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Galactose.}}{C_6H_{12}O_6}$$

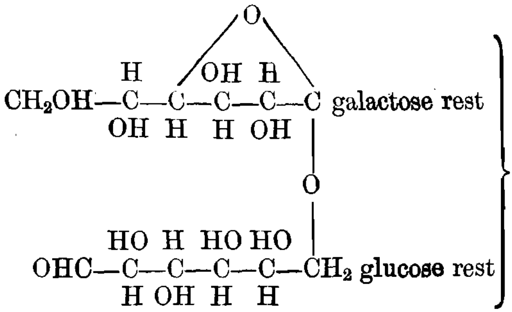
These sugars can then undergo the second stage in the transformation into lactic acid.

Bourquelot has stated that lactose can be converted into lactic acid without previous hydrolysis into glucose and galactose, but von Freudenreich has shown that the lactic acid bacteria can produce these two sugars, which are then converted further into lactic acid.

The disaccharides lactose and maltose are acted upon by the enzymes maltase and emulsin in a way similar to the α- and β- glucosides. They can therefore be regarded as glucosides: lactose as the β-galactoside of glucose, and maltose as the α-glucoside of glucose. The following formulæ demonstrate the above facts:—



maltose



lactose

Trehalose, or mycose, a disaccharide occurring in Syrian manna, is decomposed by trehalase into two molecules of glucose:—

$$\underset{\text{Trehalose.}}{C_{12}H_{22}O_{11}.2H_2O} = \underset{\text{Glucose.}}{2C_6H_{12}O_6} + H_2O$$

The enzyme trehalase, which produces this decomposition, was discovered by Bourquelot in *Aspergillus niger* and other moulds.

Melibiose, obtained from melitriose as already mentioned, is converted, according to Fischer and Lindner, and Bau, by a bottom fermentation yeast extract, but not by a top fermentation yeast extract, which contains the enzyme melibiase, into glucose and galactose:—

$$\underset{\text{Melibiose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Galactose.}}{C_6H_{12}O_6}$$

It is also hydrolysed by lactase (Fischer and Armstrong).

Gentiobiose was lately obtained from gentianose by Bourquelot and Hérissey, but not in a crystalline form; according to these observers, it is a definite compound resembling maltose, and is hydrolysed by an extract of *Aspergillus niger* into two molecules of glucose:—

$$\underset{\text{Gentiobiose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose.}}{2C_6H_{12}O_6}$$

The osones of the disaccharides maltose and melibiose have quite recently been shown by Fischer and Armstrong to be capable of undergoing hydrolysis by ferments in the same way as these disaccharides themselves, with the formation of the same products; these compounds can thus be used in the place of their disaccharides for testing their behaviour with regard to the action of ferments.

These investigators have also synthesised three new disaccharides, which have been called glucosidogalactose, galactosidoglucose, and galactosidogalactose, since they think that their constitution is very similar to that of the glucosides. These three disaccharides are decomposed by emulsin into their constituents, but only two of them—galactosidoglucose and glucosidogalactose—are fermented by bottom yeasts; top yeasts have no action on them at all. Galactosidoglucose in properties very closely resembles melibiose, and it is very probable that these two disaccharides are identical.

Fischer and Armstrong have further obtained a fourth diasccharide, isolactose, by the action of lactase upon a mixture of glucose and galactose. This is another case of synthetical ferment action. As regards its behaviour to ferments, this disaccharide occupies a position between lactose and melibiose. Another disaccharide was formed when lactase acted upon a solution of glucose alone, the synthesising power of this enzyme being here again shown.

These investigators have shown that emulsin also is capable of exerting a synthetic action, since they obtained a disaccharide as the result of its action upon a mixture of glucose and galactase, as in the case of lactase.

CHAPTER IV.

CHANGES IN THE MONOSACCHARIDES.

THE members of the first group of monosaccharides undergo various changes as the result of fermentation. The oldest known change is that called alcoholic fermentation, which glucose especially undergoes, together with fructose, both of which are formed, as before stated, by fermentation from cane sugar. The history of this fermentation process is very interesting, as the whole subject begins with it.

From the very earliest times it has been known that sweet liquids, if exposed to the air and allowed to stand, become cloudy and turbid, and bubbles of gas rise to the surface, which give the appearance of boiling to the liquid. The term "fermentation" was first applied to this change, on account of this appearance of boiling, but it was soon also applied to all changes where any effervescence took place, especially to the process of putrefaction, the terms *putrefactio* and *fermentatio* being used synonymously. A froth forms on the liquid, and a sediment, or "lees," in it; later, the whole settles to the bottom, leaving the liquid clear. After the process of apparent boiling is over, the liquid will no longer be sweet, but will have a spirituous smell and taste, owing to the presence of the substance now known as alcohol. The earliest idea of the nature of this process is found in the writings of Basil Valentine, about the end of the fifteenth century, and was to the following effect: The scum, which is formed in the process, imparted an internal inflammation to the liquid, so that a purification was thereby determined; from this it is seen that he supposed that the alcohol was present in the liquid before the fermentation began. The gas evolved, which gives the boiling appearance, was thought to be the same as ordinary air; but van Helmont, in the following century, pointed out that this gas resembled that sometimes found in caves and wells, and he termed it "gas sylvestre." In 1682 Becher observed that this change occurred only in saccharine liquids, and he considered the alcohol to be a product of the reaction. He thought that the presence of air was necessary to start the process, which, in his opinion, was analogous to combustion; a distinction was also made between fermentation and putrefaction, and between true alcoholic fermentation and acid fermentation, *e.g.* the production of lactic acid. Some twenty years before this observation, Willis had put forward a new view of this change. It had already been found that the scum was necessary for the process, and this scum had been called a ferment; this ferment was supposed to be possessed of a motion peculiar to itself, which it transmitted to the fermentable matter. Stahl, a little later, also held this view, but considered, in addition, that the fermentable matter consisted of an unstable union of salt, oil, and earth; by the internal motion caused by the ferment, these particles were separated from one another, only to combine together again to form more stable molecules, consisting of the same principles, but in other proportions.

Another century elapsed before any real light was cast upon the nature of this process; then Lavoisier took up the investigation of the changes, which was the first work of a really scientific character upon the subject. Instead of using the juices of fruits which had been employed before, he worked with a solution of cane sugar. This substance he regarded as a vegetable oxide made up of two bases, carbon and hydrogen, brought into the state of an oxide by a certain proportion of oxygen; these three elements were in such a feeble state of equilibrium that only a slight force was necessary to destroy their connection. He made analyses of the sugar, the alcohol, and the carbon dioxide, the results of which are to be found in his "Elements of Chemistry." The change is explained as a mere separation of the elements into two portions, the one being oxygenated at the expense of the other, in order to form carbon dioxide, whilst the other is deoxygenated and converted into the combustible substance called alcohol. The conclusion is that, if it were possible to reunite the alcohol and the carbon dioxide, sugar would be again formed. Though his analyses were far from correct, yet he approached very nearly to the truth.

Twenty years later, Gay Lussac and Thénard, and De Saussure, made more accurate analyses of cane sugar, which supported Lavoisier's view. The formula $C_{12}H_{12}O_{12}$ was given to sugar, and the following equation expressed the change:—

$$C_{12}H_{12}O_{12} = 2C_4H_6O_2 + 4CO_2$$

It was pointed out in 1828, by Dumas and Boullay, that Gay Lussac's results were not quite accurate; this was acknowledged by Gay Lussac, who stated that he modified them to agree with the equation. If, however, it was considered that a molecule of water took part in the process, the results would be accurate, and so Dumas and Boullay rewrote Gay Lussac's equation thus:—

$$C_{12}H_{11}O_{11} + HO = 2C_4H_6O_2 + 4CO_2$$

In 1832 Dubrunfaut discovered that cane sugar, before it could undergo fermentation, was transformed into an uncrystallisable sugar. Berthelot showed that this change is produced by a soluble ferment which is present in yeast, and that alcoholic fermentation takes place only after the transformation; he also stated that the above equation is experimentally realised.

In 1847 Schmidt discovered that succinic acid was also a product of this fermentation; but this fact was scarcely noted at the time, and became practically forgotten.

The most important work upon the subject is that of Pasteur, who, about 1860, published the results of his early work on the question, and showed that succinic acid and glycerine, besides alcohol and carbon dioxide, are products of this fermentation; that the glycerine and succinic acid are formed at the expense of the sugar; and that a portion of the sugar is used up by the ferment: the amounts of succinic acid and glycerine produced varied slightly, according to the temperature, the acidity, and the rate of the fermentation. He gave the following equation to represent the formation of succinic acid, glycerine, and carbon dioxide:—

$$49(C_{12}H_{11}O_{11} + HO) \text{ or } 49C_{12}H_{12}O_{12} + 60HO$$
$$= \underset{\text{Succinic acid.}}{12C_8H_6O_8} + \underset{\text{Glycerine.}}{72C_6H_8O_6} + 60CO_2$$

but considered it to be only a very approximate expression of the numerical results which he obtained by analysis.

Acetic acid is another product of alcoholic fermentation. It was first noticed by Béchamp, and attributed by Pasteur to a subsequent fermentation; its production will be considered later.

Higher alcohols, *collectively known as fusel oil,* are formed also in alcoholic fermentation, but the process of their formation is not yet known. This fusel oil consists of a mixture of propyl, isobutyl, amyl, caproyl, œnanthyl, and capryl alcohols in various proportions; amyl alcohol occurs sometimes in somewhat large quantities.

It is upon the exciting cause of fermentation that the most varied and interesting theories have been held. In 1680 Lœwenhoek examined the scum on fermenting liquids with his microscope, and found that it consisted of small spherical or ovoid globules. Fabroni, in 1787, considered these particles to be what he called a "vegeto-animal" substance; and Thénard, three years later, thought that the substance was of an animal nature from its nitrogenous chemical composition, but as to its organisation, he made no allusion. Astier, in 1813, still considered these particles to be of animal origin, and thought that they could only live at the expense of the sugar. Desmazières, in 1826, stated the same view. In 1810 Gay Lussac concluded from his experiments that oxygen was necessary to start fermentation, but was not required for its continuation; in his "Mémoire sur la Fermentation" he alludes to Appert's method of preserving articles of food for an indefinite time by boiling the vessel in which they are contained, and then closing it up; if air were allowed to enter, fermentation was set up, and hence his idea that oxygen was the cause of fermentation.

In 1837 Cagniard de Latour discovered the true nature of yeast, that it was composed of living organised cells, consisting of a cell envelope and granular contents; this discovery was made about the same time by two other investigators, Schwann and Kützing, independently of one another. Kützing further discovered the cause of the acetic acid fermentation which takes place at the same time in alcoholic solutions. Each of these investigators put forward a theory, which was formulated in very much the same words—that the process of alcoholic fermentation depended upon the presence of the yeast, or sugar fungus, which extracted from the sugar and the nitrogenous matter also present the materials necessary for its nutrition, and that the elements not taken up in this way united together to form alcohol.

This theory was strongly opposed by Liebig, whose views were not essentially different from those put forward by Willis and Stahl; the yeast, as well as all animal and vegetable matter, was supposed to be in state of internal motion, which was transmitted to the bodies with which it was in contact. Pasteur, on the other hand, by a long series of experiments, definitely proved that the process of fermentation was dependent on the life of the yeast-cell, and that all fermentation was dependent upon life. Pasteur's results have been summed up by Professor Frankland as follows: "No fermentation without organisms; for each kind of fermentation a particular organism."

Besides this and similar fermentations, other changes were known which took place without the presence of living organisms, such as the conversion of starch into sugar, and of amygdalin into benzaldehyde, prussic acid, and glucose by emulsin. Liebig thought that the yeast contained a ferment which brought about the decomposition of the sugar, but would not acknowledge that the fermentation was brought about by the life-processes of the yeast-cell. Quite recently this has been shown to be the case by Buchner, who has been able to extract from yeast a liquid which can set up alcoholic fermentation. Liebig's idea was expounded in a slightly different manner by Traube in 1858. Fermentation, according to him, is instigated by the enzymes or soluble ferments formed by the vitality of organisms, which are either excreted by them or retained within them to effect intracellular digestion. This view was accepted by Berthelot and Hoppe-Seyler, and it is the one which is most generally held at the present time. Traube's theory thus combines both the vitalistic theory of Latour, Schwann, and Kützing, and the mechanical theory of Liebig.

Another view was suggested by Berzelius, that, namely, of catalytic forces; the process being due to an act of contact, and yeast being considered to be an amorphous principle. Mitscherlich supported this view, but he recognised the organised nature of yeast.

Naegeli was an advocate of Liebig's mechanical theory, holding that the molecules in the enzyme possessed a considerable vibration; these had the power of giving up part of their movement to the slower movement of the other molecules, and thus caused a decomposition.

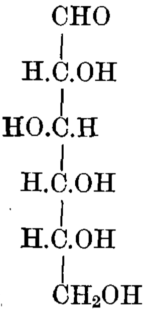
Since Pasteur, no further important investigations have been made upon the chemical changes which sugar undergoes as the result of fermentation; Buchner's extract containing *zymase* is the only recent discovery of any importance. This discovery of Buchner's is challenged by Schunck and Manassein; the former observer showed, in 1854, that erythrozyme, the enzyme of madder, could set up alcoholic fermentation; and the latter, in 1871, found that the presence of yeast cells was not necessary for the process of fermentation, which must therefore be regarded as due to an enzyme.

In 1892 O'Sullivan showed that the *hydrolytic* functions of yeast followed the same course as a simple chemical interchange, the time curve being like that for invertin, and a straight line; in the case of maltose and dextrose, the rate of alcoholic fermentation was found to be proportional to the amount of these sugars, and different to the rate of the hydrolytic action of yeast upon cane sugar. A. J. Brown has found that there is no direct proportion between the weight of yeast formed and the amount of sugar fermented, and that the fermentation proceeds better with oxygen than without. This is the reverse of the view held by Pasteur, that fermentation was a starvation phenomenon, *i.e.* a phenomenon of life without air.

Quantitative experiments upon the fermentation of mixtures of dextrose and lævulose, and of maltose and dextrose, have been made by Prior and Schulze, who find that the process takes place within the yeast-cell, and is dependent on the diffusion of the sugar solution through the cell-wall; the rate of diffusion varies for the different species of yeast, and the amount of each sugar fermented varies with its osmotic pressure. Knecht has found that two varieties of yeast ferment dextrose more quickly than lævulose, and he concludes that the rapidity of the fermentation of the different sugars is dependent on physical laws as stated by Prior.

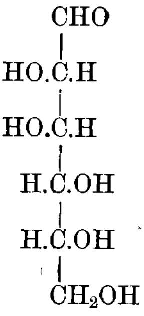
During the process of alcoholic fermentation heat is evolved; its amount has been measured by Bouffard, who found that for 180 grams of sugar 23·5 Cals. were evolved, whereas the calculated amount is 32·07 Cals.

By the far-reaching investigations of E. Fischer, the number of sugars belonging to the monosaccharides has been very largely increased; these contain three, four, five, six, seven, eight, or nine atoms of carbon in their molecules, but only those containing carbon atoms which are a multiple of three, the hexoses and the nonoses, have been shown by him to be capable of undergoing alcoholic fermentation. A mixture of the trioses glyceraldehyde and dihydroxyacetone, which are obtained from glycerol by oxidation, was stated by some authors to be fermented, but Emmerling has shown that the pure substances are not acted upon, and he suggested that during the oxidation with platinum black the glycerose is converted into acrose, or d- + l-fructose. Of the aldohexoses, twelve out of a possible sixteen of which have been synthesised, only three can be fermented, and of the ketohexoses only one. These are d-glucose, d-mannose, d-galactose from the first; and d-fructose from the last. The corresponding lævo- compounds are not attacked, and d-galactose is fermented only with difficulty. From these facts Fischer concluded that the structure of these bodies played a very important part with regard to their capability of fermentation; if the configuration of these four sugars be written down side by side, the small differences in their configurations can be readily seen:—



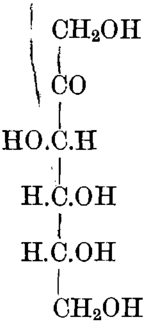
$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{HO.C.H} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

d-glucose.



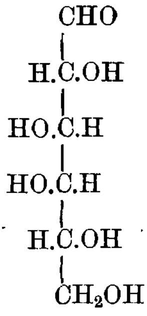
$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HO.C.H} \\ | \\ \mathrm{HO.C.H} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

d-mannose.



$$\begin{array}{c} \mathrm{CH_2OH} \\ | \\ \mathrm{CO} \\ | \\ \mathrm{HO.C.H} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

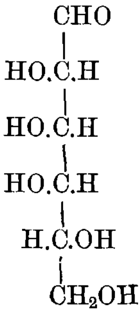
d-fructose.



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{HO.C.H} \\ | \\ \mathrm{HO.C.H} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

d-galactose.

d-glucose, d-mannose, and d-fructose are thus very similar in their structure, at least three of their asymmetric carbon atoms having the same configuration; but d-galactose has only two asymmetric carbon atoms with a configuration like that in the other three sugars, which corresponds with the greater difficulty of fermentation. If the structure of the sugar is still further removed, as in d-talose, only one carbon atom having the same configuration, no fermentation at all takes place:—



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HO.C.H} \\ | \\ \mathrm{HO.C.H} \\ | \\ \mathrm{HO.C.H} \\ | \\ \mathrm{H.C.OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

d-talose.

That structural differences are, in all probability, the cause of the behaviour of these sugars with regard to fermentation, is seen again amongst the class of bodies known as glucosides, which behave in a very similar manner towards the enzymes maltase and emulsin.

CHAPTER V.

CHANGES IN THE GLUCOSIDES.

SINCE the majority of the substances mentioned at the end of Chapter IV. are derivatives of glucose, the changes which take place in them as the result of fermentation are most appropriately described here, before those which take place in the monosaccharides themselves, and which result in the formation of various products.

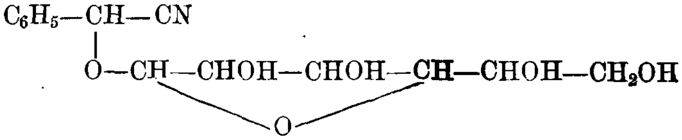
The glucosides are very widely distributed in nature, and a large number, which have not been found as naturally occurring, have been prepared artificially. These latter compounds have been very thoroughly investigated by Fischer, who has found that some very interesting relations exist with regard to their decomposition by the ferments known as emulsin and maltase, thus emphasising his theory of the importance of configuration as being a factor of chemical change by fermentation.

The glucoside amygdalin was the first to be prepared in a crystalline form by Robiquet and Boutron in 1830, from the seeds of the bitter almond. The change which it undergoes was found by Liebig and Woehler to be due to the action of the enzyme emulsin, which exists in the kernel of the almond, and was termed synaptase by

Robiquet. These observers discovered that prussic acid and sugar were amongst the products. It is now known that benzaldehyde, prussic acid, and glucose are the products of this change, which is brought about by emulsin, and the following equation shows their formation:—

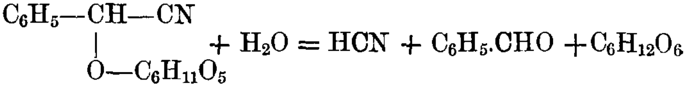
$$C_{20}H_{27}NO_{11} + 2H_2O = C_6H_5.CHO + HCN + 2C_6H_{12}O_6$$

It has been discovered by Fischer that amygdalin undergoes a different transformation when it is acted upon by the ferment maltase; in this reaction only one molecule of glucose is split off, and a new glucoside is formed which may be regarded as an artificial product. Fischer named this new compound "mandelnitrilglucosid," and gave it the formula:—



$$\begin{array}{l} C_6H_5-CH-CN \\ \qquad | \\ \qquad O-\underbrace{CH-CHOH-CHOH-\mathbf{CH}}_{O}-\mathbf{CHOH}-\mathbf{CH_2OH} \end{array}$$

Prussic acid does not appear in this transformation, but if this new glucoside be treated with emulsin, prussic acid, benzaldehyde, and glucose are formed:—



$$\begin{array}{l} C_6H_5-CH-CN \\ \qquad | \qquad\qquad + H_2O = HCN + C_6H_5.CHO + C_6H_{12}O_6 \\ \qquad O-C_6H_{11}O_5 \end{array}$$

From these results Fischer concluded that the sugar group in amygdalin was present in the form of maltose, and amygdalin is therefore a maltoside.

Emmerling has quite recently obtained amygdalin from mandelnitrilglucosid and glucose, by the action of yeast maltase, which is another instance of synthetic action by enzymes; he obtained, however, only a small quantity of amygdalin, but it was analysed, and it agreed in every respect with the natural product.

Many other natural glucosides are also decomposed by emulsin. These are—

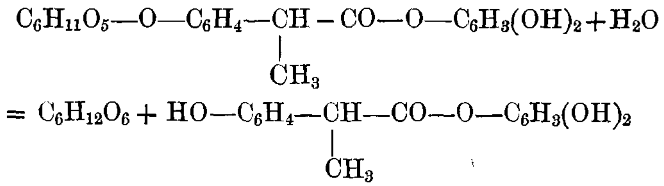
1. Salicin into glucose and saligenin, or salicylic alcohol:—

$$C_6H_{11}O_5—O—C_6H_4—CH_2OH + H_2O = C_6H_{12}O_6 + HO—C_6H_4—CH_2OH$$

2. Helicin into glucose and salicylic aldehyde:—

$$C_6H_{11}O_5—O—C_6H_4—CHO + H_2O = C_6H_{12}O_6 + HO—C_6H_4—CHO$$

3. Phloridzin into glucose and phloretin, or the phloroglucin ester of p-oxyhydratropic acid, by the emulsin of *Aspergillus niger* (Hérissey):—

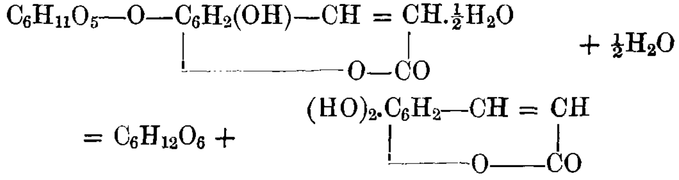


$$C_6H_{11}O_5—O—C_6H_4—\underset{\displaystyle CH_3}{\underset{|}{CH}}-CO—O—C_6H_3(OH)_2+H_2O = C_6H_{12}O_6 + HO—C_6H_4—\underset{\displaystyle CH_3}{\underset{|}{CH}}—CO—O—C_6H_3(OH)_2$$

4. Arbutin into glucose and hydroquinone:—

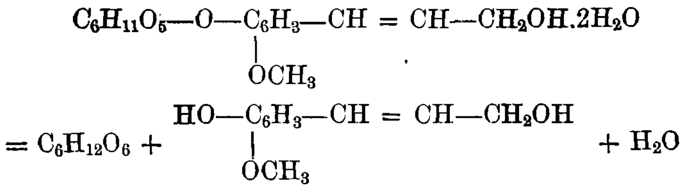
$$C_6H_{11}O_5—O—C_6H_4—OH+H_2O=C_6H_{12}O_6+C_6H_4(OH)_2$$

5. Æsculin into glucose and æsculetin, or 4, 5-dioxycoumarin:—



$$C_6H_{11}O_5—O—\underbrace{C_6H_2(OH)—CH = CH}_{O—CO}.\tfrac{1}{2}H_2O + \tfrac{1}{2}H_2O = C_6H_{12}O_6 + (HO)_2.\underbrace{C_6H_2—CH = CH}_{O—CO}$$

6. Coniferin into glucose and coniferyl alcohol:—



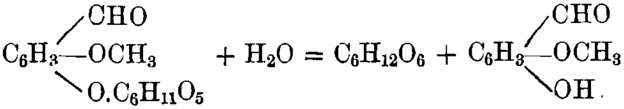
$$\underset{\displaystyle OCH_3}{C_6H_{11}O_5—O—C_6H_3}—CH = CH—CH_2OH.2H_2O$$

$$= C_6H_{12}O_6 + \underset{\displaystyle OCH_3}{HO—C_6H_3}—CH = CH—CH_2OH + H_2O$$

7. Populin into glucose and benzoylsaligenin, only, however, by certain extracts which most probably contain emulsin, *e.g.* of *Aspergillus niger* (Hérissey):—

$$C_{13}H_{17}(C_7H_5O)O_7.2H_2O = C_6H_{12}O_6 + C_7H_7(C_7H_5O)O_2 + H_2O$$

8. Glucovanillin into glucose and vanillin:—



and glucovanillic acid into glucose and vanillic acid.

9. Indican, the glucoside of indigo, is converted by a soluble ferment, according to Bréaudat, into indigo white and indiglucin; the latter, especially in alkaline solution, is oxidised by another ferment to indigotin; in woad, however, according to Beyerinck, isatan is present, which is decomposed by isatase into free indoxyl, which was formerly thought to be present. Indoxyl is considered by Hazewinkel to be an intermediate product in the formation of indigo, and this explains how in some processes indigo red is formed.

10. Daphnin is converted into daphnetin, and piceïn into piceol, and in each case glucose.

Hérissey states that the emulsin of almonds decomposes gentiopicrin from *Gentia lutea,* syringin and phyllirin, and has a slight action upon ononin and helleboreïn; the emulsin from aspergillus, however, acts easily upon these two last glucosides.

Among other glucosides which are chemically changed by ferments other than emulsin, the following may be mentioned:—

1. Sinigrin, or potassium myronate, the constituent of black mustard seeds, is converted by the enzyme myrosin into glucose, potassium hydrogen sulphate, and allyl sulphocyanate, or mustard oil:—

$$C_{10}H_{18}NS_2O_{10}K = C_6H_{12}O_6 + KHSO_4 + C_3H_5NCS$$

Apparently a molecule of water is not required for this change, but Gadamer states that the glucoside contains a molecule of water with which it crystallises and loses at 100° in a vacuum. This observer finds that allylsulphide, allylcyanide, and carbon bisulphide are produced as secondary products in the fermentation. He considers that sinigrin is the potassium salt of an ethereal sulphate derived from the hypothetical imidoxythiocarbonic acid, in which the three hydrogen atoms are replaced by SO_2OK, $C_6H_{11}O_5$, and C_3H_5, thus:—

$$C_3H_5.N:C.(S.C_6H_{11}O_5).OSO_3K$$

2. Sinalbin, contained in white mustard, is decomposed also by myrosin into glucose, sinapin hydrogen sulphate, and oxybenzyl mustard oil:—

$$C_{30}H_{44}N_2S_2O_{16} = C_6H_{12}O_6 + C_{16}H_{24}NO_5.HSO_4 + C_7H_7O.NCS$$

Gadamer gives the formula $C_{30}H_{42}N_2S_2O_{15} + 5H_2O$ to this glucoside, and the change is hydrolytic as in the case of sinigrin.

3. Rubian, discovered by Schunck in 1852, is said by

him to be converted into alizarin and other compounds by an enzyme which he has called erythrozyme.

This observer thinks that three molecules break down, one giving alizarin; the second giving rubiretin, verantin, and water; whilst the third gives glucose, rubiafin, and rubiagin.

4. Xanthorhamnin is, according to Marshall Ward and Dunlop, decomposed by the enzyme rhamnase into rhamnose, probably also galactose and rhamnin or rhamnetin—

$$C_{48}H_{66}O_{29} + 5H_2O = 4C_6H_{14}O_6 + 2C_{12}H_{10}O_5$$

C. and G. Tanret have obtained a trisaccharide, rhamninose, by the action of rhamnase on xanthorhamnin. This carbohydrate yields, on hydrolysis with acids, two molecules of rhamnose and one molecule of galactose, and the glucoside therefore apparently does not contain glucose in its molecule.

5. Gaultherin is hydrolysed by gaultherase, or betulase, into glucose and methylsalicylic acid:—

$$C_{14}H_{18}O_8 + H_2O = C_6H_{12}O_6 + C_6H_4(OH)COOCH_3$$

6. Tannin is hydrolysed by tannase into glucose and gallic acid:—

$$C_{27}H_{22}O_{17} + 4H_2O = C_6H_{12}O_6 + 3C_7H_6O_5$$

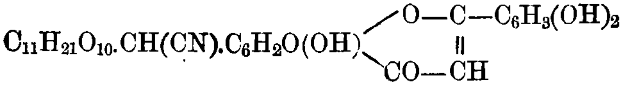
The compound of tannin with gelatine, tannates, methyl- and phenyl- salicylates, are also said to be changed by this enzyme.

7. A glucoside in *Echallium elaterium* is, according to Berg, decomposed by an enzyme (which he has named elaterase) into elaterin.

8. Lotusin, a glucoside obtained by Dunstan and

Henry in 1900, is said by these observers to be transformed by an enzyme, lotase, *into glucose, hydrogen cyanide, and* lotoflavin. Acids convert it into two molecules of glucose, one molecule of hydrogen cyanide, and one molecule of lotoflavin.

Lotusin is a lotoflavin ester of maltose cyanohydrin :—



$$C_{11}H_{21}O_{10}.CH(CN).C_6H_2O(OH)\begin{array}{l} \diagup O—C—C_6H_3(OH)_2 \\ \qquad\quad \| \\ \diagdown CO—CH \end{array}$$

$$[OH = 4 : 2' : 4']$$

and the observers point out that amygdalin and lotusin are the only glucosides which yield hydrogen cyanide on hydrolysis.

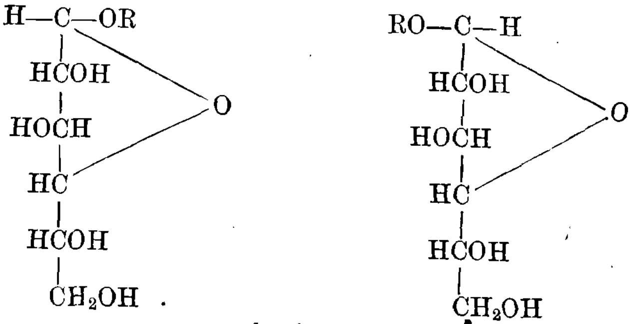
9. Kolanin, the glucoside of caffeine, yields under the action of a ferment, glucose, caffeine, theobromine, and kola-red; the same products are given by cacaonin. These glucosides are considered by Schweitzer as made up of: (*a*) kolanin : one molecule of kola-red, three molecules *of glucose, and one molecule of caffeine with the* elimination of four molecules of water, with the formula $C_{40}H_{56}N_4O_{21}$; (*b*) cacaonin: one molecule of cacao-red, $C_{17}H_{12}(OH)_{10}$, six molecules of glucose, and one molecule of theobromine, with the loss of eight molecules of water, with the formula $C_{60}H_{86}O_{15}N_4$.

Knox and Prescott, however, do not think that caffeine and kola red are products of one glucoside.

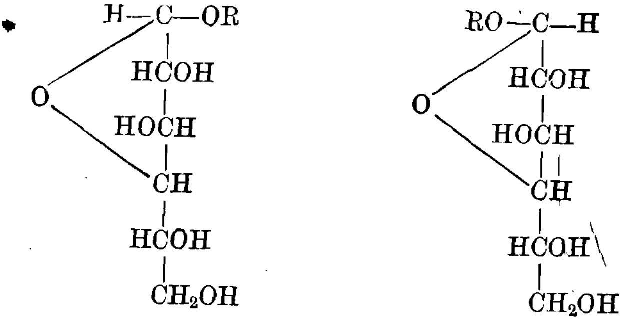
We now come to the artificial glucosides, with regard to which so many important facts have been discovered by Emil Fischer. By this investigator not only have derivatives of glucose been prepared, but also of mannose, galactose, maltose, etc. In general, two derivatives are obtained by the action of hydrochloric acid upon the sugar and alcohol, and these are termed α- and β-glucosides. It is among the methyl-glucosides that the majority of observations have been made with regard to their behaviour towards ferments, especially emulsin and maltase.

It has been found that the α- compounds are only hydrolysed by maltase, and not at all by emulsin, which attacks only the β- compounds. The ease with which these compounds are attacked also varies; whereas the glucosides are easily hydrolysed, the galactosides are attacked with difficulty, and the mannosides not at all. Fischer, noting these differences, concluded that the structure of these compounds played a very important part with regard to their behaviour to these enzymes, and that the enzymes themselves must be built up in a manner correlative to that of these glucosides, like lock and key.

If the formulæ of these compounds be written down, it is easily seen that one of the asymmetric carbon atoms is different in the α- and β- compounds:—



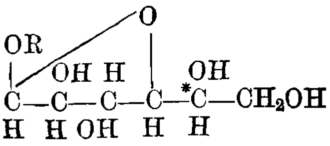
α- and β- glucosides.



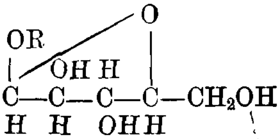
α- and β- galactosides.

This difference in all probability accounts for the different action of these enzymes.

Very remarkable evidence of this configurative difference, as being the cause of the enzyme action, exists in the case of the xylosides, whose structure is so very similar to that of the glucosides:—



d-glucoside.



d-xyloside.

From the formulæ it will be seen that one of the asymmetric carbon atoms fails in the d-xyloside, and apparently it is this carbon atom in the d-glucoside (which is marked with an asterisk) which defines the action of the enzyme, and which influences the entire configuration of the d-glucosides.

The disaccharides maltose and lactose, as already mentioned, also show similar differences in their susceptibility to these enzymes, and the idea of configuration therefore gains further support from these examples.

Quite lately Fischer and Armstrong have shown that the β-methyl-, β-ethyl-, and β-phenyl-galactosides are also hydrolysed by kefir-lactase.

CHAPTER VI.

CHANGES IN THE MONOSACCHARIDES.—Continued.

THE chief of the chemical changes other than alcoholic fermentation, which occurs in the monosaccharides, is known as lactic acid fermentation, and it takes place principally in the hexoses, glucose, fructose, and galactose.

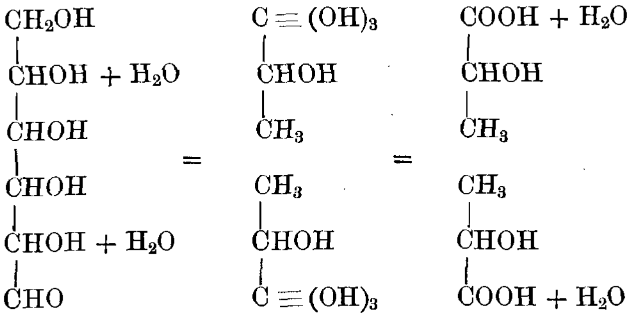
Pasteur was the first to definitely show that this fermentation was produced by micro-organisms, though Blondeau, in 1847, first observed them without discovering their significance. It is very probable that an enzyme is the cause of this change, but as yet it has not been isolated from these micro-organisms. In the organs of animals, especially in muscles, lactic acid is always present, and Nasse has pointed out the analogy of its formation to enzyme actions. Oppenheimer has recently investigated the subject with regard to the presence of a lactic acid-forming enzyme, but his experiments are not conclusive as to its existence.

It was formerly supposed that lactose and other polysaccharides could be directly converted into lactic acid, sometimes, perhaps, with difficulty; but it is now known that hydrolysis of these substances into monosaccharides, as already described, takes place before lactic acid can be produced.

This change can be expressed by the equation:—

$$C_6H_{12}O_6 = 2C_3H_6O_3$$

Apparently no water is required for this process, but, as in the case of alcoholic fermentation, Baeyer considers that two molecules of water may take part in the transformation, thus:—



$$\begin{array}{lclcl}
CH_2OH & & C\equiv(OH)_3 & & COOH + H_2O \\
| & & | & & | \\
CHOH + H_2O & & CHOH & & CHOH \\
| & & | & & | \\
CHOH & & CH_3 & & CH_3 \\
| & = & & = & \\
CHOH & & CH_3 & & CH_3 \\
| & & | & & | \\
CHOH + H_2O & & CHOH & & CHOH \\
| & & | & & | \\
CHO & & C\equiv(OH)_3 & & COOH + H_2O
\end{array}$$

The reaction, however, is not so simple as expressed in the equation, as acetic acid and other products are also generally formed at the same time.

This method of producing lactic acid has been known for a very long time, in fact, it is probably the oldest acid known, and was first prepared in this way by Scheele. In order to prepare it, calcium carbonate must be added to the solution to neutralise the free acid as fast as it is formed, as the specific micro-organisms cannot grow in an acid medium, and the production of lactic acid, therefore, necessarily would cease. If the acidity is confined to 0·02—0·5 per cent., so as to prevent the secondary formation of butyric acid, 98 per cent. of sugar can be converted into lactic acid (Claflin).

The lactic acid produced in this way is generally inactive, but certain micro-organisms form d-lactic acid, and

Schardinger has obtained l-lactic acid in one instance. It is very probable that the inactive lactic acid is formed first, and is then converted into one of its active varieties, as shown by Frankland and MacGregor in the case of the formation of d-lactic acid.

β-hydroxypropionic acid, $CH_2OH—CH_2—COOH$, the isomer of ordinary lactic acid or α-hydroxypropionic acid, was obtained by Hilger, by the fermentation of inosite; it was identified by oxidation to malonic acid. Vohl has repeated this experiment, but he obtained α-hydroxypropionic acid; this acid (generally termed lactic acid of fermentation) is thus always produced in fermentation.

Lactic acid is also a product of the fermentation of certain polyhydric alcohols, *e.g.* sorbite, inosite, mannite, and dulcite; also of rhamnose (Tate) and other pentoses; it can also be formed by fermentation from malic acid, carbon dioxide being evolved during the process:—

$$C_4H_6O_5 = C_3H_6O_3 + CO_2$$

Wehmer, in 1892, showed that certain monosaccharides, but especially glucose, could undergo yet another change as the result of fermentation, citric acid being the product formed. Two species of micro-organisms were found capable of producing this result, carbon dioxide being evolved during the process. The citric acid obtained is identical with that found in lemon juice, and is the principal product. For this process air is a necessity, the carbon dioxide evolved during the fermentation even hindering the action of these micro-organisms, and consequently it must be allowed to escape. Over 50 per cent. of the glucose can be transformed into citric acid; but if the acidity of the solution reaches 20 per cent., it becomes deleterious to these micro-organisms, and must be removed by neutralisation, in order that the total quantity can be obtained; if the fermentation is allowed to go too far, the citric acid itself is decomposed. Wehmer has since obtained three other micro-organisms which can produce this fermentation.

Oxalic acid is another product obtained by the fermentation of sugar solutions, which may consist of glucose, galactose, cane sugar, lactose, maltose, dulcitol, mannitol; glycerol can also be converted into oxalic acid in the same way. The micro-organisms which produce this change are hindered in their action by excess of the acid, and a good yield of oxalic acid is only obtained when it is neutralised as fast as it is formed by calcium carbonate, which can be added to the solution. According to Banning, fifteen species of micro-organisms are now known which can produce oxalic acid, all of them from glucose, and the majority from arabinose; glycerol and erythritol by about half of them, as also pyrotartaric acid and glycollic acid. This investigator gives a list of what substances and by how many of these micro-organisms each of the substances is converted into oxalic acid.

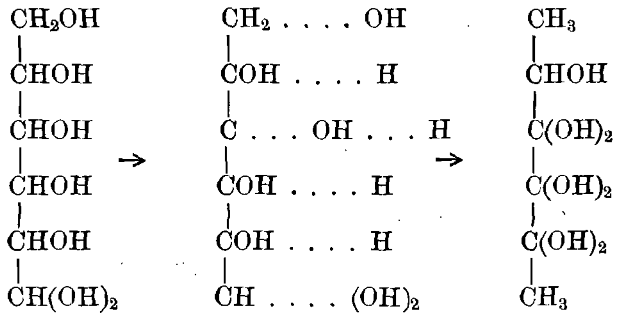
The curious phenomenon, observed in wines and saccharine liquids, known as "ropiness," is the result of a fermentation, and is due, according to Pasteur, to a particular organism. It is observed sometimes in the manufacture of cane sugar, and the result is a peculiar viscosity. The change that occurs is probably caused by two organisms, since Pasteur has observed larger and smaller particles of a different kind in the liquid; one of these produces the peculiar gum which resembles dextrin, as on oxidation it yields oxalic, not mucic acid, and the other produces mannite. During the process, carbon dioxide is evolved; that the process is in all probability due to two organisms is further seen by the varying quantities of gum and mannite produced, that of the gum being very often much larger. Monoyer has proposed the following equations for these two changes:—

$$13C_{12}H_{24}O_{12} + 12H_2O = 24C_6H_{14}O_6 + 12CO_2$$
$$12C_{12}H_{24}O_{12} = 12C_{12}H_{20}O_{10} + 24H_2O$$

The gelatinous matter, called by Scheibler dextran, is probably a derivative of cellulose, and it can be formed by several micro-organisms, *e.g. Streptococcus hornensis,* which produces it from media containing 20 per cent. of glucose (Boekhout). A gum, resembling in properties a galactan, since it yields mucic acid on oxidation, is produced by *Bacillus lactis aërogenes* in solutions of lactose or galactose, but not glucose (Emmerling).

These changes, which sugar can undergo, are thus very various. Nef has compared the action of enzymes with the action of those reagents which cause the decomposition of methylene compounds, and regards the enzymes as substances exerting a directive influence on the decomposition of the sugars. Very many other theories have been advanced to explain the processes of the metamorphosis of sugar into alcohol and carbon dioxide, but the suggestion brought forward by Baeyer, in 1870, that water is the active agent, seems to be the most reasonable. He considered that addition of the elements of water took place at certain points, and that by alternate separation and addition in a different order, an accumulation of oxygen atoms at certain parts of the molecule might take place; at these points there would be weakness, and disruption would occur.

The following formulæ of Baeyer's make this clear—



$$\begin{array}{lclcl}
\mathrm{CH_2OH} & & \mathrm{CH_2 \;.\;.\;.\;.\; OH} & & \mathrm{CH_3} \\
| & & | & & | \\
\mathrm{CHOH} & & \mathrm{COH \;.\;.\;.\;.\; H} & & \mathrm{CHOH} \\
| & & | & & | \\
\mathrm{CHOH} & & \mathrm{C \;.\;.\;.\; OH \;.\;.\;.\; H} & & \mathrm{C(OH)_2} \\
| & \rightarrow & | & \rightarrow & | \\
\mathrm{CHOH} & & \mathrm{COH \;.\;.\;.\;.\; H} & & \mathrm{C(OH)_2} \\
| & & | & & | \\
\mathrm{CHOH} & & \mathrm{COH \;.\;.\;.\;.\; H} & & \mathrm{C(OH)_2} \\
| & & | & & | \\
\mathrm{CH(OH)_2} & & \mathrm{CH \;.\;.\;.\;.\; (OH)_2} & & \mathrm{CH_3}
\end{array}$$

where the separating elements are indicated by dots.

In 1895 Armstrong drew attention to the fact that accumulation of oxygen atoms might take place at non-contiguous carbon atoms, and he considers that this is a special feature; different effects would thus be produced by different enzymes, since they would affect different systems, and the direction of the attack would depend on the asymmetry of both hydrolyte and hydrolyst. This accounts for the different action of enzymes, as in the case of the glucosides.

CHAPTER VII.

CHANGES RESULTING IN THE FORMATION OF OPTICALLY ACTIVE PRODUCTS.

As has already been pointed out, enzymes and living ferments are very selective in their action upon the carbohydrates and organic acids. Pasteur, in his classical experiments upon the tartrates and racemates, in 1848–1853, was the first to point out the selective action of micro-organisms. He grew a penicillium upon the ammonium salt of racemic acid, and obtained lævo-tartaric acid, the dextro- variety being assimilated by the organism.

This method of Pasteur's has been employed in many cases for the separation of optically active compounds; the separation of inactive mandelic acid by Lewkowitsch, in 1882, into its constituents, and the resolution of glyceric acid by Frankland and Frew, in 1891, by the *Bacillus ethaceticus*, which was grown upon its calcium salts, are two very good examples of this selective action.

Frankland has investigated the action of his *Bacillus ethaceticus*, and of the pneumococcus of Friedländer, upon several carbohydrates, *e.g.* maltose, lactose, raffinose, dextrin, mannitol, glycerol, glucose, and arabinose; the chief products were alcohol and acetic acid. Upon erythritol, glycol, and dulcitol, *Bacillus ethaceticus* had no action; the author emphasises this curious behaviour with regard to mannitol and dulcitol, and considers this to be a case of the selective action of these organisms. He has obtained only one organism which can ferment both mannitol and dulcitol; the same products are obtained by the action of this *Bacillus ethacetosuccinus*, but rather more succinic acid is formed.

Grimbert has shown that, by the action of Friedländer's pneumococcus, mannitol gives lactic acid, and dulcitol gives succinic acid. In conjunction with Ficquet, he has found that *Bacillus tartricus* acts upon mannitol, and not dulcitol.

Lactic acid of fermentation is inactive, but by some organisms, *e.g. Bacillus acidi lævolactici*, the lævo-acid is produced from dextrose, cane sugar, lactose, and glycerine. Schardinger has shown that alcohol, acetic acid, and lævo-lactic acid are the principal products.

Tate has obtained lævo-lactic acid from dextrose and mannitol, but rhamnose gave inactive lactic acid when acted upon by the lævo-lactic ferment.

Linossier has resolved inactive lactic acid by *Penicillium glaucum*, and has obtained dextro-lactic acid similar to that obtained from muscles.

Frankland and MacGregor have also obtained d-lactic acid from calcium lactate, by the action of a particular organism.

In fermentation, the inactive and the two active varieties of lactic acid are therefore produced; according to some observers the variety obtained depends on the micro-organism, but according to Kayser, the medium upon which the micro-organism is cultivated is also concerned. Péré's results show that the lævo variety is in most cases produced by two colon bacteria from various carbohydrates; and Harden has obtained the same result with the *Bacillus coli communis,* which attacks mannitol, but not dulcitol. The amount of alcohol produced from mannitol is twice as great as from glucose, and this result is ascribed by Harden to the presence of the group $CH_2OH—CHOH$, which is contained once in glucose, $CH_2OH—(CHOH)_4—CHO$, but twice in mannitol, $CH_2OH — (CHOH)_4 — CH_2OH$. This view, that the alcohol produced originates from this particular group, is confirmed by the action of this organism on glycerol, $CH_2OH—CHOH—CH_2OH$, the chief products of this action being alcohol and formic acid. As regards the formation of the lactic acid, two molecules of mannitol or glucose break down in the fermentation, and the middle groups of $CHOH—CHOH—CHOH$ are supposed to give rise to it by the interchange of a hydrogen and an oxygen atom between the two terminal radicles of one group or between those of the two groups, derived from the two molecules of the sugar. The unaltered asymmetric group, $—CHOH—$, in the centre would remain asymmetric in the new molecule of lactic acid, but the change, being brought about in the presence of the asymmetric agent—whether the organism itself or possibly an enzyme—would take place entirely in one direction or more rapidly in one direction than the other, and an active acid, independent of the previous configuration of the group, but dependent on the nature of the organism, would result. This view is based upon the theories of Fischer and of

Marckwald regarding the influence of configuration of the agent causing the fermentation.

In 1892 Buchner investigated the action of *Penicillium glaucum* and *Aspergillus niger* on fumaric and maleic acids. Although these acids are so closely allied to each other, only the acid ammonium salt of fumaric acid was affected, that of maleic acid being entirely unaffected.

In the same year Purdie and Walker grew *Penicillium glaucum* upon the acid ammonium salt of inactive ethoxysuccinic acid obtained by the action of alcohol on fumaric acid, and found that the lævo- variety was consumed, the dextro- variety being left unaltered.

These are two other cases of selective fermentation by these moulds which were first shown by Pasteur to have this selective action.

Emmerling has very recently investigated certain of the amino-acids with regard to their use as food-material by micro-organisms, and he has found that only the α-amino-acids can be employed, and only by certain moulds; γ-aminobutyric acid was assimilated by all, whereas α-aminobutyric acid was of little value. Most remarkable was the action upon serin and isoserin; the former was very good, and the latter useless as a food-material.

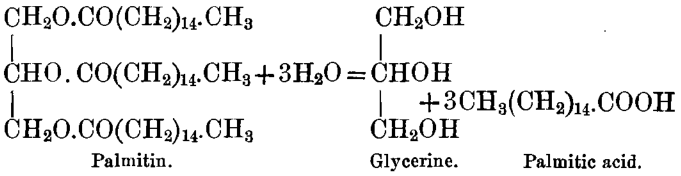
Carbon compounds, as food-material for bacteria, have been studied by Bokorny, and his results are of interest with regard to the assimilation of various alcohols; whereas methyl, ethyl, and amyl alcohols could be employed, propyl alcohol was good for fungi, but not for bacteria, and benzyl alcohol was a strong poison. Acids were also investigated; of these acetic, lactic, succinic, tartaric were good, and oxalic, and valerianic acids were of no use.

CHAPTER VIII.

CHANGES IN ESTERS.

THE decomposition of fats and oils by the enzyme known as lipase or steapsin, is another hydrolytic process; it may conveniently be described here before the other processes produced by oxidation, etc., are considered.

It was discovered by Claude Bernard that fats taken as food by animals were not assimilated in that form, but that they were transformed, by an enzyme present in pancreatic juice, into free fatty acid and glycerine. This change that the fats, or the esters of glycerine with palmitic, stearic, and oleic acids, generally known as palmitin, stearin, oleïn, undergo, may be represented by the equation :—



$$\begin{array}{lll} CH_2O.CO(CH_2)_{14}.CH_3 & CH_2OH & \\ | & | & \\ CHO.CO(CH_2)_{14}.CH_3 + 3H_2O = & CHOH & \\ | & | & + 3CH_3(CH_2)_{14}.COOH \\ CH_2O.CO(CH_2)_{14}.CH_3 & CH_2OH & \\ \text{Palmitin.} & \text{Glycerine.} & \text{Palmitic acid.} \end{array}$$

An enzyme of this nature is also present in plants, which can produce a similar change in oils. These are transformed by the plant into starch, and were in 1859 considered by Sachs to be converted directly. In 1871 Müntz showed that free fatty acid was formed from the oil; and in 1876 Schützenberger proved that glycerine was a product of this change as well as fatty acid. It was then supposed by Detmer, in 1880, that fatty acid gave rise to starch, and he suggested the equation—

$$C_{18}H_{34}O_2 + 27O = 2C_6H_{10}O_5 + 6CO_2 + 7H_2O$$

to represent this change.

It was then supposed that the glycerine gave rise to the starch, but it has been since shown by Mazé that sugar can be formed from the oil, and that this is consequently the antecedent of starch. These changes are probably all produced by the action of ferments in the plants.

The rancidity of butter, caused by the action of micro-organisms, is a common example of this decomposition, and an enzyme has been obtained by Laxa from moulds which produces this change; it also decomposes mono-butyrin and glycerides of insoluble acids, those of the highest molecular weight being first attacked.

Recent work upon the action of the enzyme known as lipase shows that it is also capable of hydrolysing esters other than those of glycerine; *e.g.* acetic ester, succinic ester, benzoic ester, salicylic ester. This power of lipase of being able to hydrolyse esters was in 1900 confirmed by Kastle and Loevenhart, who have also shown that the higher the molecular weight of the acid (in the acetic series), the more readily is it hydrolysed by lipase. The rate of the hydrolysis was shown to be dependent on the concentration of the enzyme, but independent of that of the ester; the velocity was not constant, but gradually diminished as the hydrolysis proceeded. The hydrolysis has been shown by these observers to be an incomplete and a reversible action, as they have obtained butyric ester from butyric acid and alcohol. Butyric ester was also used to determine the activity of solutions of lipase.

Kastle has also investigated the action of lipase upon the salts of certain acid esters, and has found that they are not hydrolysed, whereas the neutral esters are hydrolysed; *e.g.* diethylfumarate, diethyloxalate, diethylphthalate are hydrolysed, but not sodium ethylfumarate, potassium ethyloxalate, barium ethylphthalate. Apparently, therefore, there is no hydrolysis when the ester is electrolytically dissociated, and he assumes that hydrolysis is preceded by combination of the ester with the enzyme, and that combination cannot take place with ions, but only with undissociated molecules.

Hanriot has also found that the lipase of horse's serum can exert a synthetical action, and he has also shown that the acids of higher molecular weight are hydrolysed more quickly than those of lower. Excess of acid was found to arrest the hydrolysis, and Hanriot supposes that an unstable compound of the enzyme with the acid is formed, as the enzyme can be regenerated when attenuated by chemical action, and can return to its former activity. The hydrolysis is explained by the supposition that the ferment is a weak base which combines with the fatty acid to form a readily dissociated salt, comparable to ferric oxide and alumina, which also form unstable salts with organic acids, and can promote a slight hydrolysis of glyceryl esters.

Together with Camus, Hanriot has invented a method of quantitatively estimating lipase; monobutyrin, which was first shown by Berthelot to be hydrolysed by this enzyme, is used, and the butyric acid liberated is titrated.

CHAPTER IX.

CHANGES IN UREA AND URIC ACID.

THE last of the changes resulting from fermentation, in which the process is hydrolytic and the products are definitely known, is the conversion of urea into ammonium carbonate.

This change has been known for many years, and was recognised by van Helmont as a process of fermentation, but the cause of the change has only of late years been determined. It is a process which takes place whenever urine is allowed to stand; the acid reaction disappears, and it then becomes alkaline, and smells strongly of ammonia.

The equation $CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3$ represents this change, which was first explained by Dumas in 1830.

Micro-organisms were shown by Pasteur to be the cause, and van Tieghem succeeded in isolating a particular one, known as *Bacterium ureæ.* Since then many other bacteria have been found to possess this power.

Miquel supposed that an enzyme was the cause of this change, and Musculus has obtained an extract from certain bacteria which can produce this conversion. It has been termed urease by Miquel; the enzyme, according to Lea, is excreted at the death of the microbe.

Uric acid is stated to be changed by the same organisms into the same product, with the evolution of carbon dioxide. F. and L. Sestini have suggested the following equation—

$$C_5H_4N_4O_3 + 8H_2O + 3O = 4(NH_4)_2CO_3 + CO_2$$

to represent the result which they obtained.

E. Gérard, in 1896, found that uric acid, when dissolved in a solution of disodium phosphate, was converted by micro-organisms into urea and ammonium carbonate; he suggested that the ammonium carbonate was a secondary product resulting from the urea by the action of a urophagic micro-organism, the urea being the true product of the fermentation of uric acid. This, a little later, he was able to prove, and he gave the following equation to represent this change:—

$$C_5H_4N_4O_3 + 4H_2O = 2CO(NH_2)_2 + C_3H_4O_5$$

It was shown by Magnier de la Source that when uric acid was boiled with water, dialuric acid was formed, which on hydrolysis gave urea and tartronic acid, and hence its up to the present hypothetical formation in the fermentation of uric acid.

According to Burri, hippuric acid in the form of its calcium salt is fermented, but with greater difficulty than uric acid, into the same product, namely, ammonium carbonate. Van Tieghem states that *Bacterium ureæ* converts hippuric acid into its constituents, benzoic acid and glycocoll, thus:—

$$C_6H_5.CO.NH.CH_2.COOH + H_2O$$
$$= C_6H_5COOH + CH_2(NH_2).COOH$$

This acid and also taurocholic acid can be hydrolysed by a ferment excreted by many bacteria. That the decomposition is caused by a ferment is shown by the fact that the same hydrolysis occurs when the bacteria are destroyed with ether (Hoppe-Seyler).

CHAPTER X.

CHANGES OCCURRING AS THE RESULT OF OXIDATION.

THE formation of acetic acid by fermentation is a process differing from those changes already described, by not being hydrolytic, but produced by oxidation. This process is used on the large scale for the production of vinegar, and has been known for a long time. Acetic acid, the principal constituent, was one of the first acids known.

Before the time of Lavoisier, it was not known that oxygen was necessary for this conversion of alcohol into acetic acid, which takes place in wines when they are allowed to stand in the air; it was, however, generally supposed that air was necessary. Lavoisier showed that the oxygen of the air was absorbed in the process.

In 1821 Davy discovered that alcohol could be converted into acetic acid when platinum black was moistened with alcohol; it was subsequently shown, in 1823, by Döbereiner, that oxygen was used up in this change, and that the alcohol was converted into acetic acid and water.

In this process a skin is formed on the surface of the liquid, which was named *Mycoderma aceti*, or, mother-of-vinegar, by Persoon, in 1822. Kützing, in 1837, re-investigated this scum, and concluded that it was of a living nature. This mycoderma was held by Liebig to be non-living, and porous like platinum black; its action depended on the pores in its substance, in which oxygen was mechanically contained; the formation of acetic acid was due to the movement set up by the decomposing matter, oxidation being thus caused.

The subject was carefully investigated by Pasteur, but he did not at once recognise the *rôle* played by the mycoderma, which was supposed to act like platinum black; he found that the change was only effected during the life of the organism, and that if the action were continued, carbon dioxide and water were produced from the acetic acid. In 1873 it was shown by von Knieriem and Ad. Mayer that these two processes of producing acetic acid were not comparable, since the mycoderma could only decompose weak alcoholic solutions at a temperature not above 40°; the platinum black could effect the transformation of strong alcohol, even at a high temperature. It is now generally supposed that the change is due to the direct action of living organisms, since no enzyme as yet capable of producing this change has been obtained from them.

The most recent and careful observations were carried out by A. J. Brown, in 1886, with two particular organisms which he had isolated. He has confirmed the older observation of Pasteur's, that if the process be continued beyond the complete oxidation of the alcohol to acetic acid, carbon dioxide and water are formed from the latter.

In this process Brown has shown that the alcohol passes through two stages: aldehyde is formed first, and is subsequently converted into acetic acid, thus:—

$$2CH_3.CH_2OH + O_2 = 2CH_3.CHO + 2H_2O$$
$$2CH_3.CHO + O_2 = 2CH_3.COOH$$

Brown has made several other investigations upon the oxidising action of these two micro-organisms; propyl alcohol was converted into propionic acid, but the oxidation of methyl, isoprimary butyl, and amyl alcohols could not be effected. Henneberg has also shown that propylalcohol can be oxidised to propionic acid by the acetic acid micro-organisms.

Glucose was oxidised to gluconic acid in the presence of calcium carbonate, added to neutralise the free acid formed,—a confirmation of Boutroux's result of 1880.

The following equation is given by Brown to represent this change—

$$2CH_2OH.(CHOH)_4.CHO + O_2 = 2CH_2OH.(CHOH)_4.COOH$$

the oxidation thus affecting the aldehyde group.

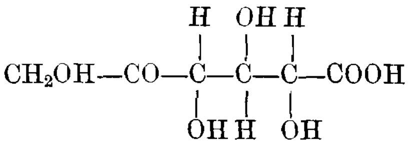
Further, mannitol is oxidised to lævulose; since mannitol can be obtained from d-glucose by reduction with sodium amalgam, and mannitol can be converted by the *Bacterium aceti* into lævulose, a new method of converting d-glucose into lævulose has been discovered by Brown.

In the vinegar plant, a membrane is formed which contains cellulose, and Brown shows that this can be formed from dextrose, mannitol, and lævulose, but not from cane sugar, starch, or alcohol. He proposes the name of *Bacterium xylinum* for this microorganism.

The oxidation of sorbitol to sorbose by the sorbose bacterium, which is probably identical with *Bacterium xylinum*, has been described by Bertrand, who has also shown that it can oxidise mannitol to lævulose, and glycerol to dihydroxyacetone. Here the carbon atoms in the molecules are a multiple of 3; but this is not a necessity, since the micro-organism can also oxidise erythritol, arabitol, and perseïtol; in each case two hydrogen atoms are removed. Bertrand states that only those alcohols seem to be oxidised which contain a CHOH chain where no hydrogen atom exists on the same side as the hydroxyl group, which is attacked in the oxidation. He has also shown that xylose can be oxidised to xylonic acid, aldose to a monobasic acid, arabinose to arabonic acid, glucose to gluconic acid, and galactose to galactonic acid.

Vincent and Délachanal obtained Bertrand's results with regard to the oxidation of mannitol to lævulose a year earlier, and Emmerling has recently confirmed the oxidations of sorbitol and glycerol by the sorbose bacterium.

Boutroux has obtained a hydroxygluconic acid by the oxidation of gluconic acid in the presence of calcium carbonate, and he gives the formula—



$$\mathrm{CH_2OH{-}CO{-}\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}{-}\underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}}{-}\underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}}{-}COOH}$$

to this acid from the results which he obtained by oxidising it with dilute nitric acid.

CHAPTER XI.

CHANGES OCCURRING AS THE RESULT OF OXIDATION.—Continued.

IN nature, both in the animal and the vegetable kingdom, changes are produced by enzymes, known collectively now as oxydases, which are the result of oxidation.

Yoshida, in 1883, noticed that the juice of the lac tree of South-Eastern Asia underwent the peculiar changes of turning black and setting hard, producing the material used in lacquer varnish. He thought that this was due to an enzyme, and he investigated the change from this point of view. The juice was found to consist of four substances—urushic acid, to which he gave the formula $C_{14}H_{18}O_2$, gum, water, and a nitrogenous body which contained an enzyme. The change caused by this enzyme is produced in the urushic acid, which is oxidised, forming oxyurushic acid, $C_{14}H_{18}O_3$. That oxidation was the cause of the change was shown by the transformation of urushic acid into oxyurushic acid by chromic acid. Some ten years later Bertrand reinvestigated this change, and confirmed Yoshida's result. He gave the name laccase to the enzyme, but he called urushic acid laccol, as it resembles in many of its properties the polyatomic phenols. This observer found that an alcoholic solution of laccol turned brown at once, and ultimately became black under the influence of the ferment. Bertrand has also investigated the action of this enzyme upon various phenols, but especially hydroquinone and pyrogallol. The hydroquinone became rose-red and green crystalline scales, with a metallic lustre separated out, consisting of quinhydrone, the odour of quinone being apparent. When performed in a sealed tube, it was found that oxygen had been absorbed.

The change may be expressed by the equation:—

$$2C_6H_4(OH)_2 + O_2 = 2H_2O + 2C_6H_4O_2$$

The pyrogallol was converted into purpurogalline, which sublimed on heating, giving orange-red needles, soluble in alcohol and acetic acid.

An alcoholic solution of gum guaiacum becomes blue in the presence of air and a very small quantity of laccase, but if the proportion of laccase is large, the blue colour becomes green and then yellow; this solution serves as a convenient test for laccase, which has been found to be of very general occurrence in vegetables and fungi, and probably plays a part in the respiration of plants.

Bertrand has investigated the action of laccase upon dihydric and trihydric phenols, amidophenols and diamidobenzenes, and he has found that the ortho- and para- derivatives are readily oxidised by oxygen or air in the presence of laccase, whilst the meta- compounds are not affected, or only with difficulty. The rate of oxidation seems to depend upon the tendency of the compound to form a quinone. The author concludes that laccase acts most readily, if not exclusively, upon compounds of the benzene series which contain not fewer than two hydroxyl or amidogen groups in the ortho- or para-positions.

Other compounds oxidised by laccase are pyrocatechuic acid, gallic acid, tannin, and hexahydric phenol, $C_6(OH)_6$; carbon dioxide is liberated in the cases of gallic acid and tannin. The simple monatomic phenols are not acted upon.

The activity of this enzyme is curiously associated with the presence of manganese, and Bertrand states that the activity is proportional to the amount of manganese, since the absorption of oxygen in the presence of manganese was found to be considerably greater than when no manganese was present. He has also found that the manganese cannot be replaced by other metals.

Bertrand has suggested that the oxydase may be a special combination of certain proteids containing acid groups with manganese, the proteid group varying for each enzyme. Under such conditions, the manganese can be kept in solution, the oxygen being conveyed by the manganese, and the proteid constituting the enzyme.

Bourquelot and Bertrand have further shown that the blue colours produced in fungi when they are cut open, are formed by the action of an oxidising enzyme, which acts only when the air has direct access to the part; in all probability laccase is the enzyme which produces this colouration. Schönbein considered that the colouration was due to the action of ozone upon a chromogen in the fungus, and that the fungus contained a substance capable of forming ozone from the air. That a definite chromogen is present in fungi was shown by Ludwig, in 1872, who has thus confirmed Schönbein's supposition of its existence.

Certain other fungi, *e.g. Russula nigricans,* produce a red and finally a black colour. This change has also been investigated by Bertrand, who has been able to isolate tyrosine from the juice of this fungus and from the root of the beet, and he has shown that the red colour is produced from this body by another enzyme, isolated from the fungus, which he has called tyrosinase; it is not brought about by laccase nor by oxidising agents. A solution of tyrosine, when acted upon by this ferment, becomes red and then black, and finally an amorphous precipitate is formed in the solution.

Bourquelot has investigated the changes produced by the oxidising enzymes in the extracts of mushrooms, and he has found that all the cresols, resorcinol, guaiacol, and eugenol, which gives a white precipitate of vanillin, are acted upon; also m-toluidine, xylidine, and aniline "for red," which is converted into a colouring matter like magenta.

Even in solutions containing 50 per cent. of methyl or ethyl alcohol, these extracts are able to readily oxidise tyrosine. He has, therefore, tried the oxidising power of these extracts upon insoluble phenols, with the result that the o-, p-, and m-xylenols, thymol, carvacrol, α- and β-naphthol, are all acted upon; α-naphthol becomes blue-violet, and a dull blue precipitate is formed, whereas β-naphthol gives a white precipitate which turns yellow; phenolic esters are also oxidised.

Since laccase and tyrosinase exist together in the extracts of certain fungi, it is not quite certain whether the above oxidations are produced by tyrosinase, though in all probability it is this enzyme which is the cause of these changes; this oxydase is also said to occur in animals and in bacteria (Roux, Von Fürth and Schneider, Lehmann).

Sarthou states that the latex of *Schinus Molle,* which is a milky fluid, becomes blue when it is exposed to the air, and that this change is effected by an enzyme termed schinoxydase. This ferment can oxidise quinol, resorcinol, pyrogallol, in the presence of atmospheric oxygen; the ash of the enzyme, which was isolated, contained iron, and its oxidising power is almost proportional to the amount of iron. He supposes that iron plays a part in this change like manganese in the changes produced by laccase.

Certain wines undergo a peculiar disorder known as "casse" or "cassure." This has now been proved to be due to the presence of an oxydase enzyme named œnoxydase by Cazeneuve, which can decolourise the wine and produce a red-brown precipitate. Bouffard, who has studied this disorder, has found that a temperature of 60° prevents it, and that it can also be prevented by a small amount of sulphurous acid. It is probably upon the tannins in the wine that this enzyme acts, as the change does not take place if they are extracted with ether; perhaps the œnoxydase is removed together with the tannin.

This enzyme has also been found in the juices of apples, pears, etc., and is the cause of their browning when they are cut open; according to Lindet, it is the tannin in the apple-juice which is oxidised by this enzyme.

The "bouquet" of wines is stated by Tolomei to be caused by an oxidising enzyme in the yeast, and he showed its existence in several species; an oxydase also exists in beer yeasts, and is the cause of an extract becoming brown. According to Grüss, the oxydase of yeast oxidises tetramethyl-p-phenylenediamine, but not guaiacol, and he proposes to divide oxydases into guaiacol-oxydases and amino-oxydases, as he has found oxidising enzymes, similar to that in yeast, in plants.

The odour of valerian is another change caused by an oxydase enzyme (Charles).

In the manufacture of indigo, indigo-white is the product obtained, which on exposure to the air becomes blue; an enzyme present in the original glucoside has, according to Bréaudat, this oxidising action upon the indigo-white.

In the animal organism, several oxidation processes occur as the result of oxidation by fermentation; for instance, phenol and benzoic acid or hippuric acid are the products obtained when benzene and toluene are taken as food.

An alkaline solution of p-phenylenediamine mixed with α-naphthol, is coloured blue when extracts of organs are added to it. Ehrlich first showed that this was due to the formation of indophenol. It serves as a test for the presence of oxidising ferments together with the other colour reactions, with guaiacum tincture and guaiacol. Salicylic aldehyde and benzylalcohol have been used by Schmiedeberg as a means of quantitatively estimating the ferment, the amounts of salicylic acid and benzoic acid produced being determined. Formaldehyde was used by

Pohl, and arsenious acid by Spitzer, for the same purpose. The most important of the oxydases of the animal organism is the one named salicylase by Abelous, which oxidises salicylic aldehyde to salicylic acid, and it occurs in most of the organs of the animal body.

Medvedeff, who has investigated the oxidising action of liver extracts on salicylaldehyde, states that the amount of oxidation is proportional to the square root of the concentration of the aldehyde, since this is the proportion of the substance dissociated in solution, but directly proportional to the concentration of the ferment.

According to Abelous and Biarnès, oxygen is consumed and carbon dioxide is liberated when salicylic aldehyde is oxidised to salicylic acid by liver extracts.

Spitzer has been able to convert the nuclein bases into uric acid by extracts of the liver and the spleen in the presence of air which was bubbled through the solution, putrefaction being carefully excluded; xanthine and hypoxanthine were almost quantitatively converted into uric acid; adenine and guanine, however, were oxidised to a lesser extent.

A third oxidising ferment, according to Abelous and Biarnès, has no action on salicylic aldehyde, but turns guaiacum tincture blue.

Cavazzani infers the existence of an oxidising ferment —cerebro-spinase—in the cerebro-spinal fluid, as it gives with pyrogallol and sulphuric acid a crystalline deposit of purpurogalline; with tannic acid it gives a brown, with quinol a rose colouration, and with o-toluidine a rose-violet precipitate.

The disappearance of sugar from blood which has been shed, is another case of oxidation in animal tissues. Seegen has shown that it is not caused by micro-organisms, as the same process takes place in the presence of chloroform; it is due to a glycolytic ferment produced by the leucocytes, or white blood corpuscles, and it exists not only in blood, but in various organs. It is not yet definitely known what change the sugar undergoes : the experiments of Oppenheimer have led to no definite conclusions, since he could not detect alcohol or acetone, though he obtained traces of iodoform; nor as yet has he been able to prove the formation of lactic acid, which is very probably produced in the process.

The light-producing organs and the eggs in the ovary of the female glowworm give a blue colour with guaiacum tincture. Dubois considers that the active agent in the production of light is an enzyme which he has named luciferase, but he believes it is not a result of oxidation.

Waters rich in iron in the form of ferrous hydrogen carbonate, contain several species of micro-organisms, which are able to oxidise this compound after it has passed by osmosis through their cell-walls, and convert it into ferric hydroxide with the evolution of carbon dioxide, thus:—

$$2FeH_2(CO_3)_2 + H_2O + O = Fe_2(OH)_6 + 4CO_2$$

This change can be followed in the deposited layers in the sheaths of these microbes. At first they are pale yellow, and can be decolourised by water containing carbon dioxide; but later on they become brown-red, and are only decolourised by dilute hydrochloric acid; finally this reagent cannot decolourise them. We have to thank Winogradsky for the majority of the investigations upon these iron bacteria, which can only grow when ferrous carbonate is present, ferric oxide even being useless as a form of iron. Molisch states that manganese can replace the iron. The deposits of ferruginous ochre and bog iron ore are said to be the result of the action of these bacteria.

Hydrogen sulphide in well-waters is oxidised by the sulphur bacteria to sulphur, in accordance with the equation—

$$H_2S + O = H_2O + S$$

oxygen being necessary for this transformation. This change was definitely shown in 1886 by Winogradsky, and further investigations were carried out by Jegunow, who has shown that the sulphur in a higher level of the bacterial cell is oxidised to sulphuric acid, which is excreted. The reverse process of reduction of sulphates will be described in the next chapter.

CHAPTER XII.

CHANGES OCCURRING AS THE RESULT OF REDUCTION.

De Rey-Pailhade, in 1889, observed that hydrogen sulphide was formed from sulphur, when it was mixed with yeast, and he attributed this change to the action of a reducing enzyme, which he termed philothion. Sostegni and Sannino have obtained the same result; they added flowers of sulphur to a solution of sugar, which was fermented with a pure cultivation of yeast, and found that hydrogen sulphide was evolved. Salkowski has noticed the production of hydrogen sulphide in wine, but he attributes its formation to the action of bacteria on the sulphur compounds present in it.

Philothion, sometimes alone and sometimes with laccase, has been found by De Rey-Pailhade to be contained in germinating seeds; he has shown that it contains a proteid radicle which can enter into loose combination with manganese or hydrogen in the same way as Bertrand showed in the case of laccase.

The power possessed by philothion, of forming hydrogen sulphide from sulphur, is stated by Cossettini to be lost when it is filtered through a Chamberland filter, and Pozzi-Escot finds that philothion does not give the blue colour with guaiacum tincture and hydrogen peroxide, which is the usual test for these enzymes. Hydrogen peroxide, however, is decomposed with the evolution of oxygen.

This observer identifies philothion with catalase, an enzyme stated by Loew to be of universal occurrence, and to whose presence the power of decomposing hydrogen peroxide, possessed by all enzymes, is due; this observer states that catalase exists in an insoluble and a soluble form, termed α- and β-catalase respectively.

Pozzi-Escot finds that philothion can also cause the hydrogenation of selenium and phosphorus, but not, or only very slightly, of tellurium and arsenic.

The production of hydrogen sulphide by micro-organisms has been investigated by Zelinsky, who isolated a bacterium—*Bacterium hydrosulfureum ponticum*—from the ooze at the bottom of the Black Sea; it can produce hydrogen sulphide not only from proteid matter, but also from inorganic salts, such as sulphates, sulphites, thiosulphates, and even ammonium thiodiglycollate when they are added to the nutrient medium. *Vibrio hydrosulfureus* is shown to be active in the same way. Beyerinck has also obtained a micro-organism which transforms sulphates into hydrogen sulphide; but Saltet finds that this *Bacillus desulfuricans* only partially reduces sulphates to sulphites or other oxygenated sulphur compounds which are then reduced by other bacteria to hydrogen sulphide. The cycle which sulphur compounds undergo in nature is thus completed; for in the previous chapter (Chapter XI.) the oxidation changes which sulphur underwent were briefly described.

Compounds of arsenic, in wall-papers especially, are acted upon by moulds, and arsenical vapours, formerly thought to be arseniuretted hydrogen, though this was denied by some investigators, are evolved. Biginelli has recently shown that the vapours consist of diethylarsine. Arsenious oxide in small quantities is completely volatilised by *Penicillium brevicaule*, which produces the arsenical odour even from 0·01 and often from 0·001 milligram of arsenic; this mould serves as a good biological test for arsenic.

These various investigations have been carried out by Gosio, Emmerling, Abel and Buttenberg, and by Galli-Valerio and Strzyzowski.

König has shown that tartaric acid is reduced to succinic acid by *Bacterium termo*, and Emmerling that malic acid is converted into succinic acid almost quantitatively by *Bacillus lactis aërogenes.*

Basile states that glucose is reduced to mannitol in certain Sicilian wines, and thus confirms Malbot, who thinks that it may be caused by the butyric ferment which liberates hydrogen. Gayon and Dubourg find that an enzyme can reduce lævulose to mannitol. This mannitol ferment decomposes the sugars not fermented by yeast, and thus affords a means of detecting lævulose in the presence of fermentable carbohydrates.

Certain colouring matters, *e.g.* indigo-carmine, are bleached by micro-organisms when they are cultivated in solutions to which the colours have been added; this reduction is said not to be due to the action of nascent hydrogen.

In the animal organism processes of reduction, as well as oxidation, also occur. Abelous and Gérard have made extracts of various organs of the animal body, and have found that nitrates can be reduced to nitrites, butyric acid to butyric aldehyde, and methylene blue decolourised by these extracts; further, nitrobenzene can be reduced to aniline. Pozzi-Escot states that fibrin contains a reducing enzyme, or reductase; and that there is also one present in urine; he uses hydrogen peroxide for detecting their presence.

CHAPTER XIII.

NITRIFICATION AND DENITRIFICATION.

Processes of oxidation and reduction are continually taking place in the soil: these are now known to be the result of the action of several micro-organisms, which are able to bring about these changes.

Formerly it was thought that the change of ammonium salts into nitrates in the soil was a purely chemical process, analogous to that which takes place when air and ammonia are heated together in the presence of spongy platinum; by the porosity of the soil, which mechanically contained the oxygen of the air, the change was supposed to take place—a kind of catalytic action, similar to that of platinum black. In 1877 it was pointed out by Schloesing and Müntz that the process took place very slowly at 0° C., that it had an optimum temperature at 37° C. and that above 60° C. the change no longer took place. These results strongly suggested the action of micro-organisms, the above observers holding that only one of these was necessary. In 1890 researches were undertaken by several investigators, among whom may be mentioned Frankland, Warington, and especially Winogradsky.

From the results obtained, it is now known that the change takes place in two stages. In the first stage the ammonium salts are converted into nitrites by the action of the nitrous organisms, or nitrose bacteria; only two types of bacteria, of each of which there are several species, are able to bring about this oxidation, and they are known as Nitrosomonas (Europe, Asia, Africa) and Nitrosococci (South America and Australia). They have no further action upon nitrites which can be produced in solutions of asparagine, urea, etc. In the second stage nitrites are converted into nitrates by the action of the nitric organisms, or nitro-bacteria, of which there is only one genus; this process does not take place in the presence of ammonia, and not until all the ammonium salts are converted into nitrites. Stutzer and Hartleb state that 89–93 per cent. of the nitrogen as nitrites can be converted into nitrates by the nitrate bacteria.

Experiments by Omeliansky show that the nitrogen of organic compounds is not attacked, but must be first converted into ammonia; substituted amines, however, are attacked, according to Demoussy; monomethylamine sulphate is converted into ammonia, and then into nitrite and nitrate; trimethylamine is attacked more slowly; there is no intermediate formation of monomethylamine, the three methyl groups being all attacked at the same time. Aniline, pyridine, and quinoline are attacked still more slowly, and the oxidation takes place apparently with greater difficulty when the molecule is more complex.

These bacteria derive their carbon from carbon dioxide;

they will not grow on gelatine, nor on the ordinary culture media, but require a mineral solution for their cultivation.

The reverse process of reduction of these substances—called denitrification—also takes place; in some cases nitrogen can be liberated from both nitrates and nitrites, *e.g.* by *Bacterium denitrificans II.* (Burri and Stutzer); in other cases nitrates are converted only into nitrites, and Maasen states that eighty-five micro-organisms are able to convert potassium nitrate into potassium nitrite. Loew has observed reduction as far as ammonia.

Ampola and Ulpiani, from the results of their experiments on denitrification by means of *Bacteria denitrificans V.* and *VI.*, conclude that the process can be expressed by the equation:—

$$5C_6H_{12}O_6 + 24KNO_3 = 24KHCO_3 + 6CO_2 + 18H_2O + 12N_2$$

Asparagine and nitromethane are not attacked, and ethyl nitrate only very slowly, and the authors conclude that denitrification only takes place with oxygen compounds of nitrogen, and in those which are electrolytes and therefore dissociated in solution. The various micro-organisms are also divided into three classes: (1) those which destroy nitrites, but not nitrates; (2) those which destroy nitrates, but not nitrites; (3) those which destroy both nitrites and nitrates. Denitrification was also found to take place more rapidly with salts in which the metal is more electropositive and of lower atomic weight.

In this way free nitrogen is liberated, but it can again be assimilated by another set of bacteria occurring in the roots of the leguminosæ, and converted into albuminous bodies.

The cycle of changes which nitrogen passes through is, as we have seen, very complicated: albuminous substances are converted into urea by animals; urea is converted into ammonium carbonate, which in turn becomes nitrite and nitrate; finally nitrogen is liberated, and this is assimilated and converted into albuminous substances.

CHAPTER XIV.

VARIOUS CHANGES OCCURRING AS THE RESULT OF FERMENTATIONS.

LACTIC acid, which itself is a product of fermentation can undergo two changes in the same way. The first of these—butyric acid fermentation—has been known, perhaps, as long as that of lactic acid, since it takes place at the same time. Special organisms, *e.g.* *Bacillus amylobacter*, are the cause of this fermentation, and the change of lactic acid into butyric acid can be expressed by the equation :—

$$2C_3H_6O_3 = C_4H_8O_2 + 2CO_2 + H_2$$

Carbon dioxide and hydrogen are generally produced in the process, and the hydrogen may cause the reduction of other organic substances which are generally present during the fermentation.

Not only, however, can butyric acid be produced from lactic acid, but also from the substances which can give rise to lactic acid; these have already been enumerated, and, in addition, higher acids, such as malic, tartaric, citric, and mucic, can be fermented with the formation of butyric acid as a product. From mucic acid, butyric acid is only slowly formed, and it ought to be considered as a secondary product.

The homologues of butyric acid, *e.g.* propionic, valerianic, and acetic acids, and also succinic acid, are often produced at the same time; they are, perhaps, formed by the action of the nascent hydrogen.

Mannite, quercite, and glycerine are decomposed by various micro-organisms, with the production of butyric acid; from glycerine, methylalcohol, acetic acid and traces of formic acid are also formed in the presence of calcium carbonate.

Schattenfroh and Grassberger have described two very similar micro-organisms which form lactic acid as well as butyric acid, with the evolution of carbon dioxide and hydrogen. One of these micro-organisms is mobile, and almost completely converts lactose into butyric acid; dextrose, cane sugar, and starch are not so completely converted. The other micro-organism is non-mobile, and produces both butyric and lactic acids. The lactic acid formed is sometimes inactive and sometimes dextrorotatory.

The second of these changes—propionic acid fermentation—takes place in the calcium salt of lactic acid; the principal product is propionic acid, but acetic and valerianic acids have also been found. Strecker first noticed this change in 1854, but the most careful study has been made by Fitz, who has isolated the micro-organism which produces it.

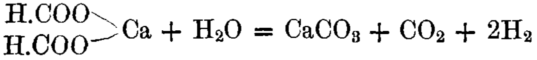
The lactic acid splits up into two parts of propionic acid and one of acetic acid, together with carbon dioxide and water. No hydrogen is evolved, and it thus differs from butyric acid fermentation. This change can be represented by the equation:—

$$3C_3H_6O_3 = 2C_3H_6O_2 + C_2H_4O_2 + CO_2 + H_2O$$

Malic acid, according to Fitz, is decomposed by another bacillus, with the production of propionic acid in rather larger quantities ; butyric acid and alcohol are formed at the same time, but only in traces.

The production of butyl alcohol is another very important chemical change resulting from fermentation. It can be obtained from glycerol and mannitol by the action of the *Bacillus butylicus* obtained by Fitz; and Emmerling, who has investigated this change, finds that butyl alcohol is not obtained from dextrose, but that the products are ethyl alcohol and a solid fatty acid, probably palmitic acid. Beyerinck's *Granulobacter butylicum*, however, forms butyl alcohol from dextrose, but not from glycerol.

Calcium formate is converted into calcium carbonate and hydrogen by certain bacteria, though probably an enzyme is the cause of this change, since it continues after the micro-organisms have been destroyed. The following equation represents this change :—



$$\begin{matrix} H.COO \\ H.COO \end{matrix} > Ca + H_2O = CaCO_3 + CO_2 + 2H_2$$

Pakes and Jollyman have recently investigated the products obtained from sodium formate by the action of certain bacteria, and they express the reaction as follows :—

$$HCO_2Na + H_2O = NaHCO_3 + H_2$$

$$\text{or} \quad HCO_2H = CO_2 + H_2$$

the total amount of carbon dioxide, evolved as gas and in combination, being equal in volume to the amount of hydrogen evolved.

Gonnermann has quite recently investigated the hydrolysis of acid amides and anilides by ferments; *e.g.* pepsin, trypsin, and emulsin, and by extracts of organs; and he finds that acetamide, acetanilide, formanilide, and others are hydrolysed; ptyalin, invertin, and maltase had no action.

E. Gérard has described the formation of creatine from creatinine by a kidney extract, and he refers to a similar dehydrating effect obtained by Abelous and Ribaut in the formation of hippuric acid by kidney extract.

Grimbert has obtained acetylmethylcarbinol from dextrose by the action of *Bacillus tartricus;* it was identified by its osazone and osotetrazone; it was also obtained from other sugars.

Various compounds have been obtained by the action of bacteria; *e.g.* esters by *Bacillus esterificans fluorescens* (Maasen); quinone by *Streptothrix chromogena* (Beyerinck). Went describes the formation of carbon disulphide by *Schizophyllum lobatum.*

In this chapter it will be convenient to shortly describe the colouring matters produced by bacteria.

The best known is that produced by *Bacillus cyanogenus.* The result of its action is the production of a blue pigment often seen in milk, especially in warm weather. Erdmann considered this colouring matter to belong to the aniline group, and it is probably triphenylrosaniline. The blue colouration in cheese is due to another micro-organism, named *Bacillus cyaneo-fuscus* by Beyerinck. This organism also gives rise to black glue.

The blue pigment formed by *Bacillus pyocyaneus* has been termed pyocyanine $C_{14}H_{14}N_2O$. It can be extracted by chloroform, and was first obtained crystalline by Fordos. According to Boland, a reddish-brown colouring matter is found with the pyocyanine, which is insoluble in chloroform but can be extracted by alkalies; this is supposed to be formed from the pyocyanine in the culture of the bacillus. A green fluorescent colouring matter is produced by this microbe when it is cultivated in bouillon.

The oldest known chromogenic micro-organism is *Micrococcus prodigiosus* or *Bacterium prodigiosum*, which is the cause of the so-called "bleeding bread," "red snow," etc. The red pigment, termed prodigiosin, is insoluble in water, but is soluble in alcohol, chloroform, and carbon bisulphide. Griffiths gave it the formula $C_{38}H_{56}NO_5$, but Scheurlen has obtained analyses which do not agree with this. It has been suggested that this colouring matter is identical with fuchsine, but this statement has been contradicted by Helm and Bordoni-Uffreduzzi.

The red, yellow, and rose-coloured pigments were termed lipochromes by Zopf, the red being termed liporhodines, and the yellow lipoxanthines. They are converted by sulphuric acid into deep blue crystals of lipocyanine. They are closely related to the lipochromes of fat, yolk of egg, etc., and to the carotine of carrots.

A violet colouring matter is produced by *Bacterium violaceum*, and an indigo blue one by *Bacterium indigonaceum.* Lankester has named a bacterial colouring matter bacterio-purpurin, which pigment is also found in the sulphur bacteria.

Lastly, green fluorescent pigments are produced by bacteria, *e.g.* by *Bacilli fluorescens liquefaciens* and *non-liquefaciens:* it has been termed bacterio-fluorescein. Jordan has found that phosphorus and sulphur are essential for its formation, and he has arranged a number of organic compounds in the order of their fluorescegenic value: asparagine, succinic, lactic, citric, tartaric acids, etc.; and he points out that the presence of a methylene group is favourable to fluorescence, but not coincident with dibasicity, nor with the presence of two methylene groups as asserted by Lepierre.

In conclusion, phosphorescence and heat can be produced by bacteria.

CHAPTER XV.

CHANGES IN BLOOD.

Among the albumins and allied substances, the transformation which blood undergoes when it is shed, is undoubtedly the change which has been known for the longest time. This change is commonly called coagulation, or "clotting," and it is the result of the action of a ferment.

When blood is drawn and allowed to stand, it quickly becomes gelatinous, but after some hours it is found to consist of a yellowish liquid containing a fibrinous substance, which is red in colour, owing to the mechanical entanglement in it of the red blood corpuscles. If diluted blood be looked at under the microscope, these corpuscles are seen to run together, forming rouleaux, and it was formerly thought that coagulation consisted in the running together of these bodies. In 1772 Hewson showed that these red blood corpuscles took no part in the process, and that the fibrinous substance was formed from the blood plasma, or *liquor sanguinis.* This observer isolated from the plasma "coagulable lymph," which is now termed "fibrinogen." Buchanan, in 1835, made several observations upon coagulation; he found that hydrocele fluid and pericardial fluid could not coagulate of themselves, but if he added to them a solution which he obtained from lymphatic glands or from blood clot, coagulation at once set in. He compared this action to that of a ferment, and was therefore the first to suggest that this process was due to a ferment. Denis, in 1859, obtained by another method a substance which he called plasmine, and which on solution could undergo coagulation; this substance was no doubt identical with Hewson's coagulable lymph, or fibrinogen, as it is now called.

Much more complete investigations were carried out by Alexander Schmidt of Dorpat, who rediscovered the facts observed by Buchanan, which had been forgotten. From hydrocele fluid he prepared fibrinogen; and from serum, fibrinoplastin. Of themselves these two substances would not coagulate, but on adding one to the other coagulation resulted. He found that fibrinoplastin contained another substance, whose presence was necessary for the coagulation, but only in 1872 was he able to isolate this by precipitation with alcohol; he called it fibrin ferment, and considered that coagulation was caused by the action of fibrinoplastin on fibrinogen under the influence of fibrin ferment. Fibrinoplastin was subsequently named serum-globulin.

The next experiments were performed by Hammarsten, who showed that fibrin could be formed from fibrinogen and fibrin ferment without the presence of serum-globulin, which had no effect when pure, but contained a factor, when impure, which was necessary for the process; this could be replaced by impure casein or by calcium salts. The fibrinogen is split up during the change, and does not combine with serum-globulin, as was formerly supposed.

Subsequently, Green found that fibrin ferment contained calcium sulphate, and that calcium sulphate was necessary for the change. Ringer and Sainsbury have shown that any calcium salt can replace the sulphate, and that barium and strontium salts can also be used. Experiments by Horne, in 1896, show that these salts, in the proportion of 0·5 per cent. and upwards, retard the coagulation, barium salts being the most powerful, and calcium salts the least; their influence is overcome by dilution with distilled water, or by the addition of a suitable amount of potassium oxalate; solutions of sodium and potassium chloride of 0·07 per cent. and upwards also retarded coagulation.

Calcium salts are therefore necessary for coagulation; this factor is often made use of when blood is wanted in an uncoagulated condition, by precipitating them as oxalates.

Fibrinogen and calcium salts will not coagulate by themselves, but fibrin ferment must also be present as a third factor, in order that coagulation may take place.

Fibrin ferment is a nucleoproteid; and Pekelharing and Halliburton, who have extracted nucleoproteids from various organs, have shown that these can cause the coagulation of fibrinogen in the presence of calcium salts. The former has therefore suggested that the nucleoproteid transfers calcium with which it combines to fibrinogen, whose calcium salt is insoluble, and constitutes fibrin; the nucleoproteid then combines with more calcium and transfers it again to fibrinogen. This view is urged by Frederikse, who finds that the presence or absence of serum-globulin in such mixtures makes no difference in the amount of fibrin formed. He also confirms Hammarsten's statement, that fibrinogen is split up into fibrin or coagulated proteid and a soluble globulin by the action of fibrin ferment; the same decomposition is said to be brought about by the action of dilute acetic acid.

Lilienfeld regards the active agent in coagulation as a nucleo-albuminous substance, named nucleohiston, which arises from the leucocytes, especially from their nuclei, and from platelets. In this substance the nuclein hastens while the histon hinders the coagulation, the separation into nuclein and histon being effected by calcium salts. From fibrinogen he has obtained a substance, thrombosin, by precipitation with acetic acid and nuclein, or rather nucleic acid, which acts in the same way; thrombosin is coagulated by calcium salts, and the fibrin so formed is regarded as a calcium compound of thrombosin. Schäfer has criticised these assertions of Lilienfeld's, stating that further evidence is necessary to show that thrombosin and fibrinogen are different substances; also that Lilienfeld does not prove that nucleo-albumin does not enter into the reaction, for it is impossible to prepare fibrinogen quite free from nucleo-albumin.

Hammarsten and Cramer also consider that thrombosin is the same as fibrinogen. In the same publication, Hammarsten shows that calcium salts are not essential for the formation of fibrin from fibrinogen, since they both contain the same amount of calcium, and he confirms Pekelharing, that calcium salts act in the genesis of fibrin ferment, *i.e.* in the change of prothrombin, the precursor of fibrin ferment, into thrombin, the fibrin ferment.

The formation of fibrin from fibrinogen is usually regarded as a process of hydrolysis, the fibrinogen being converted into fibrin and fibrinoglobulin (Arthus). Hammarsten has found that the elementary composition of these substances is very similar, and he brings forward the view that the change may consist in an intramolecular rearrangement in the fibrinogen, part of it appearing as fibrin, and part remaining in solution as fibrinoglobulin.

Under several other conditions, besides that of the removal of calcium salts, blood can be prevented from clotting, *e.g.* by leech extract, albumoses, peptones; and many experiments have been made in order to elucidate the manner in which fibrin is formed, but these do not throw any more light upon this important change.

The following facts, however, still remain proved: nucleoproteid (or prothrombin) combines with calcium to form fibrin ferment, which then acts upon fibrinogen, and causes the formation of fibrin, which constitutes the coagulum.

The facts can be tabulated thus:—

$$\left.\begin{array}{l}\text{Fibrinogen}\\ \left.\begin{array}{l}\text{Nucleoproteid}\\ \text{Calcium salt}\end{array}\right\} = \text{fibrin ferment}\end{array}\right\} = \text{fibrin}$$

CHAPTER XVI.

CHANGES IN MILK, MUSCLE, AND IN THE LIQUID OF THE PROSTATE GLAND.

A TRANSFORMATION, very similar in many ways to the coagulation of blood, is carried out daily in the manufacture of cheese from milk. This change is produced by the action of rennin, a soluble ferment or enzyme contained in calves' stomachs, from which it can be extracted to form rennet.

Milk contains three proteids—caseinogen, lactalbumin, lactoglobulin, the two latter only in small quantities; also milk, sugar, fat, calcium phosphate, and other salts. Cheese is obtained from the caseinogen, which forms the curd, which contains the fat mechanically; the other proteids, the sugar, and the salts are contained in the liquid which constitutes the whey.

The action of the rennin was supposed in 1859, by Liebig, to be the conversion of lactose into lactic acid; this neutralised the alkali which kept the caseinogen in solution, thus causing its precipitation. This idea evidently arose from the fact that acids, when added to milk, produce a precipitation of the caseinogen. Soxhlet held a very similar view, but he took into account the presence of the calcium phosphate; this was converted by the lactic acid, obtained from lactose by the action of rennin, into acid calcium phosphate, which precipitated the caseinogen.

The most important work upon the coagulation of milk is that of Hammarsten, who has shown that the lactose takes no part in the change, and that the clot can even be formed in an alkaline medium, as first pointed out by Selmi. The presence of calcium was found essential for the conversion, and Ringer states also that other calcium salts than the phosphate can be used.

The production of casein is a double process; in the first instance the caseinogen is converted into soluble casein by the rennin, and secondly the casein is precipitated by calcium, probably as calcium salt or caseate of lime (Halliburton).

Hammarsten considers that in the process the caseinogen is split into two parts—the insoluble casein and the whey proteid, which is soluble.

This change can be tabulated thus—

$$\left.\begin{array}{l}\left.\begin{array}{l}\text{Caseinogen}\\ \text{Rennin}\end{array}\right\} = \text{soluble casein}\\ \text{Calcium salt}\end{array}\right\} = \text{casein}$$

and is therefore slightly different from that which occurs in blood, where the calcium takes part in the first stage of the change.

The similarity between the formation of fibrin in the clotting of blood and the production of *rigor mortis* in muscle, was first pointed out by Kühne, and he made the first investigations upon this change, about the year 1860. From frog's muscle at a temperature of 0° C., he obtained a plasma which possessed the power of coagulation; in very much the same way as in blood and milk, this clot contracted and gave a serum, but, instead of being fibrinous, this coagulum remained gelatinous and soft. The term "myosin" was given to it.

Halliburton has obtained the same results with plasma obtained from the muscles of rabbits and other animals, and he has shown that the myosin obtained, if redissolved after coagulation, could be made to clot even three or four times. He states that a ferment, which he has isolated, is the cause of this change, and he has called it myosin ferment. From the plasma he has obtained four proteids, which he has named paramyosinogen, myosinogen, myoglobulin, and myoalbumin; and he has shown that the coagulation is caused in the myosinogen, which carries down with it on coagulation the paramyosinogen. He considers that the myosinogen does not split up into two proteids, as is supposed to happen in the case of fibrinogen; in the coagulation, however, sarcolactic acid is formed and carbon dioxide is evolved, thus:—

$$\left.\begin{array}{l}\text{Myosinogen}\\ \text{Myosin ferment}\end{array}\right\} = \text{myosin} + CO_2 + CH_3\text{—}CHOH\text{—}COOH$$

Von Fürth has also carried out investigations upon the subject, and has obtained two proteids from muscle plasma:—(1) Paramyosinogen, constituting 17–22 per cent., and (2) myosinogen or myogen, constituting 77–83 per cent. of the plasma; this latter corresponds to Halliburton's myosinogen. These two proteids enter into the formation of the clot; paramyosinogen passes directly into myosin fibrin, but myosinogen or myogen passes through an intermediate soluble stage into myogen fibrin, thus :—

Paramyosinogen ————————→ myosin fibrin
Myosinogen → soluble myogen → myogen fibrin

He thus confirms Halliburton, that two proteids enter into the formation of the clot, and he regards Halliburton's myoglobulin as part of the myosinogen which is not coagulated.

Stewart and Sollman have obtained the same results, but they regard Von Fürth's soluble myogen as part of the paramyosinogen. Both the proteids are converted into insoluble myosin, the myosinogen passing first into a modification similar or identical to paramyosinogen, thus :—

Paramyosinogen ————————→ myosin
Myosinogen → paramyosinogen → myosin

They confirm Halliburton also, that coagulation is accompanied by an increase of acidity.

Another case of coagulation is said by Camus and Gley to take place in the fluid of the vesiculæ seminales, which is somewhat viscid. If a drop of the liquid from the prostate gland of the same animal, guinea-pig, rat, or hedgehog, is added to the first liquid, coagulation sets in. This change is stated by the observers to be due to an enzyme which they have named vesiculase, on account of the fact that the property is lost when the prostatic liquid is heated to 70° C. for fifteen minutes. Beyond these observations nothing further is known of this transformation.

CHAPTER XVII.

CHANGES OCCURRING IN ALBUMINS AS THE RESULT OF THE ACTION OF PEPSIN.

By far the most important changes which albumins and allied substances undergo as the result of fermentation are those produced by the digestive ferments of the animal organism, pepsin and trypsin. In plants similar ferments, bromelin and papain, have been found, which resemble trypsin in their action very closely.

The change which takes place in the albumins is known as proteolysis, and it consists in a breaking up of the very complex albumin molecule into smaller ones, the size and constitution of some of which, especially those obtained from trypsin digestion, are known. Since some of these later changes are known to take place by hydrolysis, it is very probable that the complex molecule is broken up in a similar way; especially so as the same results can be obtained with mineral acids or superheated steam. The changes are thus quite comparable to those produced in the carbohydrates, smaller molecules being obtained at each stage; here, however, the end-products are very much more various, and consist chiefly of amino-acids.

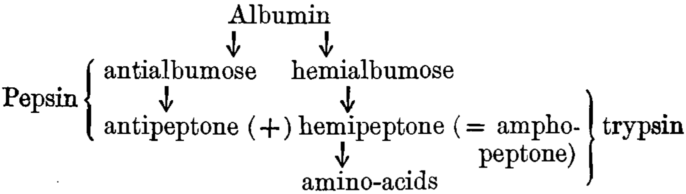
In the animal organism, albumins taken as food are first acted upon by the gastric juice of the stomach, which contains pepsin; and it is therefore most convenient to describe the changes produced by this ferment before going on to those produced by trypsin.

Pepsin, in order to produce proteolytic changes, must always be accompanied by an acid; in the gastric juice hydrochloric acid is always present in about 0·2–0·5 per cent. In this connection, observations have of late years been made by Mayer, Hahn, and Wroblewsky in particular, upon which acids are the most favourable to peptic action; and it has been found that oxalic acid produces the most speedy solution of the albumin. Next in order comes hydrochloric acid, then nitric, phosphoric, tartaric, lactic, citric, malic, sarcolactic or d-lactic, sulphuric, and acetic acids, in the order mentioned. A dilution of 0·01–0·5 per cent. was the most favourable; pepsin will not act in an alkaline solution, and even neutral salts retard its action.

Very many investigations have been made upon the products of the action of pepsin upon albumins, fibrin and egg-albumin especially. The earliest were those of Meissner and his pupils, in 1859–62, who obtained the following substances: parapeptone, metapeptone, dyspeptone, α-peptone, β-peptone, γ-peptone. Brücke, who made a determination of the products about the same time, could, however, only obtain two products, which corresponded to parapeptone and γ-peptone.

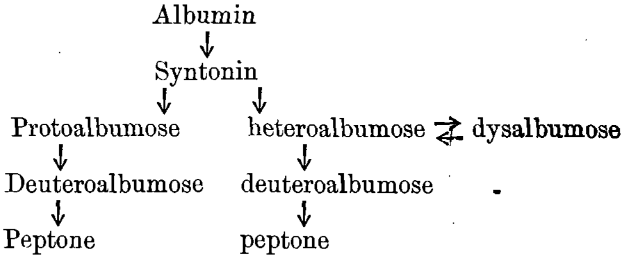
Kühne, some years later, studied the action both of pepsin and trypsin, and found that the only real difference was that trypsin produced a more complete hydrolysis.

He obtained, by using pepsin, albumose and peptone; this he called amphopeptone on account of the fact that trypsin only converts half the peptone further into amino-acids. He considered the albumin molecule to be split up into two halves, antialbumose and hemialbumose, and devised the following diagram to represent these changes:—



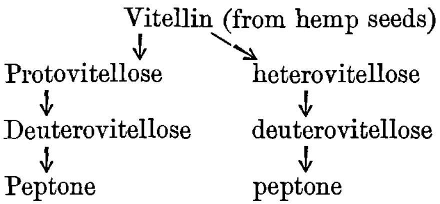
Fränkel and Langstein have recently shown that Kühne's amphopeptone consists of two substances; (1) a peptone; (2) a substance identical with the carbohydrate complex of albumin—albamin.

Chittenden, Neumeister, and others have somewhat recently studied the products of pepsin digestion very carefully, and they have come to the following conclusions: fibrin or egg-albumin is converted firstly into syntonin, chiefly by the action of the acid, and then this is split up into two primary albumoses, which are called protoalbumose and heteroalbumose. Heteroalbumose, on standing under water, or on drying, is converted into dysalbumose, which can be reconverted by acids into heteroalbumose. These two albumoses then give deutero-albumose, and subsequently peptone. The diagram shows these results more plainly:—



Some interesting conclusions from the results of molecular weight determinations of peptone and protoalbumose by Sabanejeff as given by Neumeister seem appropriate here. Peptone was found to possess a molecular weight of about 400, and protoalbumose of 2400; thus on decomposition six peptone molecules would be obtained from one protoalbumose. Taking the albumin molecule as 15,000, then albumin would give altogether 40, and heteroalbumose would give 34 molecules of peptone.

The other albumins, when subjected to pepsin digestion, give very similar products; these may be tabulated as follows:—



Chittenden and Mendel, who have investigated the changes produced in this substance, find that there is a gradual falling off of carbon in the percentage composition of these substances formed in the digestion; they suppose that the four substances above named are only the main steps in the hydration process, and that there are still other links in the chain which cannot as yet be separated, and which show the same general chemical behaviour.

Globulin → globuloses → peptone
Egg-albumin → ovalbumoses → peptone
Myosin → myosinoses → peptone

The compound proteids are firstly split up into their constituent parts, one of which only undergoes peptic transformation.

The behaviour of casein in peptic digestion, especially with regard to the phosphorus, has been studied carefully by Salkowsky; though frequently stated that the phosphorus is not dissolved, but is separated as insoluble paranuclein, he finds that much of it is dissolved, only about 15 per cent. being contained in the paranuclein; the more favourable the conditions of digestion, the less paranuclein is obtained, and paranucleic acid is obtained from the paranuclein. The phosphorus in solution is in the form of a phosphorised albumose, in which it is not combined, however, as phosphoric acid; if dry hard lumps of casein are avoided, it is completely dissolved, and this is done by making a solution of casein before adding the digestive fluid, which acts best in the proportion of 500 to 1 of casein. Lindemann confirms this, and states that caseinogen is more digestible than casein. The first product of the digestion of casein is caseoproteose, which contains all the phosphorus of the original proteid; paranucleic acid, together with paranuclein, are formed in the digestion, and Salkowski has prepared the iron salt of this acid; thus:—

$$\begin{tikzcd}[row sep=tiny] & & \text{paranuclein} \arrow[r] & \text{paranucleic acid.} \\ \text{Casein} \arrow[r] & \text{caseose} \arrow[ur] \arrow[dr] & & \\ & & \text{peptone} & \end{tikzcd}$$

$$\begin{tikzcd}[row sep=tiny] & \text{hæmatin} & & \\ \text{Oxyhæmoglobin} \arrow[ur] \arrow[dr] & & & \\ & \text{albumin} \arrow[r] & \text{albumoses} \arrow[r] & \text{peptone} \end{tikzcd}$$

$$\begin{tikzcd}[row sep=tiny] & \text{carbohydrate} & & \\ \text{Glucoproteids} \arrow[ur] \arrow[dr] & & & \\ & \text{albumin} \arrow[r] & \text{albumoses} \arrow[r] & \text{peptone} \end{tikzcd}$$

The decomposition of the albuminoids has been carefully studied by Chittenden, with the following results:—

$$\begin{tikzcd} \text{Collagen} \arrow[d] & \text{Elastin} \arrow[d] \\ \text{Hydrate = gluten or gelatine} \arrow[d] & \text{Protoelastose} \arrow[d] \\ \text{Protogelatose} \arrow[d] & \text{Deuteroelastose} \\ \text{Deuterogelatose} \arrow[d] & \\ \text{Gelatinpeptone} & \end{tikzcd}$$

No peptone is formed from elastin, and keratin is not attacked at all.

Umber has obtained from crystallised egg-albumin and serum-albumin, the primary proteoses, three deuteroproteoses, A, B, C, and peptone; and Zunz finds, as primary products of peptic proteolysis, acid albumin, proto- and heteroproteose, and deuteroproteose B; as secondary products three other deuteroproteoses, two peptones, and unknown substances which do not give the biuret reaction, ammonia and amino-substances.

Pick has investigated the proto- and hetero- albumose obtained from fibrin, and finds that they differ from one another in solubility and in the amount of nitrogen in a basic form which they contain, heteroalbumose containing 39 per cent. and protoalbumose 25 per cent.; heteroalbumose yields a large quantity of leucine and glycocoll, whereas protoalbumose yields only a little leucine and no glycocoll. They arise together from fibrin, but not from one another, and they both yield, on further peptic digestion, deuteroalbumoses A and B and peptone B. This confirms the results of Zunz.

As regards the nature of the end-products of gastric digestion, a great deal of work has lately been done. Pfaundler obtained from serum-albumin leucine and a diamino-acid, probably histidine, but from fibrin there was no leucine; he finds that the principal end-products are apparently substances intermediate between peptone and amino-acids; they do not give the biuret reaction, and contain more than one carbon nucleus, in the case of serum-albumin at least there being a leucine and diamino-nucleus present. Langstein has obtained from crystallised egg-albumin the following substances: leucine, tyrosine, glutamic acid, aspartic acid, cystine, lysine (in small amount), pentamethylenediamine, hydroxyphenylethylamine, and "a polymeric carbohydrate containing nitrogen;" also a base giving skatole and two unknown acids which give the biuret reaction; he did not find the intermediate substances of Pfaundler, perhaps because the digestion had gone too far. Malfatti has found that tryptophane is formed by the prolonged action of pepsin-hydrochloric acid on Witte's peptone, and also small quantities of leucine, tyrosine, and hexone bases.

V. Harlay has found a very interesting substance among the products of pepsin digestion: it is a chromogen, as it gives, on treatment with the enzyme tyrosinase, a red colour; on decomposition with trypsin it yields tyrosine and tryptophane, a body first observed by Neumeister among the products of trypsin digestion. According to Lawroff, pepsin breaks up proteids as much as trypsin does.

Sawjaloff has recently advanced the theory that peptonisation of proteid matter is not to make it more easy for absorption, as there is a reverse process of building up of proteids of larger molecular weight from the products of peptonisation, and this action is attributed to the rennet ferment; a substance termed plastein is thus synthesised, which appears to be the same, whatever the proteid may be which is digested. It resembles Kühne's anti-albumid in properties; Krüger's results, that the digestive products inhibit the action of pepsin, help to support this view.

CHAPTER XVIII.

CHANGES OCCURRING IN ALBUMINS AS THE RESULT OF THE ACTION OF TRYPSIN.

CLAUDE BERNARD, and in 1857 Corvisart, who refers to Purkinje and Pappenheim as having made experiments with pancreatic juice in 1836, were really the first to begin the scientific study of the digestion of albumins with pancreatic juice. The former considered bile to be necessary, but Corvisart showed that the juice by itself could dissolve albumins; and that they were converted into substances similar to those of gastric digestion. These results were at first denied, but later they were confirmed by Meissner, Danilewsky, and Kühne; this last observer showed that the action was due to an enzyme to which he gave the name of trypsin. Until Kolbe discovered that disinfectants could prevent the action of those micro-organisms which are always present in the intestine, the products of digestion by trypsin were very much confused and complicated; Kühne, in his experiments, made use of this fact, and was therefore the first to investigate the real products of trypsin digestion.

Kühne discovered that the peptone which is formed is only partially further broken up by trypsin, and he gave the name of amphopeptone to the total quantity, antipeptone to that part not attacked, and hemipeptone to that part which was further broken down. He surmised that the splitting of the albumin molecule took place earlier in the process, and he made attempts to isolate intermediate substances, which he called amphoalbumoses. That part of the albumin not attacked was called antialbumid; very strong trypsin converts it into antipeptone, as was observed by Schützenberger in 1875. It is really, however, converted into antialbumose or deuteroalbumose, and Kühne gave the following diagram to show these results:—

$$\begin{tikzcd}
\text{Albumin} \arrow[d] \arrow[dr] & \\
\text{Antialbumose} \arrow[d] & \text{Hemialbumose} \arrow[d] \\
\text{Antipeptone} & \text{Hemipeptone} \arrow[d] \\
& \text{Amino-acids}
\end{tikzcd}$$

Now, however, it is known that no antialbumose is formed, but only antipeptone.

The characteristic changes produced by trypsin have been more carefully studied with fibrin. As with pepsin, this substance has also the property of taking up the ferment, and a method of obtaining trypsin in a relatively pure condition is thus obtained.

The fibrin first dissolves, but does not swell up as it does in peptic digestion, being rather eroded from the outside. The solution has the property of a globulin, but no globulin is formed from serum-albumin, etc., also no syntonin nor a product corresponding to that formed in peptic digestion. Primary albumoses are not formed, but deuteroalbumose is obtained directly, then amphopeptone, and finally antipeptone and hemipeptone, of which the former constitutes rather more than half of the total quantity of the peptone. The further action of trypsin consists in the conversion of hemipeptone only into amino-acids, and a chromogen, which was first observed by Tiedemann and Gmelin in 1831, and called tryptophane by Neumeister. This chromogen forms a very suitable substance for determining the progress of tryptic digestion.

According to Nencki, tryptophane, called proteinchromogen by Stadelmann, can be brominated, when it gives rise to two substances. These, on removal of the bromine, have similarities on the one hand to hæmatoporphyrin and to bilirubin, and on the other hand to melanin; he therefore considers them the mother substances of the animal colouring matters, even of hæmoglobin.

Hopkins and Cole have quite recently isolated tryptophane, and have given it the formula $C_{11}H_{12}O_2N_2$; it gives the Adamkiewiez reaction, and yields skatole and indole when heated; it is probably indoleaminopropionic acid or skatoleaminoacetic acid.

The chief acids obtained are leucine, tyrosine or p-oxyphenylaminopropionic acid, aspartic acid, and glutamic acid; besides these, Drechsel and Hedin have found lysine or α, ε-diaminocaproic acid, and lysatinine, which is now known to be a mixture of lysine and arginine or δ-guanidine-α-aminovalerianic acid; Kutscher has obtained histidine, $C_6H_9O_2N_3$.

Glycocoll, long known as a decomposition product of gelatine, has been found by Spiro among the decomposition products of albumins.

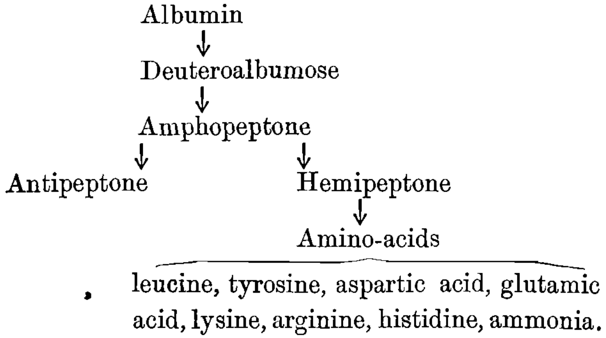
Emerson has obtained p-hydroxyphenylethylamine, which is formed from the tyrosine, which loses carbon dioxide, its amount decreasing as the quantity of this substance increases.

Ammonia, according to Hirschler and Stadelmann, is given off in small quantities when trypsin acts upon albumins. Its amount has been determined by Dziergowski and Salaskin, who obtained it both in peptic and tryptic digestion. For the different albumins its amount varies, but in every case it is greater than that liberated by dilute acid or alkali, and it never corresponds to the total amino-nitrogen of the albumin.

Lea states that the removal of the products has an influence upon the digestion by trypsin, which is more rapid and complete than that of pepsin.

Nencki has stated that he has obtained xanthine, guanine, hypoxanthine, and adenine in the digestion of fibrin by trypsin; but Kossel denies this, saying that they are products of nucleins resulting from the entanglement of leucocytes in the fibrin.

The following diagram shows the formation of the principal products:—



Lately, investigations have been carried out as to the nature of antipeptone. Siegfried and Balke considered it to be a chemical individual, and the former investigator regarded it as identical with his carnic acid, $C_{10}H_{15}O_5N_3$. Kutscher, on the other hand, maintains that antipeptone is not a chemical entity, because he has obtained from it a mixture of substances which can be separated by phosphotungstic acid into an acid part and a basic part; the basic part consists of histidine, arginine, and another unknown base, and from the acid part aspartic acid was obtained. Siegfried states that antipeptone can be obtained pure from impure antipeptone by precipitation with alcohol, and he has since isolated two acids from antipeptone which he designates α-antipeptone, $C_{10}H_{17}O_5N_3$, and β-antipeptone, $C_{11}H_{19}O_5N_3$; they were not equal in amount, and they do not give quite the same products on hydrolysis with stannous chloride and hydrochloric acid; neither of them is identical with his carnic acid; he thus shows that antipeptone is not a mixture of substances, as stated by Kutscher, though his two acids can give those substances on hydrolysis.

The proteids are generally quickly decomposed by trypsin into their components, and the albumin digested like fibrin; *e.g.* casein is almost completely digested, giving tyrosine, casein albumose, and casein antipeptone. Biffi, who investigated this decomposition, found that the phosphorus was partially converted into phosphoric acid, and partially into another compound not precipitated by magnesia mixture.

The nuclein of nucleoproteids is peptonised with the separation of nucleic acids, and then of nuclein bases, which Bokay states resist the action of trypsin, though Kutscher, as above mentioned, found xanthine, hypoxanthine, and guanine.

Of the albuminoids, only collagen and elastin are attacked by trypsin ; the native collagen, however, is only changed when it has been first transformed into gelatine, or gluten, by the action of steam or acids, by the latter of which it is changed in peptic digestion.

Chittenden and Solley state that gelatine is converted into protogelatose and deuterogelatose, and that no gelatine peptone is formed when putrefactive changes are very carefully prevented from occurring. Chittenden and Hart state that no peptone is formed from elastin, but that protoelastose and deuterogelatose are both formed.

From gelatine Nencki states that he has obtained gelatine peptone, leucine, glycocoll, and ammonia ; and from elastin Wälchi has obtained glycocoll, leucine, aminovalerianic acid, and ammonia.

The lecithines are also decomposed by pancreatic juice like fats, and are converted into their constituents, viz. choline, glycerophosphoric acid, and fatty acid. The choline, according to Hasebroek, is converted into carbon dioxide, marsh gas, and ammonia, the same products being also produced by bacteria when no oxygen is allowed to be present.

Kossel and Matthews have investigated the action of pepsin and trypsin on protamines (salmine and sturine), and have found that they are only attacked by trypsin, which converts them firstly into protones, and then partially only into hexone bases (lysine, arginine, and histidine); the existence of an anti-group is said by them to be present on account of this fact, and also an intermediate stage between protones and hexones is probable.

Gulewitsch finds that only p-diacetylamidophenol, among many other less complex chemical compounds, is acted upon by trypsin, with the formation of acetic acid. He cannot confirm Nencki and Blank, who stated that hippuric acid was decomposed, and considers that their trypsin contained the fat-splitting ferment of the pancreas.

Baldwin and Levene find that the diphtheria and tetanus toxines are digested by trypsin, and thus rendered inert; the activity of tuberculine is only destroyed by the prolonged action of trypsin.

According to Cohnheim, the passage of peptone through the intestinal wall depends on its further decomposition into simpler compounds caused by a ferment which he has termed erepsin; it acts rapidly on proteoses and peptones, producing ammonia, leucine, tyrosine, lysine, histidine, arginine; it acts feebly upon histones, and not upon proteids except caseinogen; protamines are, however, acted upon.

Matthews even suggests that the centrosome in the unfertilized eggs of *Arbacia* plays the part of a proteolytic enzyme, as he has observed localized areas of liquefaction in the protoplasm.

CHAPTER XIX.

PROTEOLYSIS BY FERMENTS OTHER THAN PEPSIN AND TRYPSIN.

A PROTEOLYTIC ferment has been discovered by Babcock and Russell in the ripening of cheese, which they have named galactase. It has an action like trypsin, alkaline media being the best, but it is not identical with it, and it is found in the milk of all animals. Albumoses, peptones, amino-acids, and ammonia are formed, these nitrogen compounds being fairly evenly divided. It readily decomposes hydrogen peroxide, and it liquefies gelatine. These results have been confirmed by von Freudenreich.

A phenomenon, first described by Salkowski and termed autodigestion, occurs in the organs after death; it is not due to putrefaction, as it takes place in the presence of chloroform and sodium fluoride. Biondi has confirmed Salkowski in his observations, and shows that the enzyme which produces this action is not trypsin, as considered by Neumeister, in the following way: (1) xanthine-like substances are formed which in tryptic digestion are present in a latent condition; (2) autodigestion is not inhibited by an acid medium; (3) tryptophane is not a product, whereas it occurs in tryptic digestions.

Magnus-Levy has obtained i-lactic acid, d-lactic acid, and succinic acid; also formic, acetic, and butyric acids, hydrogen, carbon dioxide, and hydrogen sulphide from livers which were subjected to autolysis; further, glycocoll was obtained by Jacoby in autodigestion of the liver.

In yeast there is also a proteolytic enzyme, as Hahn found that an appreciable quantity of gelatine was dissolved in twenty-four hours by it, and he thus confirms Will as regards its presence in yeast; in conjunction with Geret he has shown that the albumin in yeast extract is decomposed with the formation of tyrosine and leucine. These investigators have further shown that it can digest added albumin in addition to that in the extract, with the formation of the same products; hypoxanthine is also found, its amount increasing during the digestion. They have termed this enzyme endotrypsin, and it resembles pepsin in requiring an acid medium, and trypsin in the nature of its products. In addition to the above products, Kutscher has isolated histidine, arginine, lysine, aspartic acid, and a compound $C_8H_6O_4N_4$.

A similar ferment has also been found in *Aspergillus niger* and other fungi by Bourquelot and Hérissey; this enzyme of *Aspergillus niger* has been found by Malfitano to act upon gelatine, nucleo-proteids, globulins, and albuminates, but not on egg-albumin. Emmerling and Reiser have investigated the action of *Bacillus fluorescens liquefaciens* on gelatine. Twenty-five per cent. of the nitrogen was eliminated as ammonia; and methylamine, trimethylamine, choline, and betaïne were identified in the solution. Fibrin gave peptone, arginine, leucine and aspartic acid when acted upon by this micro-organism.

Peptonising bacteria are also found in milk which produce an enzyme very similar to trypsin; in the fermentation of casein aromatic acids are formed as well as peptone, leucine, and tyrosine; and cane sugar can be inverted, but not lactose (Kalischer).

Proteolytic enzymes are also probably the cause of bacteriolysis, the term used for their autodigestion.

The vegetable ferment bromelin was discovered by Marcano in 1891, and the products of its action have been examined by Chittenden and his pupils. Except for a small residue, fibrin is completely dissolved, and is converted into the various albumoses and peptones, and finally into amino-acids. The small residue is called anti-albumid. The other albumins behave similarly.

Papain, the proteolytic enzyme of the papaw tree, was first investigated by Wurtz in 1879, who said it converted albumins into peptones and leucine. Then, in 1883 and 1884, Martin made a complete examination, and found a milk-curdling ferment as well as a proteolytic one in the juice of this tree. The products of the digestion of fibrin were a globulin-like body, peptone, leucine, and tyrosine, more of the former than of the latter being formed.

Vegetable proteids are converted in a similar way, a small anti-albumid residue being left. Halliburton states that no peptone is formed from vegetable albumins by this enzyme, the process stopping short at the proteoses.

Harlay considers it intermediate in power between pepsin and trypsin, and he has obtained the same chromogen by its use as he obtained with pepsin.

Chittenden, who made very complete observations, states that the nitrogen in the albumoses produced by vegetable ferments is less than in those produced by trypsin.

Emmerling has quite recently published the results he has obtained by the digestion of fibrin with papain; the fibrin dissolved giving peptone, which, by prolonged action, was converted into tyrosine, arginine, glycocoll, alanine, leucine, aspartic acid, and phenylalanine. Mendel and Underhill find that papain can act in both alkaline and slightly acid media, giving products corresponding closely to those obtained with pepsin; leucine, tyrosine, and tryptophane do not occur if bacteria are excluded; bromelin, however, readily forms leucine, tyrosine, and tryptophane, even in acid media.

A proteolytic ferment is also contained in the germinating seeds of the Lupin and other plants; it acts upon conglutin, giving leucine and tyrosine, but not asparagine, which is not regarded by Schulze as a primary product of the decomposition of albumin; Bokorny has observed the same decomposition by malt enzymes.

A theory to account for the action of these enzymes, especially papain, was published by Wurtz in 1881; he has compared the process with that which takes place in the formation of ether from alcohol and sulphuric acid. In this process, ethylsulphuric acid is first formed; this is then acted upon by alcohol, giving ether and sulphuric acid; a small quantity of sulphuric acid can thus transform large quantities of alcohol into ether. The enzyme is supposed to have the property of combining with the albumin, forming a compound which is decomposed by a further quantity of albumin, thus:—

$$C_2H_5OH + H_2SO_4 = C_2H_5.HSO_4 + H_2O$$
$$C_2H_5.HSO_4 + C_2H_5OH = (C_2H_5)_2O + H_2SO_4$$

This theory evidently arose from the fact that pepsin, trypsin, etc., can be taken up by fibrin as before mentioned.

CHAPTER XX.

THE CHANGES AND PRODUCTS OCCURRING AS THE RESULT OF PUTREFACTION.

THE last of the changes resulting from fermentation is known as putrefaction; here several different changes are without doubt taking place, but only in a very few instances have they been definitely determined.

Putrefaction is a process which has been known from early times to take place in organic substances when they are exposed to the air, and it was considered by the earliest observers to be a kind of fermentation, similar to the production of alcohol from sugar solutions. It was Pasteur, again, who showed that micro-organisms were the cause of these changes, and he found that air was not necessary for the majority of the organisms producing putrefaction, which are now known to be anærobic; it is now thought to be very probable that enzymes are secreted by those organisms which produce many of these changes.

Analogous processes also take place in the intestines of animals both normally and abnormally, and the products resulting are very similar to those occurring in putrefaction; it is, therefore, convenient to describe the changes occurring in the intestines and in the air together.

The first stages in the decomposition of albumins consist in their solution and conversion into peptone; this, according to Salkowski, is an enzyme action; but Kühne has denied that this enzyme is trypsin. The quantity of albumoses and peptones obtained is always small, as they are immediately further broken down. In this connection Neumeister has made some very interesting experiments; he finds that native albumins are not attacked until peptone is no longer present, or until it is completely removed from the mass of decomposing matter; but gelatine is an exception, as he has found peptone to be formed from it under the same conditions of putrefaction, the quantity increasing instead of decreasing as in the former cases. In the intestine it is found that bacteria are not necessary for digestion, but *also that they do no harm* (Nuttall and Thierfelder).

In tryptic digestion, the aromatic radicles in the albumin molecule yield chiefly tyrosine, but in putrefaction various aromatic bodies are formed. These are produced partly by the action of bacteria on the tyrosine, and partly by their action on the albumin directly.

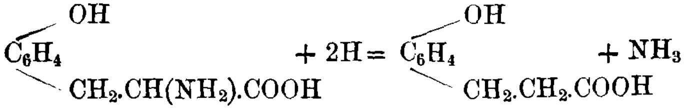
The chief aromatic compounds obtained by putrefaction are :—

(*a*) Tyrosine and its derivatives—

1. Hydroparacumaric acid, or p-oxyphenylpropionic acid.
2. p-oxyphenylacetic acid.
3. Phenylpropionic acid.
4. Phenylacetic acid.
5. p-cresol.
6. Phenol.

According to Baumann, these are produced from tyrosine in the following way:—

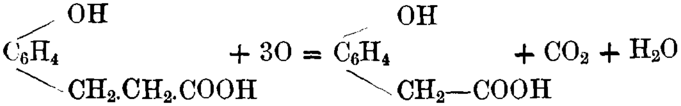
1. Reduction of the tyrosine with nascent hydrogen—



$$\mathrm{C_6H_4}{<}\begin{matrix}\mathrm{OH}\\\mathrm{CH_2.CH(NH_2).COOH}\end{matrix} + 2\mathrm{H} = \mathrm{C_6H_4}{<}\begin{matrix}\mathrm{OH}\\\mathrm{CH_2.CH_2.COOH}\end{matrix} + \mathrm{NH_3}$$

Tyrosine. Hydroparacumaric acid.

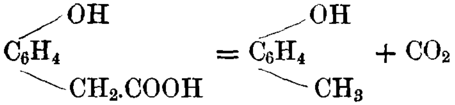
2. Oxidation of the hydroparacumaric acid—



$$\mathrm{C_6H_4}{<}\begin{matrix}\mathrm{OH}\\\mathrm{CH_2.CH_2.COOH}\end{matrix} + 3\mathrm{O} = \mathrm{C_6H_4}{<}\begin{matrix}\mathrm{OH}\\\mathrm{CH_2{-}COOH}\end{matrix} + \mathrm{CO_2} + \mathrm{H_2O}$$

p-oxyphenylacetic acid.

3. Splitting off of carbon dioxide—



$$\mathrm{C_6H_4}{<}\begin{matrix}\mathrm{OH}\\\mathrm{CH_2.COOH}\end{matrix} = \mathrm{C_6H_4}{<}\begin{matrix}\mathrm{OH}\\\mathrm{CH_3}\end{matrix} + \mathrm{CO_2}$$

p-cresol.

4. Oxidation of the p-cresol—

$$\mathrm{C_6H_4(OH)CH_3} + 3\mathrm{O} = \mathrm{C_6H_5.OH} + \mathrm{CO_2} + \mathrm{H_2O}$$

Phenol.

(*b*) Those produced from albumin directly—

1. Indole (Nencki and Kühne).
2. Skatole.
3. Skatole-carboxylic acid (Salkowski).
4. Skatole-acetic acid (Nencki, Salkowski).

These products, according to Baumann, are not primary products of bacterial action on albumin, but they are formed in the decomposition of an intermediate substance which is not known.

From these products it is seen that there must be several aromatic nuclei in the albumin molecule, the majority of which are split off as tyrosine by fermentation.

From the fatty series no aromatic body as yet has been found as a product of bacterial action, although this would, according to Neumeister, be quite possible, since it is not known why bacteria give indole and skatole, and other decomposing agents only tyrosine, and *Proteus vulgaris* practically only indole. But the selective action of these organisms, on the other hand, as has been shown in several other cases, may be sufficient to account for these results.

In the body these various products combine chiefly with sulphuric acid, and are excreted as sulphuric acid esters; hippuric acid is formed from phenylpropionic acid and glycocoll, the phenylpropionic acid first undergoing oxidation; phenaceturic acid $C_6H_5—CH_2CO—NH—CH_2COOH$ is formed from phenylacetic acid and glycocoll.

The aliphatic products of intestinal putrefaction are leucine, aspartic, and glutamic acids, also θ-aminovalerianic acid (Salkowski); the ammonium salts of caproic, valerianic, and butyric acids; marsh gas and hydrogen; in addition to these succinic and glutaric acids, probably from the aspartic and glutamic acids. From the sulphur in the albumin molecule, sulphuretted hydrogen and methylmercaptan are formed.

Elastin and gelatine give the same products, but in addition glycocoll.

These changes are very probably hydrolytic in character.

Besides these products, outside the organism certain basic substances are formed, which in their reactions closely resemble the vegetable alkaloids; they belong chiefly to the fatty series, whereas the true plant alkaloids are pyridine derivatives. In the living body these bases are seldom formed, oxygen being necessary for their formation. Dupré and Bence Jones in 1866, and Selmi in 1873, who suggested the name of Ptomaine for the whole group, first hinted at their existence, but they were unable to isolate them.

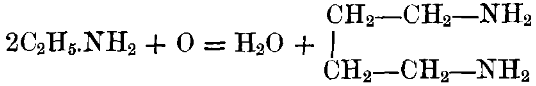
The recognition of these substances has been chiefly studied by Brieger, who has isolated the following bases:—

1. Monamines: methylamine, ethylamine, dimethylamine, diethylamine, trimethylamine, triethylamine.

2. Diamines: putrescine, cadaverine.

A diamine of the constitution $C_6H_{16}N_2$, which is probably hexamethylenediamine, was obtained by Garcia in 1893 from putrefying horseflesh; also saprine, $C_5H_{16}N_2$, and neuridine, $C_5H_{14}N_2$ (Ehrenberg).

According to Baumann and Udransky, the diamines are produced synthetically from monamines, thus:—



$$2C_2H_5.NH_2 + O = H_2O + \begin{array}{l} CH_2—CH_2—NH_2 \\ | \\ CH_2—CH_2—NH_2 \end{array}$$

Collidine, $C_8H_{11}N$, has been obtained by Nencki from gelatine; and hydrocollidine, $C_8H_{15}N$, and parvoline, $C_9H_{13}N$, are generally produced from horse and cattle flesh which are undergoing putrefaction.

Choline also is found, which most probably originates from lecithine, and from which, perhaps, dimethyl-, trimethyl-, and triethylamine are formed.

Mydatoxine and mydine have been obtained by Brieger from putrefying human corpses.

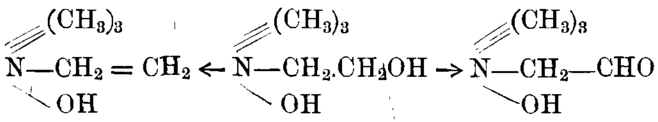
All these substances are not poisonous, or only poisonous in large doses, and for the following bases, which are very poisonous, Brieger suggested the name Toxine.

1. Peptotoxine, found by Brieger in many peptones.

2. Neurine, $C_5H_{14}NO$, found in putrefying flesh.

3. Muscarine, $C_5H_{13}NO_2$, found by Brieger in putrefying fish; the poison of the fly agaric.

These two bodies very probably originate from choline, thus:—



$$\underset{OH}{\overset{(CH_3)_3}{N}}-CH_2 = CH_2 \leftarrow \underset{OH}{\overset{(CH_3)_3}{N}}-CH_2.CH_2OH \rightarrow \underset{OH}{\overset{(CH_3)_3}{N}}-CH_2-CHO$$

Neurine. Choline. Muscarine.

4. A base, $C_2H_4(NH_2)_2$, isomeric with ethylenediamine, found in putrefying fish.

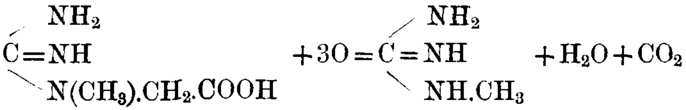
5. Mydaleine, from human corpses (Brieger).

6. Mytilotoxine, $C_6H_{15}NO_2$, obtained by Brieger from edible mussels.

7. Tyrotoxicone, found in milk and cheese by Vaughan.

8. Tyrotoxine, found in cheese by Dokkum.

9. Methylguanidine, obtained by Brieger from putrefying horseflesh; it is probably produced by oxidation from the creatine of muscles, thus:—



$$C\begin{cases} NH_2 \\ =NH \\ N(CH_3).CH_2.COOH \end{cases} + 3O = C\begin{cases} NH_2 \\ =NH \\ NH.CH_3 \end{cases} + H_2O + CO_2$$

The creatinine group of compounds (xanthocreatinine, $C_5H_{10}N_4O$, crusocreatinine, $C_5H_8N_4O$, and amphocreatinine,

$C_9H_{19}N_7O_4$) and the uric acid group of compounds (adenine, xanthine, hypoxanthine) were called Leucomaines by Gautier in 1888; they are poisons produced from lecithine, or proteids, by the metabolic activity of the body.

Brieger has also investigated the poisonous substances produced in certain diseases; in cystinuria, cystine is formed in the organism; and in cholera, typhoid, tetanus, etc., several poisonous substances are formed. Using pure cultures of the micro-organisms of these latter diseases, Brieger isolated from cholera, putrescine, cadaverine, methylguanidine, indole, and nitrites; from typhoid, typhotoxine, $C_7H_{17}NO_2$, and the non-poisonous neuridine; and from tetanus, tetanine, $C_{13}H_{22}N_2O_4$, putrescine, tetanotoxine, spasmotoxine, and an unknown base.

Oméliansky has found that their toxines can be produced from amino-acids by the bacteria of cholera and diphtheria.

The real poisons in these diseases, however, are bodies of albumose properties, the constitution of which is not yet known; similar bodies constitute the deadly poisons of snakes, fishes, spiders, insects, plants, from which, *e.g.*, abrine, ricine, lupinotoxine have been obtained.

THE principal deductions from the mass of work on the chemical changes occurring in fermentation, which has been summarised above are as follows:—

1. That the active agent in all cases is a living organism.

2. That this organism sets up either by its own metabolism, or by bodies secreted by, or excreted from it, changes in substances of different constitution, *e.g.*, carbohydrates and albumins.

3. That these changes are principally hydrolytic, and that some are oxidative, and a few reductive.

4. That the results of these changes are generally simplifications of the original body.

5. In certain cases synthesis occurs.

LONDON, *October*, 1902.

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