Symbiosis [1].

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Synopsis [2].

ORIGIN of the idea and of the term. Differences between parasitisim and symbiosis (1).

Lichens, previously regarded as autonomous plants, are shown to be dual organisms, a symbiosis of Alga and Fungus (2). Controversy regarding the Lichen theory, and establishment of the latter by means of synthetic cultures (3).

Other cases of symbiosis known previous to 1880. Algae in the stems of *Gunnera*, and the roots of *Cycas*, in the thallus or fronds of *Anthoceros* and *Blasia*, *Azolla*, *Lemna*, &c. (4).

Extension of the idea of symbiosis: insect-fertilization, epiphytes, &c. (5).

Galls not necessarily due to insects, but may be due to the irritating action of Fungi or Bacteria. Phytocecidia of the Aleppo pine, &c. (6).

Symbiosis in animals. Green Infusoria, *Hydra*, sponges, &c. (7).

Mycorhiza, the roots of many humus plants curiously

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[2] The figures in parentheses refer to the bibliography collected at the end.

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swollen and modified owing to the presence of Fungi, which do not injure the plant, but link its roots to the decomposing leaves around. Explanation as an instance of symbiosis. Evidence partly anatomical and partly experimental (8).

'Budding' and 'grafting' are processes involving the establishment of a symbiosis.

The nodules on the roots of leguminous plants (9). Discovery and controversy as to their nature. They contain living bacteroids, which penetrate the root hairs and flourish in the living cells. Universality of these nodules on healthy roots. Hellriegel and Willfarth's cultures, and evidence as to the fixation of nitrogen (10). Laurent and Schloesing's proof that nitrogen is fixed from the air (11).

The leguminous nodules a case of symbiosis, comparable to galls.

Other instances not yet explained. Nodules on the roots of *Juncus*, *Myrica*, and other plants (12).

Symbiotic fermentations (13). All natural fermentations mixed. Pure cultures and the importance of synthetic cultures.

Kephir (14), the ginger-beer plant, and other instances of symbiotic ferments (15). Decomposition of cellulose (16). Nitrifying and de-nitrifying organisms (17). The direct alcoholic fermentation of starch by the simultaneous action of two Fungi (18).

Return to the idea of symbiosis. Necessity of limiting the term. Antibiosis (19) (antagonism). Metabiosis (20). Difficulty of distinguishing in given cases. Hypothetical considerations, and importance of further investigations.

Particular Cases.

The above may be accepted as affording general headings under which the subject of symbiosis might be treated.

For the purposes of this discussion, I proceed to consider some special cases, and limit myself—as requested to do—to certain aspects of symbiotic fermentations.

Several cases of symbiosis among Bacteria are now known. Apart from numerous instances of temporary association between pathogenic micro-organisms and animals such as earth-worms, rats, flies, ticks, and mosquitoes, which disseminate their germs and infect cattle, sheep, horses, and men (21) (reminding us of the transference of the spores of *Botrytis* by bees, which carry this parasite with the pollen and infect the stigmas of bilberries with the parasite (22)) or which act the part of intermediate hosts to the disease germs, much as certain pond snails do to the liver-fluke of sheep (23), we now know several cases of symbiosis between two species of Bacteria or of Fungi, or between a Bacterium and a Fungus, where each symbiont is incapable of carrying on alone the work which the symbiotic association is able to perform—a point which is essential to the definition of symbiosis in the narrower sense, i. e. the co-operation of two associated organisms to their mutual advantage.

A striking example is afforded by certain Bacteria concerned in the destruction of cellulose in ponds, bogs, rivers, &c. (24). Van Senus found that a certain anaërobic Bacterium, resembling, if not identical with, Van Tieghem's *B. Amylobacter* (25), though incapable of dissolving cellulose by itself, can do so if associated with another Bacterium, also incapable of itself attacking cellulose. *B. Amylobacter* can ferment pectose compounds, and is thus capable of isolating cells one from another, but cellulose is not attacked by it.

Van Senus believed that the one Bacillus destroys certain products of fermentation excreted by *B. Amylobacter*, which inhibit its cellulose-fermenting powers.

I may remark here, that if a sound potato, rhizome, or other underground organ is placed in water and the air exhausted as completely as possible, I almost invariably find its cellulose walls destroyed in a few days by a mixture of Bacteria, and with the symptoms found in many kinds of 'wet rot.' There is no reason to believe that these organs would rot if merely wet and not deprived of air, since they lie in ordinary soil—even moist soil—for weeks or months, with plenty of water in their tissues, and respire oxygen, as is well known. The presumption is that the anaërobic conditions set up in the experiment described favour certain forms of soil Bacteria, such as Van Senus worked with, and enable them to co-operate in the destruction of the cell walls.

An even more remarkable example is given by Winogradsky, who found that the anaërobic Bacterium known as *Clostridium Pasteurianum* is able, if supplied with abundance of dextrose and protected from the access of oxygen, to fix atmospheric nitrogen (26). In the cultures, and presumably in the soil, the *Clostridium* was found to work when protected by a mantle of aërobic bacteria. In fact, the nitrogen-fixing *Clostridium* was working in the meshes of the oxygen-consuming species, and forming gelatinous flocks like the well-known grains of kephir, or of ginger-beer plant.

Yet another striking instance of symbiotic association has recently been given by Omeliansky (27). In experiments on nitrification at Bonn (28), the assertion had been made that the nitrifying organisms, i. e. the Bacteria known to oxidize ammonia to nitrous acid, and nitrous acid to nitric acid, could be grown and made to do their specific work in media containing proteids or other organic nitrogenous bodies. Now this was directly contradictory of the experience of Warington (29), Winogradsky (30), and other workers, who had found that one great peculiarity of these nitrifying organisms is that they refuse to grow on such media; they are incapable of using organic nitrogen. Several workers (31) then showed that the Bonn observers had inadvertently employed mixtures of two or more species, and Omeliansky undertook a critical re-investigation of the whole subject, and has put forward the following explanation of the tangle.

If *Nitrosomonas*—the Bacterium which oxidizes ammonia to nitrous acid—and *Nitrobacter*—the Bacterium which further oxidizes nitrous to nitric acid—are sown together or separately on a medium containing organic nitrogen, no growth or change occurs.

But if a Bacterium capable of decomposing the organic nitrogenous medium, e. g. *Bacillus ramosus*, is added to the above-mentioned *Nitrosomonas* and *Nitrobacter*, the associated three organisms are able to carry out all the processes and complete the cycle of nitrification. That is to say, *B. ramosus* breaks down the gelatine and ammonia is formed, this is then oxidized to nitrous acid by *Nitrosomonas*, and the nitrous acid is further oxidized to nitric acid by the *Nitrobacter*.

If *B. ramosus* and *Nitrosomonas* only are sown together, then only nitrous acid is formed, because the latter organism is only capable of carrying the oxidation the one stage.

If *B. ramosus* and *Nitrobacter* only are used, then only ammonia is formed, because the latter organism cannot oxidize ammonia.

If we try to imagine the working of this association of organisms in the soil, and bear in mind the frequent co-existence and action of the de-nitrifying Bacteria which Gayon and Dupetit (32), Giltay and Aberson (33), Warington (34) and others have familiarized us with, a glimpse is obtained of the very complex symbioses which may be concerned in the circulation of nitrogen in Nature. Moreover, some of these de-nitrifying Bacilli appear to be anaërobic, and can only work in the surface soil if protected from the access of oxygen ; say, by an associated aërobic Bacterium.

Another interesting case is the following. Perdix a few years ago isolated from water an anaërobic Bacterium which converts starches into sugars, which with the aid of a yeast can be fermented, the whole process going on in association (34 *a*).

Other cases of symbiotic associations of Bacteria exist among the forms concerned in the reductions of sulphates (35) and the oxidation of sulphuretted hydrogen (36), the iron bacteria (37), &c. ; but I propose to mention only one or two further examples, taken from the true Fungi.

Symbiotic associations of Fungi are probably common, but only a few cases are as yet established, and these principally among the ferment-Fungi (38).

Van Laer has called attention to the symbiotic co-existence of two yeasts in many beers, explaining certain peculiar after-fermentations as due to the results of one yeast acting on the medium improved for it by the other (38 *a*).

The Japanese have long been in the habit of brewing a peculiar fermented liquor known as rice-wine, or Saké (39). Rice grains are steamed, and when cool are infected with a mould fungus now known as *Aspergillus Oryzae.* When the rice is quite mouldy, at which time it emits a peculiar odour like that of pine-apples, the starch is found to be rapidly turning to sugar, under the action of a diastatic enzyme secreted by the Fungus.

This decomposing rice is then placed in water and exposed to the action of a yeast, which rapidly ferments the sugar, and the alcoholic Saké results.

So closely is the yeast associated with the *Aspergillus*, that, in practice, the alcoholic fermentation commences soon after the enzyme of the *Aspergillus* begins to hydrolyze the starch of the rice, and for some time a controversy existed as to whether the yeast was not really part of the life-history of the *Aspergillus.* Several observers have now shown, however, that we have here a striking case of symbiosis (40).

On reviewing these examples, we shall find that very different degrees of association of the organisms are to be met with.

At the one end of the series we find two organisms merely associated for a short time, e. g. Bacilli and worms, bees and *Botrytis*-spores, and, so far as we may speak of symbiosis at all in these cases, it is merely temporary or disjunctive.

At the other end of the series we have a close permanent combination of the two organisms working in unison, e. g. the Lichens, and Winogradsky's *Clostridium* with its protective mantle of aërobic Bacteria ; also the ginger-beer plant and kephir.

But between these extremes it is possible to find all stages, the half-way house being met with in cases such as the Saké ferment, where the *Aspergillus* evidently prepares the way for the *yeast.*

It has been proposed to apply the term *Metabiosis* to such cases.

It must not be forgotten that there are extremes in another direction, where one of the two associated organisms is injuring the other, as exemplified by many parasites, but these cases I leave out of account here. This state of affairs has been termed *Antibiosis.*

It seems not impossible that the biological relationships of these cases one to another could be shown thus :—

$$\begin{tikzcd}[column sep=small]
\text{Antibiosis.} & & & & \text{Symbiosis.} \\
& \text{Facultative parasitism.} \arrow[ul] & & \text{Metabiosis.} \arrow[ur] & \\
& & \text{Disjunctive—association.} \arrow[ul] \arrow[ur] & &
\end{tikzcd}$$

The Physiology of Symbiosis.

It will be an interesting exercise to see if we can get any further glimpses into the physiology of the phenomenon of Symbiosis.

When we come to inquire as to the processes which lead to enhancement of the functional activity of one organism by another living symbiotically with it, the matter presents many difficulties; for it is at the outset quite obvious that many things are possible, and it soon becomes evident that a tangle of complexities lies before us, as always in the inter-relations between associated biological units. We need go no further than the examination of the possibilities in the inter-relations between a weed and a cultivated plant, or between two trees in a forest, for illustrations of this truth.

Confining attention for the moment to closely associated symbionts, such as those composing a Lichen, the ginger-beer plant, or a clump of symbiotic Bacteria or Fungi, researches have made it practically certain that the provision of definite food-materials by the one symbiont for the other may be an important factor; e.g. an Alga supplies a Fungus with carbohydrates, or a Fungus converts starch into the fermentable sugars which the associated yeast needs. In other cases the advantage derived is one of protection from some injurious agent—e.g. the aërobic Bacterium prevents the access of oxygen to the anaërobic one. But there is evidence which suggests that mere nutrition or protection is not the only or even the principal factor involved. It is well known that the products of fermentative actions are frequently poisons, and we all know of cases where such poisonous excreta of living cells act as stimuli to other living cells, if supplied to them in minimal doses and very gradually: I need only instance the effects of tobacco or alcohol on man, in illustration of this.

Several observers have shown that in presence of a particular food-substance the living cell is stimulated to produce and excrete a particular enzyme, while the substitution of another food stimulates the organism to excrete a totally different enzyme.

Now let us see if there is any evidence to support the hypothesis that some such stimulative action is exerted by one symbiont on another. To a certain extent we find such evidence in the remarkable vigour and large size of the algal cells in a Lichen as compared with the same cells living an independent life, and in the persistent zone of brilliant green and often hypertrophied cells of leaves in which certain Fungi are living, the gigantic cells of the nodules on leguminous roots in which the bacteroids are living, and many other cases; but since it is impossible to say how far these are cases of merely enhanced nutrition, we will pass them by and seek for other instances.

One of the earliest I can find is Hugo Schulz's demonstration in 1888 (41) that minute quantities of poisons such as corrosive sublimate, iodine, iodide of potassium, bromine, arsenious acid, chromic acid, sodium salicylate, or formic acid, when added to yeast in 10 per cent. grape-sugar solution, immediately raise the fermentative activity of the organism—

as measured by the amount of carbon-dioxide evolved. Effront, in 1894 (42), showed that hydrofluoric acid acts similarly on yeasts, butyric ferments, and *Mycoderma*, and, later, that the same is true of formaldehyde, salicylic acid, picric acid, &c.

The results of Johannsen's experiments with seeds, buds, &c., treated with ether or chloroform, look like another case in point: respiration is increased, and the whole course of metabolism so altered that in some cases buds of flowers can be stimulated to open long before their normal period (43).

The results obtained by Farmer and Waller with carbon-dioxide, which was found to induce an initial acceleration of the movement of the protoplasm in *Elodea*, may be a further instance (44).

Pfeffer has recently called attention to a still more remarkable instance—that it is possible by etherizing the living cells of *Spirogyra* to alter the type of nuclear division from *mitotic* (indirect) to *a-mitotic* (direct). Massart had shown that callus, the hypertrophied tissue developed under stimulation by mites, fungi, exposure to air, &c., is formed of cells which divide with *a-mitotic* nuclear division; and other cases occur. But it is even more to the point for my purpose that Gerassimoff, in Pfeffer's laboratory, found *Spirogyra* driven to *a-mitotic* division by associated Bacteria and other organisms, which he regards as a case of symbiosis (44 *a*).

Now it may be regarded as certain that if a cell can be thus stimulated to alter the details of so fundamental and complex a morphological process as its cell-division, by the action of associated organisms, the metabolic activities of its protoplasm are being driven into very different channels from the normal, and many physiological processes must be affected.

Of course I am here raising questions which concern the border-line between health and disease, and much investigation is still required as to the meaning of these matters; but I ought to add that according to Pfeffer the etherized cells can be again restored to their normal state if the traces of the anaesthetic are washed out, and those familiar with Klebs' experiments on other algae will appreciate the significance of this one with *Spirogyra*.

However feeble the evidence may be, we can at least say, then, that there is some evidence in support of the hypothesis that one symbiont may stimulate another by excreting some body which acts as an exciting drug to the latter—just as truly as certain drugs act as stimulants to some cell or organ of a higher animal, and no doubt in a fundamentally similar manner (44 *b*). It will be noted that such drugs are frequently excreta from vegetable cells.

But there is another, perhaps more indirect way in which one symbiont may enhance the activity of another. It has long been known that the accumulation of the products of metabolism of a cell tend to inhibit the activity of that cell, and that if by any means we can destroy or remove the metabolite as it is formed, the cell concerned can go on working. Similarly with ferments, and even with enzymes, the accumulation of the products gradually inhibits the action, as Tammann (45) showed in the case of Amygdalin and emulsin, and Brown and Morris (46) and Lea (47) in the case of starch and diastase, to mention two illustrations only.

Now suppose we have two organisms *A* and *B* living in symbiosis, and suppose that *A* is capable of hydrolysing starch by the excretion of diastase, while *B* removes the product of hydrolysis, by fermenting the sugar as fast as it is formed; in this case there is every reason to expect that *A* will push its hydrolysing action to the utmost, not only because it is of advantage to *A* to be relieved of the inhibiting sugar, but because the diminution of the sugar re-acts as a stimulus to the secretion of more enzyme.

There is yet another point to be considered. Katz, in 1898 (48), published some results confirming in many points the discoveries of Wortmann (49), Brown and Morris (50), and others, that Fungi, Bacteria, embryos, and other enzyme-secreting organisms not only vary the extent and kind of enzyme secreted, but can be stimulated to vary the enzyme according to the quantity or quality of food materials at hand.

I think this line of inquiry may lead to results in the present connexion, as it is obvious that the products of fermentation of an organism *A* must be favourable, or without effect, or deleterious to the action of another, *B*, in its immediate neighbourhood. Moreover, it is shown that a product which is, *per se*, devoid of either favourable or deleterious action, may acquire the power of exerting one or the other if the concentration increases.

Katz regards the action of sugars as not a purely chemical one, but as a physiological stimulus; and without pretending to understand the distinction in detail, we may admit the importance of the experimental facts, and not only seek for, but also hope for, more light.

Here, then, is a brief sketch of some of the salient features of symbiosis, and of some of the physiological factors concerned in the processes; and though it is far from exhaustive, it may serve our purpose to-day of starting a discussion, and of showing some lines along which further investigation is desirable.

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