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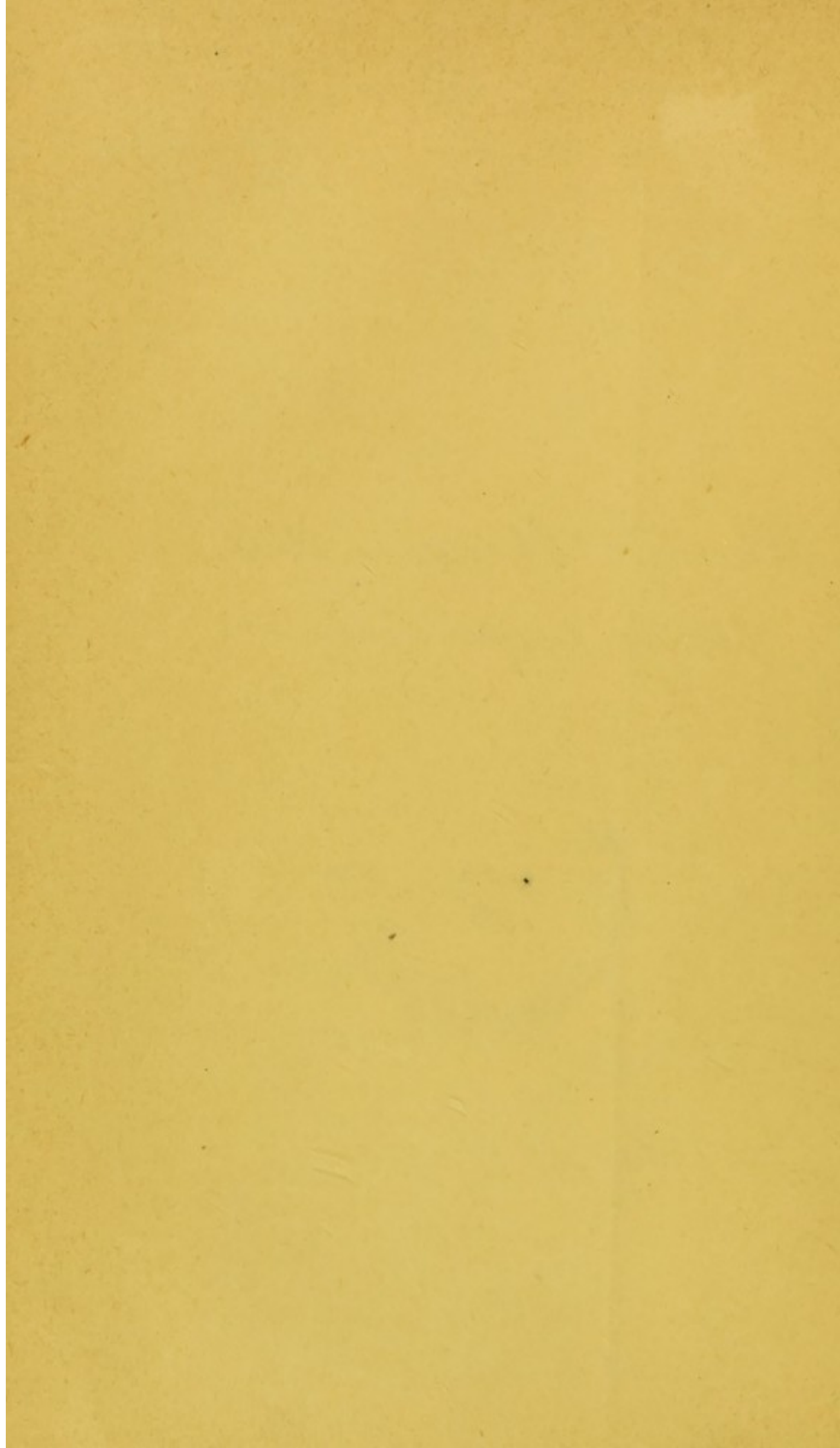Royal College of Surgeons

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Chemical Changes in their

Relation to Micro-organisms

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ON CHEMICAL CHANGES IN THEIR RELATION TO MICRO-ORGANISMS.

E. Frankland, D.C.L., M.D., LL.D., F.R.S.

[A Lecture delivered before the Chemical Society on Thursday, February 5.]

In addition to analytical and synthetical changes in chemical composition depending upon heat, light, and electricity, there are two well recognised kinds of chemical action, viz.:—

1. Chemical change effected in each of two or more substances brought into contact with each other; and,

2. Chemical change effected by contact with a substance which itself suffers apparently no alteration. Failing any satisfactory explanation, very heterogeneous changes of the latter kind have been grouped together under the designation of "catalytic actions," but a careful study of many of the reactions of this second class has transferred them to the first, and it is more than probable that the remainder, when better known, will be similarly disposed of. The chemical changes occurring in animal and vegetable organisms were, until recently, tacitly, if not formally, relegated to the second type. The plant or animal was regarded either as effecting the changes by mere contact, or by some mysterious process outside the ken of experimental inquiry. This illusion has been finally dispelled by the synthetical operations of organic chemistry, which have taught us how to produce, by purely laboratory processes, numerous compounds formerly obtainable only as the products of living organisms, and it is to be hoped that chemists and biologists will now give more attention to this hitherto neglected field of chemical action—the chemical changes which occur in animal and vegetable organisms.

In studying the present aspect of this subject, I have found it neither desirable nor possible to draw any sharp line of demarcation between living beings of the highest type and the micro-organisms to which my remarks must be chiefly directed; in the first place, because I do not find any essential difference between the chemical changes which occur in man and the mammalia, for instance, and

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those which accompany the lowest forms of life; and, secondly, because it is more than probable that many of those changes with which we are familiar in the bodies of the higher animals (and possibly in plants) are also brought about by micro-organisms.

The chemical actions induced by living organisms are of two kinds :—

1. Synthetical; and,

2. Analytical.

The first are chiefly performed by plants; the second by animals.

Of the synthetical operations the transformations of CO_2, OH_2, and NH_3 into cellulose, starch, sugar, gum, and albumen are the most conspicuous, although Hoppe-Seyler has observed synthetical changes under the influence of certain micro-organisms, as, for instance, the transformation of glycerin into hexyl alcohol and caproic acid after two years' putrefaction, 6-carbon molecules being thus produced from 3-carbon molecules. He considers that glycerin is first converted into lactic acid, and the synthesis is then performed by the combination of lactic acid residues. In his opinion, animal fats are thus produced from carbohydrates. All the first named synthetical operations must be attended by the absorption of heat; or, in other words, by the transformation of actual into potential energy.

What is the nature of the substance in the plant which decomposes CO_2 and OH_2, liberating C under the influence of solar or artificial light? Scarcely any approach has yet been made towards the answer to this question, and yet it cannot be doubted that a satisfactory reply will sooner or later be disclosed. Has the vegetable kingdom the monopoly of the substance which effects this transformation? This is scarcely probable, for although the secret has eluded chemical research half a century beyond the time when Wöhler and Kolbe found out the artificial synthesis of organic compounds, yet it cannot be doubted that it belongs to the "knowable," and will be discovered. As a step towards the solution of this problem, it was interesting to ascertain whether, under the influence of a powerful beam of light, the constituent atoms of CO_2 and OH_2 molecules become to any extent dissociated as they do under the influence of a high temperature. In other words, can the luminous impulses compel these atoms to make excursions beyond the molecular boundary of their mutual attractions? To put this to the proof, I exposed CO_2, both alone and mixed with aqueous vapour, in an extreme state of attenuation to the intense light of a powerful electric arc, but failed to detect the slightest decomposition. I then similarly exposed a mixture of carbonic anhydride and nitric oxide, but even this searching test for wandering atoms of oxygen failed to detect any. Although light has not yet been made to produce these results, it is well known that they

can be effected by electricity, and that not merely by the disruptive discharge or by electrolysis, but by the silent discharge. It is now more than a quarter of a century since I saw, in Gassiot's laboratory, the vacuum of a water-hammer impaired by silent discharges induced from without; this result having been brought about by the decomposition of the aqueous vapour inside the glass tube. Brodie has since demonstrated that CO_2 is decomposed into CO and O by the same means.
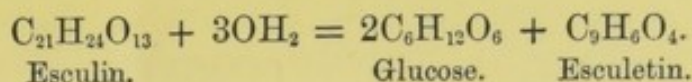
The *modus operandi* of plants in effecting chemical changes being at present entirely unknown, we cannot with profit pursue the discussion of the vegetable division of organic life. I therefore propose to confine our attention to the chemical operations of animal life. Here, however, I am met by the difficulty of distinguishing between plants and animals amongst micro-organisms. Biologists have not yet arrived at a final conclusion as to the definitions which shall completely separate the animal from the vegetable kingdom. For our present purpose the following definitions will be sufficient :—

1. A plant is an organism performing synthetical functions, or one in which these functions are greatly predominant.

2. An animal is an organism performing analytical functions, or one in which these functions greatly predominate.
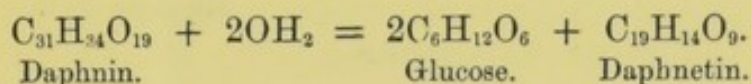
From a *physical* point of view the distinctive characteristics of plants and animals may be thus formulated—a plant is an organism which transforms actual into potential energy, whilst an animal is one which transforms potential into actual energy.

So far as I have been able to make out, all micro-organisms belong to the second class. Their life depends essentially upon the taking asunder of more or less complex compounds, resolving them into simpler molecules at the expense of potential energy. The new and simpler molecules, therefore, contain less potential energy than those from which they are derived. As micro-organisms are commonly termed ferments, and their analytical operations fermentation, it is necessary here to distinguish, sharply, between these organised ferments and certain other bodies which bring about analogous chemical changes, but which are not only unorganised, but invariably exist in solution. These latter, or "soluble ferments," as they are commonly termed, are said to act by contact; they produce certain chemical changes in organic compounds (the fermentescible substances) without themselves furnishing from their own substance any of the products of the reaction. In this way a comparatively minute quantity of the ferment can effect the transformation of a very large quantity of the fermentescible substance. As we proceed, it will be seen that these soluble ferments are essentially analytical, and that their effects are closely allied to those of the organised ferments, differing only or

chiefly from them in the absence of organised matter, and in the non-increase of the ferment during its action upon the fermentescible substance. I will, therefore, here briefly indicate those fermentations which are the results of so-called contact action, and not of micro-organisms :—

1. Transformation of starch into glucose and dextrin, or hydrated maltose by diastase—

$$4C_6H_{10}O_5 + 3OH_2 = C_6H_{10}O_5 + 3C_6H_{12}O_6.$$
Starch.   Dextrin.   Glucose.

Hoppe-Seyler gives this equation. The identity of this glucose with grape-sugar has been called in question by Nasse.

2. Transformation of cane sugar into glucose (dextrose) and levulose—

$$C_{12}H_{22}O_{11} + OH_2 = C_6H_{12}O_6 + C_6H_{12}O_6.$$
Cane-sugar.   Glucose.   Levulose.

Berthelot has shown that this change is effected by a soluble ferment accompanying yeast, and also found in the bodies of bees and in bee-bread. It has been named " Invertin."

3. The transformation of glucosides :—

(a.) Amygdalin into glucose, benzoic aldehyde, and hydrocyanic acid—

$$C_{20}H_{27}NO_{11} + 2OH_2 = 2C_6H_{12}O_6 + C_7H_6O + CNH.$$
Amygdalin.   Glucose.   Benzoic   Hydrocyanic
                                   aldehyde.   acid.

(b.) Salicin into glucose and saligenin—

$$C_{13}H_{18}O_7 + OH_2 = C_6H_{12}O_6 + C_7H_8O_2.$$
Salicin.   Glucose.   Saligenin.

(c.) Arbutin into glucose and hydroquinone—

$$C_{12}H_{16}O_7 + OH_2 = C_6H_{12}O_6 + C_6H_6O_2.$$

(d.) Helicin (product of the oxidation of salicin) into glucose and salicylic hydride (product of the oxidation of saligenin)—

$$C_{13}H_{16}O_7 + OH_2 = C_6H_{12}O_6 + C_7H_6O_2.$$
Helicin.   Glucose.   Salicylic
                                   hydride.

(e.) Phloridzin (in bark of apple and pear trees) splits into glucose and phloretin—

$$C_{21}H_{24}O_{10} + OH_2 = C_6H_{12}O_6 + C_{15}H_{14}O_5.$$
Phloridzin.   Glucose.   Phloretin.

(*f.*) Esculin (in horse-chestnut bark) into glucose and esculetin—

$$C_{21}H_{24}O_{13} + 3OH_2 = 2C_6H_{12}O_6 + C_9H_6O_4.$$

Esculin.   Glucose.   Esculetin.

(*g.*) Daphnin (in bark of the Mazereon) gives glucose and daphnetin—

$$C_{31}H_{34}O_{19} + 2OH_2 = 2C_6H_{12}O_6 + C_{19}H_{14}O_9.$$

Daphnin.   Glucose.   Daphnetin.

All these reactions are produced by emulsin or synaptase, and many of them by yeast and other soluble ferments (salicin, helicin, and daphnin). Except in the case of amygdalin, acids produce the same results.

Of the same nature, appear to be the so-called fermentations producing (*a*) essential oil of mustard $\left\{\begin{array}{l} C_3H_5 \\ S \\ CN \end{array}\right.$, (*b*) gallic acid and glucose from tannin, (*c*) alizarin and glucose from rubian, (*d*) margaric acid and glycerin in the so-called pancreatic fermentation, (*e*) and the proteïc fermentation by which proteïn bodies are dissolved in the stomach.

All these phenomena appear to be due to soluble, and consequently unorganised organic matters.

The equations show that they are all essentially of the same character. They all consist in the assimilation of water, and with one exception (which is, however, only apparent), the splitting up of the fementescible substance into two or more new molecules, one of which is a saccharine body.
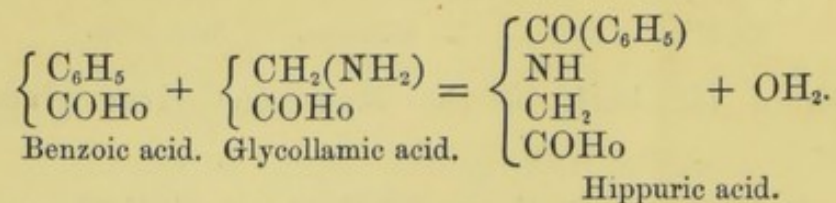
According to Müntz, these unorganised ferments can be sharply distinguished from organised by chloroform, which kills the latter, but has no effect on the former. Hamlet refutes this. P. Bert declares that compressed air in like manner distinguishes between the two.

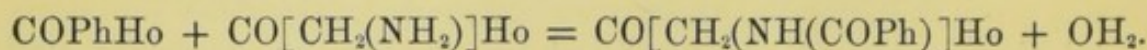### *Chemical Changes due to Micro-organisms.*

Having thus cleared the ground by the dismissal of a class of ferments which are soluble, and therefore not organised or living, we are now prepared to enter upon the chemical changes which are effected by living organisms.

In the higher animals, these transformations have not been studied in the systematic way that they deserve, nevertheless, some general and far reaching results have been established. Thus we know that the food which these animals consume is oxidised, and practically converted into carbonic anhydride, water, urea, hippuric acid, and ammonic

urate, and that benzoic and kinic acids are converted into hippuric acid :—

$$\left\{\begin{matrix} C_6H_5 \\ COHo \end{matrix}\right. + \left\{\begin{matrix} CH_2(NH_2) \\ COHo \end{matrix}\right. = \left\{\begin{matrix} CO(C_6H_5) \\ NH \\ CH_2 \\ COHo \end{matrix}\right. + OH_2.$$

Benzoic acid.   Glycollamic acid.   Hippuric acid.

or

$$COPhHo + CO[CH_2(NH_2)]Ho = CO[CH_2(NH(COPh)]Ho + OH_2.$$

With the exception of the last two, these changes are essentially processes of oxidation. In that portion of the animal world with which we are best acquainted, oxidation is the essential condition of life : it is the kind of action by which the animal changes *potential* into *actual* energy, and this actual energy is manifested in the phenomena which we term life. There are, however, many other chemical transformations in which potential becomes actual energy, and which therefore can support life. Besides such changes as are known to be thus utilised by micro-organisms, there are many others which might possibly be so utilised. It is even conceivable that animal life could be supported by allotropic or isomeric changes, analogous to the transformation of amorphous into waxy phosphorus, or of amorphous into crystalline antimony.

The changes effected by micro-organisms are essentially of the same character as those brought about by the higher orders of animals; that is to say, they are all changes by which potential becomes actual energy (as illustrated by the calorimetric determinations of Von Rechenberg), and these organisms appear to me, therefore, to be excluded from the vegetable kingdom, which is characterised by operations of the reverse order, attended with the transformation of actual into potential energy.

With one or two exceptions, the chemical changes effected by micro-organisms (unlike those produced by soluble ferments) cannot be brought about by other means. Thus the *direct* transformation of glucose into alcohol and carbonic anhydride, or into lactic acid, butyric acid, or gum, has not been accomplished except by vital operations, although some of these changes can be artificially effected by roundabout processes. Thus glucose can be resolved into its elements from which alcohol, lactic acid, and butyric acid can be constructed.

The conditions favourable and inimical to the propagation and development of micro-organisms have, of late years, received a good deal of attention. It has been established by Pasteur, that certain of these organisms, which he calls *aërobes*, can live only in the presence

of oxygen ; whilst others, *anäerobes*, continue their development only in the absence of this element, although their germs retain their vitality in aërated liquids and develop into active life, so soon as they come into an environment free from oxygen.   The late Frank Hatton stated that, of the micro-organisms spontaneously developing during the putrescence of mutton broth, many survived 10 to 20 days under atmospheres of the following gases,—air, oxygen, nitrogen, hydrogen, carbonic oxide, carbonic anhydride, cyanogen, sulphurous anhydride, nitrous oxide, sulphuretted hydrogen, nitric oxide, acetylene, and coal-gas.

Some of these gases were utilised by the bacteria, whilst others were entirely unchanged.   From air, the whole of the oxygen was absorbed and partially replaced by carbonic anhydride.   Pure oxygen was slowly absorbed, and in 15 days more than one-fifth of it was converted into carbonic anhydride.   Carbonic oxide was wholly absorbed, and partially converted into carbonic anhydride with evolution of hydrogen and marsh-gas.   Cyanogen was to a considerable extent transformed into carbonic anhydride and nitrogen.   Nitric oxide was partially absorbed and replaced by nitrogen and carbonic anhydride ; but the bacteria did not attack this gas vigorously, for, at the end of 10 days, the greater part of it remained unchanged. Hydrogen, sulphurous anhydride, nitrogen, nitrous oxide, carbonic anhydride, acetylene, and coal-gas were unchanged.

Urea in aqueous solution, mixed with potassic phosphate, was decomposed with evolution of pure nitrogen.   Solutions of salicylic acid, strychnine, brucine, morphine, and narcotine exerted no deleterious action upon these bacteria.   On the other hand, they were rapidly destroyed by the following substances :—phenol, spongy iron, alcohol, and potassic permanganate.   Kosegarten mentions thymol hydrocinnamic and phenylacetic acids, indole and scatole, as more or less inimical to the development, if not to the life, of bacteria. Most micro-organisms appear to retain their vitality under very wide ranges of temperature.   It has not yet been shown that any degree of cold, however intense, is fatal.   Animation may be suspended, but it is restored when the temperature rises.   With regard to heat, the lowest fatal temperature recorded is 40° C., but many species can withstand much higher temperatures, and their germs retain their vitality after boiling for many hours, or even after exposure to a moist heat of 143° C. (Dallinger).   They are, however, rapidly destroyed at a boiling temperature, if they have been previously softened by long maceration in warm water.

All these results lead to the conclusion that developed micro-organisms are very tenacious of life, whilst the vitality of their germs is even still more difficult to destroy.   It is very desirable, however,

that researches of this kind should be extended in other directions. The unexpected fatal effects of spongy iron would seem to indicate that there are substances destructive to bacterial life but which have no toxic effect upon more highly organised animals. It has been suggested by Dr. James Johnson, that aromatic substances of various kinds may possess these properties, and hence their reputation as prophylactics, and their use in embalming, and in the preservation of animal and vegetable substances. It is needless to add how exceedingly valuable the discovery would be of substances virulent to micro-organisms and harmless to man.

I will now pass in brief review some of the best known micro-organisms and the chemical reactions due to them.

Yeast and its Chemical Reactions.

Yeast (*Saccharomyces cerevisiæ* or *Torula cerevisiæ*) consists of slightly egg-shaped cells filled with granules. Sometimes these cells are separate and sometimes strung together in chains of six or twelve,
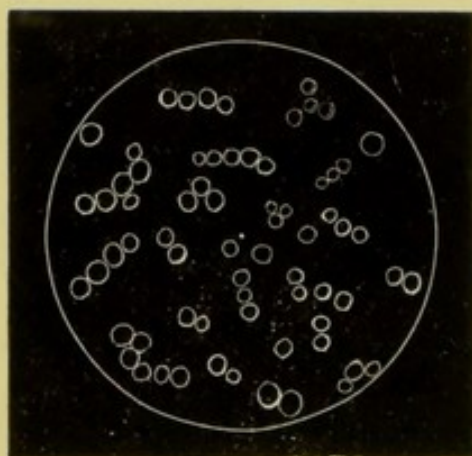
Saccharomyces cerevisiæ.
(Active cells.)
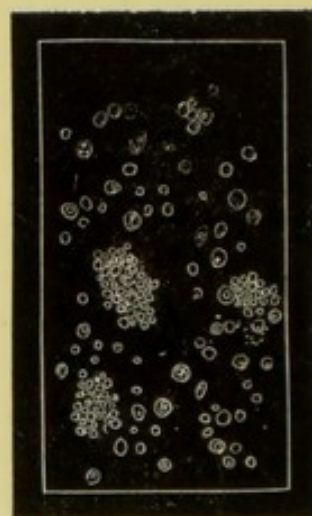
S. cerevisiæ.
(Exhausted cells.)

They are 0·009 mm. in their longest diameter and 0·008 in their shortest. They are "sprosspilze," that is, they multiply by budding, but sometimes by fructification or spores. The spores or cells are widely distributed in the air. They can propagate in water containing sugar, ammonia, and the necessary mineral constituents. Yeast is believed to obtain its carbon from sugar, and is probably incapable of decomposing carbonic anhydride. It contains no chlorophyll. Phosphorus is absolutely necessary for its development, and so is, probably, potassium. Deprived of free oxygen, yeast becomes inactive, but does not die for a long time. With excess of oxygen, it may vegetate without fermentation.

Yeast can withstand a temperature of nearly 100° C. when quite dry, but perishes at 53° when moist. It is comparatively insensitive to poisons, such as hydrocyanic acid, strychnine, antimony, phenol, and arsenious acid, but is destroyed by corrosive sublimate and sulphurous anhydride.

The following is the ultimate composition of yeast, after abstraction of ash :—

	Mitscherlich. Surface yeast.	Schlossberger.	
		Surface.	Bottom yeast.
C	47·0	49·9	48·0
H	6·6	6·6	6·5
N	10·0	12·1	9·8
S	0·6	—	—
O	35·8	31·4	35·8
	100·0	100·0	100·0
N : C =	1 : 4·7	1 : 4·1	1 : 4·9

Yeast is therefore a highly nitrogenous organism, containing a proportion of nitrogen approaching that present in proteïn, which contains $N : C = 1 : 3·8$.

The percentage of ash varies from 3·5 to 7·7, the chief constituents being potassic and magnesic phosphates.

Chemical Changes produced by Yeast.

1. *In Saccharine Bodies.*—The bodies of this class, which are *directly* subject to its action, are dextrose or glucose, levulose, maltose, and lactose or galactose.

Those which are only *indirectly* affected, that is, after transformation into glucose, are cane-sugar, melitose, trehalose, melezitose, starch, dextrin, gum, and glycogen.

The dominant, if not the sole, products of the action of yeast upon saccharine bodies are alcohol and carbonic anhydride—

$$C_6H_{12}O_6 = 2CO_2 + 2EtHo.$$

If subject to this transformation alone, 100 parts of glucose ought to give—

CO_2	48·89
EtHo	51·11
	100·00

Pasteur found that the weight of these products was only—

$$CO_2 \dots\dots\dots\dots\dots \quad 46\cdot40$$
$$EtHo \dots\dots\dots\dots\dots \quad 48\cdot51$$
$$\overline{\qquad\qquad}$$
$$94\cdot91$$

The missing 5·09 parts are resolved into one or more of the following compounds :—

Propyl alcohol.	Glycerin.
Butyl ,,	Succinic acid.
Amyl ,,	Acetic acid.
	Butyric acid.

As part of the missing 5·09 parts, the following have been accounted for :—

$$\text{Succinic acid and attendant } CO_2 \dots\dots \quad 1\cdot13$$
$$\text{Glycerin} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad 3\cdot00$$
$$\overline{\qquad\qquad}$$
$$4\cdot13$$

leaving only 0·96 for the remaining products which have been qualitatively recognised, and for sugar forming new yeast cells.

According to Monoyer, the production of succinic acid and glycerin is explained by the following equation :—

$$4C_6H_{12}O_6 + 3OH_2 = \begin{cases} COHo \\ C_2H_4 \\ COHo \end{cases} + 6 \begin{cases} CH_2Ho \\ CHHo \\ CH_2Ho \end{cases} + 2CO_2 + O.$$

Glucose.   Succinic acid.   Glycerin.
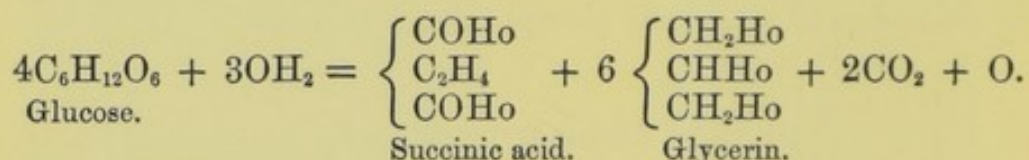
No free oxygen has been detected; it may possibly acetify a minute portion of the alcohol.

The proportion of these bye-products is increased when fermentation is slow, and Horace Brown finds that fermentation is retarded under diminished pressure, and the proportion of carbonic anhydride to ethyl alcohol much increased.
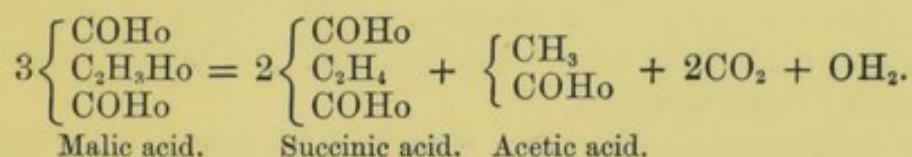
The question whether these bye-products are the work of the *Saccharomyces cerevisiœ*, or each of a special organism, is highly important to the brewer, the wine grower, and the distiller. Perhaps also to the physician; for, closely connected with it, is another question, whether uric acid and other compounds, which may be considered as the bye-products collateral with urea, are generated by distinct species of micro-organisms in the blood.

Sugar of milk or lactose is not directly fermentescible by yeast, that is to say, it is not a food for yeast; but when submitted to the action of acids it is converted into two isomers, called *galactose*, both of which are fermentescible and behave like glucose.

By another micro-organism, which has not been further investigated, sugar of milk is directly converted into alcohol. This micro-organism is developed when milk is allowed to stand in wooden vessels, and the product is the koumis of Tartary.
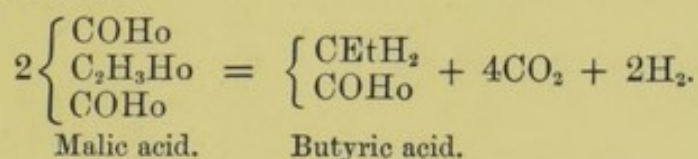
One part by weight of dry yeast transforms 100 parts of grape-sugar into alcohol and carbonic anhydride in from 6 to 10 days, or say, 10 times its weight in 24 hours. One part moist yeast transforms 2·6 parts of grape-sugar in 6 days, or about two-fifths of its weight in 24 hours.

2. *In Malic Acid.*—*Saccharomyces cerevisiæ* breaks up malic acid into succinic acid, acetic acid, carbonic anhydride, and water, according to the following equation :—

$$3\begin{cases} COHo \\ C_2H_3Ho \\ COHo \end{cases} = 2\begin{cases} COHo \\ C_2H_4 \\ COHo \end{cases} + \begin{cases} CH_3 \\ COHo \end{cases} + 2CO_2 + OH_2.$$

Malic acid.   Succinic acid.   Acetic acid.

The calcium salt is generally employed, and the most favourable temperature lies between 15° and 30° C.

Sometimes little or no succinic acid is formed, butyric acid taking its place. It has not been shown that this is not due to the prevalence of *Bacillus subtilis* :—

$$2\begin{cases} COHo \\ C_2H_3Ho \\ COHo \end{cases} = \begin{cases} CEtH_2 \\ COHo \end{cases} + 4CO_2 + 2H_2.$$

Malic acid.   Butyric acid.

Theoretically 3 lbs. of calcic malate ought to give nearly $1\frac{1}{4}$ lb. of succinic acid. Liebig actually obtained 1 lb. The transformation is therefore tolerably clean and sharp.

3. *In Glycerin.*—Retenbacher found that fermentation with yeast produced propionic and acetic acids; but this has since been denied by Roos and Brown.

Saccharomyces Apiculatus.

This is another alcoholic ferment which exists in ripe and juicy

Saccharomyces Apiculatus.

fruits, and winters in the earth. It develops in two forms, one lemon-shaped, the other more elliptical. It has only one-sixth of the activity of the *S. cerevisiæ*, and it produces beer of an entirely different flavour. It resists heat and moisture well.

Eurotium Oryzæ.

This alcohol-producing ferment is used in Japan for producing *Saki*.

Mucor Racemosus—Mucor Mucedo.

These micro-organisms also produce alcohol, but only in the absence of free oxygen. The spontaneous production of alcohol and carbonic anhydride from fruit, when excluded from the air, is probably due to one of these ferments.

These fermentations were first described by James Bell (to whom I am indebted for several of the microscopic drawings), and his observations have since been confirmed by Pasteur.

So far as experiments have gone, it would appear that the distinction between the effect of different alcoholic ferments is chiefly one of
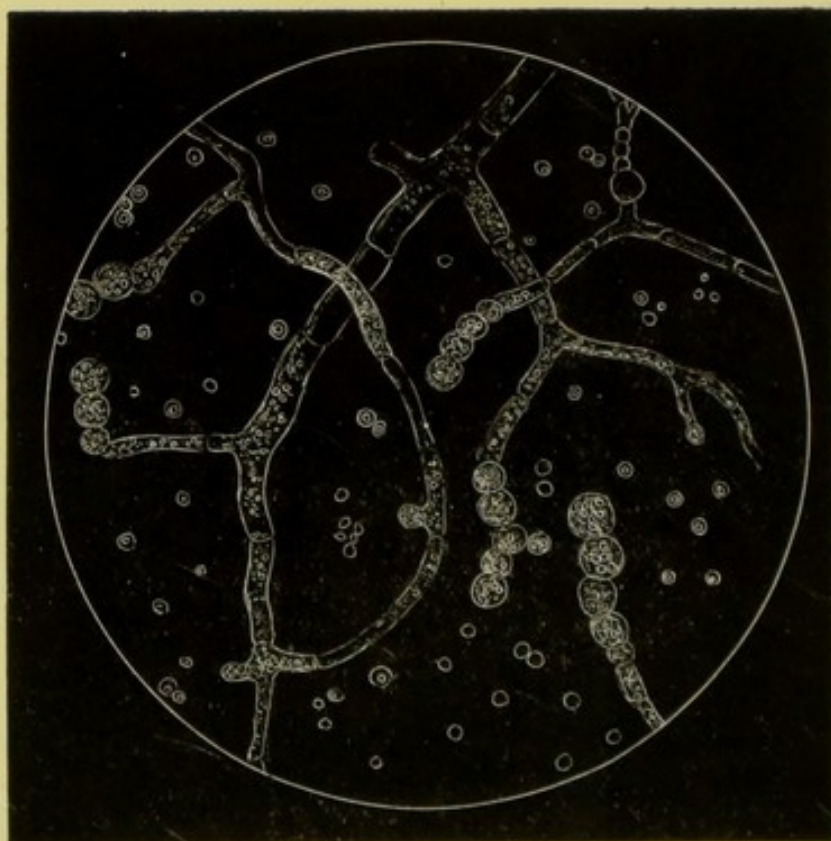
Mucor Racemosus and M. Mucedo.

degree only. The yeast organisms set to work with great energy, and diffuse themselves rapidly throughout the whole liquid, whereas the

action of the other organisms is more localised and consequently slower. These actions of the alcohol organisms, other than yeast, have, however, hitherto not been exhaustively studied as regards their subsidiary products.
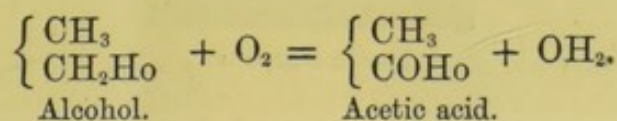
Mycoderma Aceti.

Acetic acid is one of the products of a number of fermentations. It is a product of the fermentation of calcic tartrate, malate, citrate, and mucate. It is produced from glycerin by yeast; from ethyl alcohol by muscle; from sugar by the lactic and butyric ferments, and from starch by the *leaven* ferment. It is also produced, probably by a micro-organism, during the germination of seeds.

The special organism, however, which transforms alcohol into acetic acid is the *Mycoderma aceti* (mother of vinegar), which forms a gelatinous mass resolved under the microscope into innumerable bent chain-like threads, which split by transverse section.
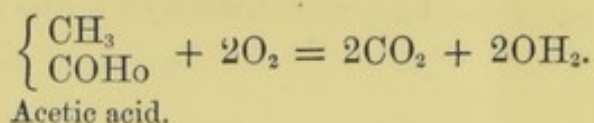

Mycoderma aceti.

These filaments are usually accompanied by spherical cellules when the liquid becomes acid. According to Pasteur, the germs of this organism are everywhere in the air, and rapidly develop on the surface of weak alcoholic liquors containing nitrogenous matters and phosphates. The *Mycoderma aceti* flourishes as a film on the surface of the liquid, and, so long as it remains there, it determines rapid oxidation of the alcohol; but as soon as it is immersed, it becomes inactive and oxidation ceases. This points decidedly to true zymotic action, as distinguished from that of a soluble ferment. The reaction is:—

$$\begin{cases} CH_3 \\ CH_2Ho \end{cases} + O_2 = \begin{cases} CH_3 \\ COHo \end{cases} + OH_2.$$

Alcohol.　　　　　　　　Acetic acid.

A little succinic acid is also formed.

Such is the oxidising power of this organism that, when all alcohol has been transformed into acetic acid, it immediately attacks what

must be regarded as its own excrement, and prolongs its existence by transforming acetic acid into carbonic anhydride and water.

$$\left\{ \begin{array}{l} CH_3 \\ COHo \end{array} \right. + 2O_2 = 2CO_2 + 2OH_2.$$

Acetic acid.

Mycoderma aceti develops readily in weak alcohol to which small quantities of acetic acid, albumen, and alkaline phosphate, have been added. An excess of alcohol or acetic acid, or the withdrawal of oxygen by the immersion of the organism, promptly arrests all further production of acetic acid.

Fresh *Mycoderma aceti* contains 95 per cent. of water and only 5 per cent. of solid matter. The latter consists of 94 per cent. of organic matter and 6 per cent. of ash, which is chiefly phosphates.

100 grams of pure alcohol require, for conversion into acetic acid, 69.57 grams of oxygen; or 1 litre of pure alcohol requires 552.4 grams of oxygen, or about 2.1 cubic metres of atmospheric air, for its oxidation; but as only about one-fifth of the oxygen is used, it follows that more than 10 cubic metres of air must pass through the respiratory organs of the *Mycoderma aceti* in the transformation of 1 litre of alcohol into acetic acid.

The Lactic Bacillus.

This micro-organism producing lactic fermentation somewhat resembles yeast. It consists of slightly viscous globules $\frac{1}{600}$ mm. in diameter, sometimes single and sometimes strung together in short chains. When single, these cells readily exhibit that molecular motion which is commonly called the Brownian movement. They appear to be a species of bacterium requiring no free oxygen for their

Lactic Bacillus.

development. They prefer a more dilute saccharine solution than that which is most favourable for yeast, and the continuous neutralisation of the lactic acid, as it is formed, also favours their development. The spores of this bacillus are everywhere in the air, and are believed

to be the cause of the souring of milk, by the transformation of milk-sugar into lactic acid in accordance with the following equation:—

$$C_6H_{12}O_6 = 2 \begin{cases} CMeHHo \\ COHo \end{cases}.$$

Lactose.   Lactic acid.
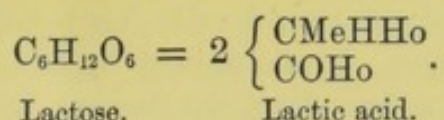
On the other hand, A. Schmidt attributes the coagulation of milk to an unorganised ferment, which is precipitable by alcohol. When this precipitate is dissolved in water and added to milk, it produces rapid coagulation.
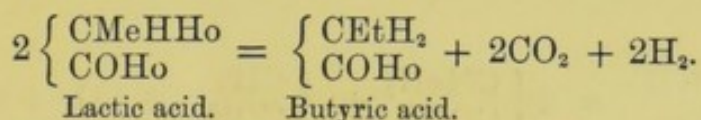
According to Bechamp, the coagulation of milk is due to micro-organisms contained normally in the liquid as it comes from the cow. They cause alcoholic, acetic, and lactic fermentation whether the organisms have developed into bacteria or not. According to this chemist, milk, as it comes from the cow, contains alcohol and acetic acid, and these increase as the milk becomes sour.

The saccharine bodies, sorbin, inosite, mannite, and dulcite, which are not attacked by yeast, yield at once to the lactic bacillus.

The lactic ferment is one of the most powerful enemies of the beet-root sugar manufacturer, as also, at times, of the wine grower.
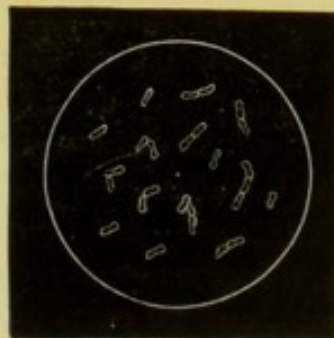
The Butyric Bacillus (B. amylobacter).

In carrying out the usual fermentative process for the preparation of butyric acid from a mixture of milk, sugar, putrid cheese, tartaric acid and chalk, it has always appeared to me that as soon as the micro-organisms have transformed these materials into calcic lactate, they begin to attack the latter and prolong their existence by converting it into calcic butyrate with disengagement of hydrogen and carbonic anhydride, according to the following equation:—

$$2 \begin{cases} CMeHHo \\ COHo \end{cases} = \begin{cases} CEtH_2 \\ COHo \end{cases} + 2CO_2 + 2H_2.$$

Lactic acid.   Butyric acid.

In this equation, for the sake of simplicity, lactic acid is substituted for calcic lactate.

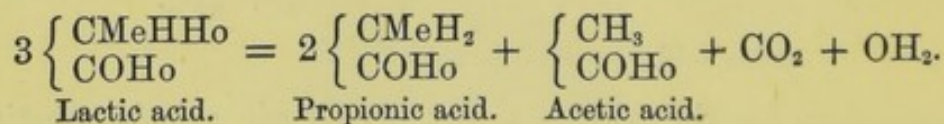Butyric Ferment (Fitz).   Butyric Ferment (Bell).

Bell and Fitz, however, describe a true butyric ferment which, according to the first named, consists of very active bacteria; but, according to the second, of round cells (arranged in garlands) 1·6 to 1·7 microms. in diameter. It is obtained by adding cow's excrement to calcic lactate. In a few days, the lactate is transformed almost entirely into normal butyrate, with traces only of acetic, caproic, and succinic acids. This is said to be the best ferment for butyric acid. I have sometimes obtained quite as good a result with cheese; but, on other occasions, a mixture of acids was produced from which it was difficult to extract any pure butyric acid.

Van Tieghem finds that *Bacillus amylobacter* always produces butyric acid, carbonic anhydride, and hydrogen, no matter what substance it operates upon. Thus, for instance, it always yields these products when it attacks cellulose, dextrin, arabin, lichenin, lactose, mannite, glycerin, calcic malate, or calcic citrate.

Bacillus Amylobacter (Tieghem).

On the other hand, when a small long bacillus, often in long bent chains, gains the victory, calcic lactate is converted into the calcium salts of propionic and acetic acids, according to the following equation :—

$$3 \begin{cases} CMeHHo \\ COHo \end{cases} = 2 \begin{cases} CMeH_2 \\ COHo \end{cases} + \begin{cases} CH_3 \\ COHo \end{cases} + CO_2 + OH_2.$$

Lactic acid.   Propionic acid.   Acetic acid.

Glycerin Fermentation.   Butyl bacillus and Ethyl bacillus.

Fitz has observed that there is a micro-organism which converts glycerin into normal butyl alcohol, normal butyric acid, caproic acid, and a small quantity of ethyl alcohol. The acids must be continuously saturated with calcic carbonate. A good result was obtained with the following mixture :—2000 parts of water, 100 of glycerin, 1 of potassic phosphate, 0·5 of magnesic sulphate, 2 parts of pepsin (as food for the ferment), and 20 parts of chalk. To this, a trace of the ferment was added, and the mixture exposed to a temperature of 40° C. The

micro-organisms increased rapidly, and after two days, energetic fermentation set in, and was finished in 10 days.

There were obtained about 7·7 of butyl alcohol per hundred parts of glycerin employed, and 12·3 per cent. of normal calcic butyrate. Also a small quantity of a non-volatile acid, probably lactic acid, and a base of the picoline type. If the butyl alcohol be distilled off, the fermentation will recommence without fresh ferment.

Fitz believes that two distinct organisms take part in this fermentation, one producing butyl alcohol, and the other ethyl alcohol. The ethyl bacillus appears to be identical with the *B. subtilis* of Cohn. It is a small thin bacillus which is coloured yellow by iodine, and is easily distinguished from the butyl bacillus, which is much broader (2 to 3 microms. broad, and 5 to 6 long), and is coloured violet, or even black, by iodine. The ethyl bacillus withstands a boiling temperature, whilst the butyl bacillus is destroyed by this temperature.
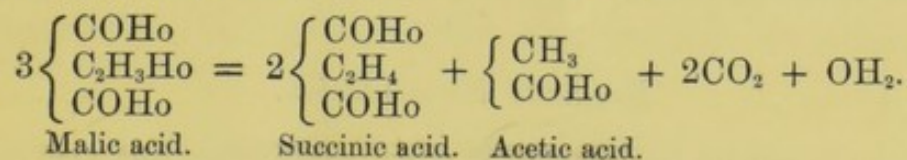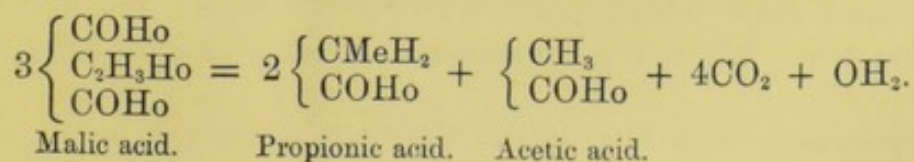

Butyl Bacillus.

Bacillus Subtilis, Ethyl Bacillus (Cohn).
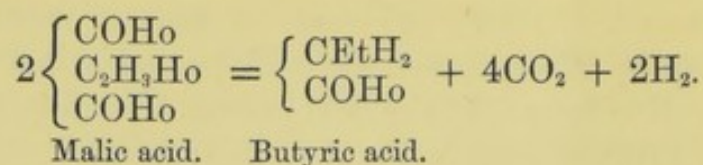
Malic Fermentation.

In addition to its fermentation by yeast, already alluded to, calcic malate is also attacked by the micro-organisms contained in putrid cheese or muscle. These organisms are "spaltpilsen," and appear to be of three different species. One of them, which has the appearance of small thin rods, produces succinic acid, thus:—

$$3\begin{cases} COHo \\ C_2H_3Ho \\ COHo \end{cases} = 2\begin{cases} COHo \\ C_2H_4 \\ COHo \end{cases} + \begin{cases} CH_3 \\ COHo \end{cases} + 2CO_2 + OH_2.$$

Malic acid.   Succinic acid. Acetic acid.

Another, which is a short cylindrical bacillus, splits the malic acid into propionic and acetic acids, carbonic anhydride, and water.

$$3\begin{cases} COHo \\ C_2H_3Ho \\ COHo \end{cases} = 2\begin{cases} CMeH_2 \\ COHo \end{cases} + \begin{cases} CH_3 \\ COHo \end{cases} + 4CO_2 + OH_2.$$
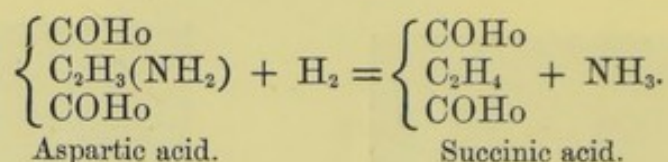
Malic acid.   Propionic acid. Acetic acid.

Whilst a third is the ordinary butyric ferment, taking the malic acid neatly asunder into butyric acid, carbonic anhydride, and hydrogen.

$$2\left\{\begin{array}{l} COHo \\ C_2H_3Ho \\ COHo \end{array}\right. = \left\{\begin{array}{l} CEtH_2 \\ COHo \end{array}\right. + 4CO_2 + 2H_2.$$

Malic acid.   Butyric acid.

It is highly probable that the transformation of maleic and fumaric acid into succinic acid, by fermentation with putrid cheese, is performed by one of these organisms :—
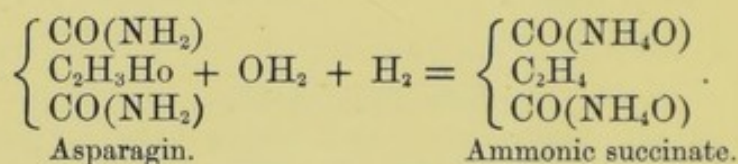
$$''C_2''H_2(COHo)_2 + H_2 = C_2H_4(COHo)_2.$$

Fumaric or maleic acid.   Succinic acid.
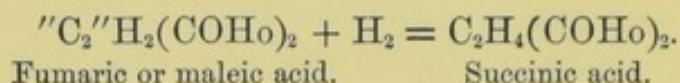
The transformation of aspartic acid into succinic acid and ammonia is also probably the work of the same organism :—

$$\left\{\begin{array}{l} COHo \\ C_2H_3(NH_2) \\ COHo \end{array}\right. + H_2 = \left\{\begin{array}{l} COHo \\ C_2H_4 \\ COHo \end{array}\right. + NH_3.$$

Aspartic acid.   Succinic acid.

The calcium salts were used by Dessaignes, who observed these reactions.

The change of aspargin into ammonic succinate is of the same type of fermentations.

$$\left\{\begin{array}{l} CO(NH_2) \\ C_2H_3Ho \\ CO(NH_2) \end{array}\right. + OH_2 + H_2 = \left\{\begin{array}{l} CO(NH_4O) \\ C_2H_4 \\ CO(NH_4O) \end{array}\right. .$$

Asparagin.   Ammonic succinate.

P. Miquel finds that asparagin is converted into carbonic anhydride, ammonia, and succinic acid by a bacterium existing in water. This organism is twice branched, 0·0015 mm. long, and 0·0008 mm. thick. It is destroyed by a temperature of 49° C.
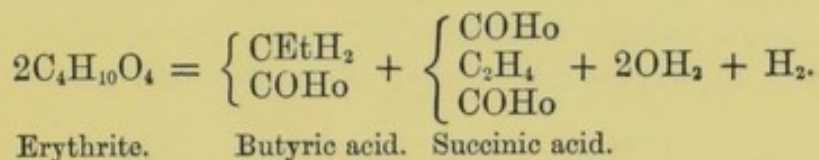
Erythrite Fermentation.

This sugar can be fermented by cows' excrement, but the ferment should be several times cultivated in fresh solutions of erythrite. Many kinds of " spaltpilsen " are produced, the most prominent being pear-shaped cells which become elliptical towards the end of the fermentation. They are 1·5 microm. broad and 2·5—2·8 microms. long.

The products from 30 grams of erythrite were—

Normal calcic butyrate .... 13·0 grams.
Succinic acid .... 12·7 „
Alcohol. ... ⎱
Acetic acid. ⎰ .... traces.
Caproic „ . ⎰

These products correspond to the following leading reaction :—

$$2C_4H_{10}O_4 = \begin{cases} CEtH_2 \\ COHo \end{cases} + \begin{cases} COHo \\ C_2H_4 \\ COHo \end{cases} + 2OH_2 + H_2.$$

Erythrite.   Butyric acid. Succinic acid.

Viscous Fermentation.

According to Pasteur, the viscous ferment consists of globules 1·2 to 1·4 microm. in diameter, strung together like garlands of roses. These organisms develop in wine and in various vegetable juices such as beetroot and onions. They cause a mucilaginous or ropy condition. They transform grape-sugar, or previously modified cane-sugar, into mannite, CO_2, and a gum similar to dextrin. If these organisms be put into a saccharine liquid containing the necessary nitrogenous food and mineral salts, the viscous fermentation soon begins.

From 100 parts by weight of sugar, there are obtained about 51·09 parts mannite, and 45·5 parts gum, besides CO_2. The chemical change has been expressed in the following equation :—

$$25C_{12}H_{22}O_{11} + 25OH_2 = 12C_{12}H_{20}O_{10} + 24C_6H_{14}O_6 + 12CO_2 + 12OH_2.$$
Cane-sugar.   Gum.   Mannite.
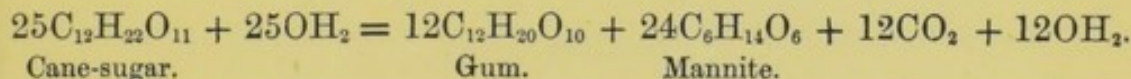
The gum thus produced is not oxidised to mucic acid by nitric acid, but to oxalic acid; 30° is the best temperature for their development.
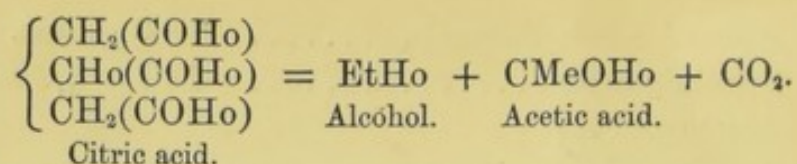
Mannite Fermentation.

Infusion of hay contains a club-shaped bacillus, which breaks up mannite, so that 100 grams give 26·3 grams of ethyl alcohol, 5·6 grams of formic acid, and a trace of succinic acid :—

$$C_6H_{14}O_6 = 2EtHo + CHOHo + CO_2.$$
Mannite.   Alcohol.   Formic acid.
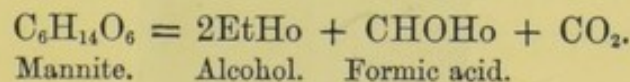
Citric Fermentation.

By the action of a peculiar small and thin bacillus contained in hay infusion, citric acid yields alcohol and acetic acid according to the following equation :—

$$\left\{\begin{array}{l} CH_2(COHo) \\ CHo(COHo) \\ CH_2(COHo) \end{array}\right. = EtHo + CMeOHo + CO_2.$$

Citric acid.　　　　　Alcóhol.　　Acetic acid.

Cellulose Fermentation.

According to Böhm, the cellulose of land or bog plants undergoes, under water, a butyric fermentation, whilst the cellulose of aquatic plants splits up into carbonic anhydride and marsh-gas.

$$C_6H_{10}O_5 + OH_2 = 3CO_2 + 3CH_4.$$

Cellulose.　　　　　　　　　　　　　Marsh-gas.
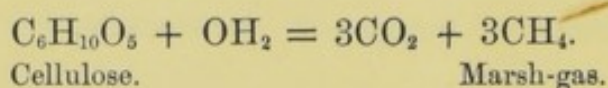
Glyceric Acid Fermentation.

A peculiar bacillus causes fermentation in calcic glycerate, and produces ethyl and methyl alcohols, formic acid, and a little acetic acid.　No non-volatile acid is produced.

Bacterium Termo.

This organism resolves tartaric acid into succinic acid, acetic acid, and carbonic anhydride ; but if the calcium salt of this acid be presented to *Bacterium termo*, it is broken up into acetic acid, traces of propionic acid, formic acid, and carbonic anhydride.

Bacillus Ureæ.

This bacillus, or vibrio, which gets its living by converting urea into ammonic carbonate by the following reaction,—

$$CO(NH_2)_2 + 2OH_2 = CO(NH_4O)_2,$$

Urea.　　　　　　　　　Ammonic carbonate.
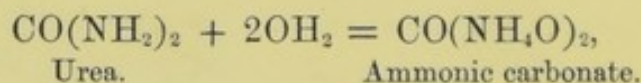
continues in active motion until the change is complete, and then becomes motionless and probably dead.

Urea Bacillus.

According to Miquel this bacillus belongs to the class of aërobes, is 0·006 mm. long and 0·0008 mm. broad, and bears a heat of 90° C. It changes to elliptical spores which, even when moist, withstand a temperature of 96° C.

In order to study the action of this bacillus upon urea, I allowed fresh urine to stand for 25 days in a clean glass vessel, observing, at short intervals, the progressive development of micro-organisms, and determining at the same time the amount of organic carbon, organic nitrogen, and ammonia in 100,000 parts of the liquid.

The following results were obtained :—

	Residue left on evaporation and drying at 100° C.	Organic carbon.	Nitrogen as urea and other organic matter.	Ammonia.	Microscopical observations.
Fresh urine	4817·0	943·81	1080·27	142·40	No bacilli.
,, ,, after 1 day..	—	940·46	1095·05	136·65	,,
,, ,, ,, 3 days.	—	928·76	1106·70	136·50	,,
,, ,, ,, 5 ,, ·	—	882·66	983·26	288·55	No observation.
,, ,, ,, 7 ,, ·	—	739·82	900·80	333·60	Sparse bacilli.
,, ,, ,, 9 ,, ·	—	682·99	784·93	485·12	No observation.
,, ,, ,, 11 ,, ·	—	621·02	744·64	534·30	Numerous bacilli.
,, ,, ,, 14 ,, ·	—	559·22	481·49	870·62	Very numerous bacilli.
,, ,, ,, 16 ,, ·	—	530·68	492·04	881·87	Vast numbers of bacilli.
,, ,, ,, 18 ,, ·	—	487·01	355·25	990·78	Mostly still.
,, ,, ,, 21 ,, ·	—	466·43	278·22	1105·75	,, ,,
,, ,, ,, 23 ,, ·	—	451·43	347·45	1017·25	All dead or still.
,, ,, ,, 25 ,, ·	2718·0	460·78	283·90	1070·50	,, ,,
After allowing for evaporation	2045·5	346·77	213·66	805·63	

On the 5th day only, was any trace of nitric or nitrous acid present, and then only 0·019 part per 100,000.

The sample analysed on the 14th day was previously filtered; the others were all analysed unfiltered.

During the experiments, the liquid became considerably concentrated by spontaneous evaporation; for, whereas at the commencement it contained only 730·0 parts of chlorine, at the conclusion the amount found was 970·0 parts per 100,000.

The results of these observations and determinations, which were made during the month of June, show conclusively that, previously to the development of *Bacillus ureæ*, the chemical composition of the urine remained practically unchanged; but with the appearance of micro-organisms, a diminution of organic carbon and a transference of nitrogen from the organic to the ammonia column immediately

began. As regards rapidity, this change marched *pari passu* with the density of population, and reached its maximum about the 12th day; for during the three days (11th to 14th) nearly 10 per cent. of carbon disappeared, whilst more than 35 per cent. of the organic nitrogen became ammonia. After the 14th day, the rate of change became much slower, on the 18th day the bacilli were mostly either dead or motionless, whilst on and after the 23rd day no more moving bacilli were seen. Altogether, the quantity of carbon converted into carbonic anhydride, after allowing for concentration of the liquid by evaporation, amounted to 597·04 parts per 100,000 of liquid, or 63·3 per cent. of the total quantity; whilst the quantity of organic nitrogen converted into ammonia was 546·19 parts per 100,000, or 50·6 per cent. of the whole. These proportions show that all the organic nitrogen contained in the urea was not converted into ammonia. It no doubt escaped as free nitrogen, in accordance with Frank Hatton's observation.

In the original urine, the proportion of organic carbon to organic nitrogen was as $1 : 1·15$, whilst, after the action of the bacilli, it was as $1 : 0·62$.

Kinic Acid ($C_7H_{12}O_6$) *Fermentation.*

Spaltpilsen, when fed with asparagin, potassic phosphate, and magnesic sulphate convert kinic into protocatechuic acid, $\left(\left\{ \begin{array}{l} C_6H_3Ho_2 \\ COHo \end{array} \right\} \right)$, but if the organism be fed with peptone, no protocatechuic acid is produced, but the molecule of kinic acid is split up into formic, acetic, and propionic acids.

Hay Bacilli.

The action of the micro-organisms which convert grass into hay or ensilage has been studied by Mr. F. Jordan and my son. These organisms exist in vast numbers in grass. In the presence of free oxygen, they absorb this gas and evolve carbonic anhydride and nitrogen, and these are the gaseous products in the making of hay. The action is sufficiently energetic to set fire to the hay, if it is stacked too soon; but it is inconceivable that the bacilli continue their action until ignition ensues, and therefore ordinary oxidation probably supervenes.

The absorption of oxygen is not necessary, for carbonic anhydride is evolved in an atmosphere of hydrogen; but, under these circumstances, very little nitrogen makes it appearance.

The fermentation goes on under water, but its character is changed. The gases, which are much augmented in volume, now consist of carbonic anhydride (84·63 per cent.), hydrogen (6·9 per cent.),

nitrogen (5·83 per cent.), and traces of other combustible gas, probably marsh-gas. Acetic acid, lactic acid, and probably butyric acid are at the same time formed. This is probably the kind of fermentation by which grass is converted into ensilage.

Nitrification.

In respect of utility to man, few species of bacteria can claim precedence over the organism which, in the presence of bases, transforms nitrogenous organic matter into nitrates. This organism is the real natural scavenger of both savage and civilised society, and its operations are all the more acceptable because it transforms foul and offensive animal matter into innocuous and inodorous mineral compounds. It carries out to completion the work commenced by the *Bacillus ureœ*, and it is the great agent in the purification of sewage by irrigation and intermittent filtration. Unfortunately, it does not seem to prey upon morbific bacilli and their spores; otherwise, low as its place is in creation, it would, after man, be the most useful organism in existence.

Warington and Storer find that this bacterium works only in the dark, which again is an advantage, as the materials it operates upon are best kept out of sight. In the presence of bases, it transforms both nitrogenous organic matter and ammonia into nitrates, the most complete form of nitrogenous oxidation. It is also found that the formation of nitrites is determined by a modification of the nitrate bacillus. It is active in more concentrated solutions and at a higher temperature than 15° C. When once formed, this modified ferment always produces nitrites. It has no action on potassic nitrite, whilst the nitrate bacterium converts this into potassic nitrate.

I must not omit to notice the extraordinary selective power exhibited by some micro-organisms, and first observed and utilised by Pasteur, who employed a micro-organism to destroy one of the optically active compounds in a mixture, and thus to isolate the compound of opposite optical activity. He operated upon racemic acid, out of which the *Penicillium glaucum* consumed all the dextrotartaric acid, but preferred to die rather than to eat the lævotartaric acid. Le Bel afterwards extended these observations to optically active amyl alcohol and propyl glycol, whilst Lewkowitsch was the first who showed that by varying the micro-organism, either the dextro- or lævo-compound (in the case of mandelic acid) could be destroyed at will.

In conclusion, I have endeavoured to show that there are two classes of operations by which chemical changes in organic matter are

brought about in a somewhat mysterious way. Superficially considered, these classes appear to be nearly allied, but on closer study they are found to be essentially different.

In the first place, although a comparatively small amount of a soluble ferment can produce a chemical change in a large mass of matter, its action is limited; whereas there is no such limit to the action of an organised ferment. Thus the distiller finds that it is necessary to add 18 per cent. of malt in mashing, in order to convert the whole of the starch of the unmalted grain into sugar. But a single yeast cell, in an albuminous liquid, will bring about the conversion of an unlimited amount of sugar into alcohol.

Secondly, soluble ferments are unaffected in their activity by chloroform or compressed air, whilst these reagents at once arrest the changes produced by micro-organisms.

Thirdly, the changes effected by soluble ferments can, with scarcely an exception, be easily brought about by purely chemical means, whilst those dependent upon the presence of micro-organisms have, with one or two exceptions, never been directly effected by other non-vital means.

I have also hazarded the proposition that there is no break in the continuity of chemical functions between micro-organisms and the higher forms of animal life. Both alike owe their vitality to the liberation of the energy stored in their food, and both go through a cycle of existence and then lose their vitality. It is true that there are apparently certain sharp distinctions between them. Thus the enormous fecundity of micro-organisms and their tremendous appetites (on the assumption that all the changed matter passes through their bodies) seem to separate them from the higher orders of animals. But this distinction is only comparative.

Thus, in regard to fecundity, the power of multiplication gradually increases as the animal descends in the scale of organisation. There are, of course, exceptions, but this is the rule. The sheep produces only one or two lambs annually. The herring in the same time multiplies itself many thousand fold, whilst the aphis produces young at such a rate, that a single specimen would, if all its progeny lived, produce in three months a weight of aphides greater than that of the whole contemporary human race. And, as to appetite, voracity is greatest in the lowest organisms. A sheep or a cow consumes about one-sixth of its own weight in 24 hours; an earthworm, a caterpillar, or a silkworm many times its own weight. The yeast organism must, therefore, taking into account its position in nature, be considered decidedly abstemious, inasmuch as it only consumes two-fifths of its own weight of sugar in the course of 24 hours. Moreover, it must be borne in mind that the sheep converts most of its food into carbonic

anhydride, water, and hippuric acid, thus utilising nearly the whole of the potential energy, whilst the micro-organism, as a rule, utilises only a small portion.   We have seen also that those micro-organisms which have been chemically studied produce, like the higher animals, perfectly definite chemical changes.   There is in this respect, therefore, no essential difference between a mass of yeast, a populous town, a herd of cattle, and a colony of snakes ; each produces its own peculiar chemical changes in the food it consumes.

The position of micro-organisms in nature is only just beginning to be appreciated.   Their study, both from chemical and biological points of view, is however of the highest importance to the welfare of mankind ; and I venture to predict that whilst there is no danger of their being spoiled by petting, or by their welfare being made the special care of sentimentalists, these lowly organisms will receive much more attention in the future than they have done in the past. Their study leads the inquirer right into those functions of life which are still shrouded in obscurity, and it is to be hoped that these investigations will not be retarded by mischievous legislation.