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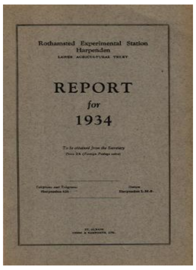

Report for 1934

The Work of the General Microbiology Department

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with lucerne was found to contain much more nitrogen than was applied as nitrate. This nitrogen must have been derived from that fixed by the lucerne. The uptake of this "fixed" nitrogen by the grass could be detected within three months of sowing. This suggests actual secretion of nitrogen compounds by the lucerne roots.[17,18]

7. *The Equilibrium between Symbiosis and Parasitism, within the Nodule.*

When a healthily growing plant bears nodules produced by an efficient strain of the nodule organism, the relationship between host and bacterium is normally one of symbiosis. A delicate equilibrium exists, however, which can readily be unbalanced in the direction of parasitism.

Such induced parasitism was first observed in the broad bean (*Vicia faba*) on plants grown in boron-deficient culture solution. Such plants bore minute nodules which fixed no nitrogen, while healthy controls bore large and active nodules.

It was found that deficiency of boron had so affected the structure of the nodules that the vascular strands which normally connect the nodule tissue with the stele, were absent or vestigial. In the centre of the nodule the bacteria had become parasitic and had destroyed the contents of the cells in which they lay.[19]

It was supposed that in this case the change to parasitism was caused by the bacteria being cut off from their supply of carbohydrates, normally brought to them along the vascular strands, and to their being reduced to obtaining their energy material from the host protoplasm.

To test this hypothesis, inoculated lucerne plants were placed in the dark so that a deficiency of carbohydrate might be produced, in this case by the stopping of photosynthesis. Nodules from these plants showed parasitic attack on the part of the bacteria quite similar to that shown in boron-deficient nodules.[20]

The change to parasitism which, in these experiments, was induced in young nodules, is a normal phenomenon in old lucerne and clover nodules towards the end of the summer. In such old nodules, parasitism was observed to commence at the base of the nodule and gradually to extend throughout the central tissue until the middle of the nodule was completely decayed.[20] Parasitism is thus an annual phenomenon, tending to extinguish the nor ma symbiotic growth.

THE WORK OF THE GENERAL MICROBIOLOGY DEPARTMENT

D. W. Cutler

The kinds of micro-organisms, both among the bacteria and the protozoa, that occur in the soil, and their activities, are determined by the soil structure ; broadly speaking, the suitability of land to agricultural purposes is correlated with the size of its population, for a soil in good tilth, with ample spaces both within and between the

(18) H. G. Thornton and Hugh Nicol—" Further Evidence upon the Nitrogen Uptake of Grass grown with Lucerne." Journ. Agric. Sci :, **24**, 1934, 540. Hugh Nicol—" The Derivation of the Nitrogen of Crop Plants, with Special Reference to Associated Growth." Biol. Rev., **9**, 1934, 383.

(19) W. E. Brenchley and H. G. Thornton—" The Relation Between the Development, Structure, and Functioning of the nodules on *Vicia faba*." Proc. Roy. Soc. London, Ser. B, **98**, 1925. 373.

(20) H. G. Thornton—" The Influence of the Host Plant in Inducing Parasitism in Lucerne and Clover Nodules." Proc. Roy. Soc. London. Ser. B, 106, 1930. 110.

crumbs for air and for water, provides an admirable habitat for bacteria and protozoa. Even where the soil structure is less desirable, and where temporary anaerobicity may occur owing to the cutting off of pore spaces by films of liquid, a large population either of facultative anaerobes, or of spores and cysts will survive.

One of the chief characteristics which makes soil under normal conditions such a suitable home for living cells is the fact that changes when they do occur only take place very slowly, and the inhabitants have time to adapt themselves to the new circumstances and this the free living bacteria and protozoa are very well able to do.

Further, in this, which is one of the oldest of all habitats, the different groups of the community must have arrived at a condition of delicate equilibrium, where any disturbance in one group may seriously influence others, and where drastic changes, either in the chemical or physical environment, may have detrimental effects on the general efficiency of the population in improving soil fertility.

Studies on the soil bacteria have been carried on for a considerable number of years and have been both quantitative and qualitative. Unfortunately the methods of counting these organisms are laborious and in no case entirely satisfactory ; while the indirect dilution methods always give an under-estimation of the numbers, since they depend upon the assumption that all types will grow on one and the same medium at the same time, the more direct methods depend upon the equally unsound assumption that all the cells observed under the microscope are viable. Neither method discriminates between the different physiological groups. Nevertheless, there can be no doubt that both types of method yield valuable results, and that they have thrown considerable light on some aspects of bacterial behaviour both in soils and in cultures. It is firmly established that the numbers of bacteria in field soils change rapidly, and that these changes are to a very great extent independent of environmental conditions. While the general level of numbers is determined by the character of the soil, and largely by the amount of organic matter that it contains, the actual daily or hourly fluctuations seem to arise from intrinsic causes in the organisms themselves. There is, however, some evidence that other things being equal, a high moisture content tends to raise the bacterial numbers, and that a soil temperature of more than 50°F. has a slightly depressing effect, although in the laboratory most soil bacteria grow well at a higher temperature than this.

In the spring and the autumn there is a definite rise in the numbers of bacteria, which again can only be ascribed to intrinsic and not extrinsic causes. It is obvious since the numbers vary so much not only from day to day but from season to season that a single estimation of the numbers of bacteria is of little or no value. Nor is it easy to correlate the numbers of bacteria with the chemical changes that they bring about in the soil. This is for the two-fold reason that under field conditions there are numerous kinds of bacteria, and also many different chemical compounds which because they are constantly changing and inter-changing make a straightforward issue impossible. When, however, a sterilized soil to which a single bacterial species has been added is, for example, given a carbohydrate such as glucose the issue is clear and there is a direct

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correlation between the numbers of bacteria and the amount of carbon dioxide evolved. When the output of carbon dioxide from untreated field soils is considered a very wide range of results is obtained ; for example, samples taken from the farmyard manured plot of Broadbalk may give an average daily carbon dioxide production of as little as 0.006 grammes per 200 grammes of dry soil, or as much as 0.022 grammes, although in both cases the average bacterial numbers, as found by the plate method, are twenty millions per gramme of soil. Parallel investigations on such plots as the unmanured, or on plots receiving dressings of minerals with nitrogen as both nitrate of soda and ammonium sulphate, compared with the dunged plot suggest that there are comparatively small differences in the average daily output of carbon dioxide as between plots. Although it may seem a far cry from the heterogeneous community and environment of the soil to the laboratory pure culture, yet work on pure cultures is useful in attacking piece by piece the intricate problem of the soil as a whole. For example, in cultures there is a general agreement between the bacterial numbers and the amount of carbon dioxide produced, but though a rise in numbers is accompanied by an increase in the output of carbon dioxide, yet there is no exact numerical relationship between them, for the individual efficiency of each bacterium varies according to the numbers present in the medium and to their physiological condition. The more bacteria present the less efficient is each individual, and on the whole, they are less efficient producers of carbon dioxide when they are rapidly reproducing. Broadly speaking the same thing is true for efficiency in ammonification, and it seems probable that it applies equally to other chemical activities of soil bacteria.

On the whole work on cultures shows that the soil bacteria are for the most part active fermenters of sugars, both monosaccharoses and disaccharoses, and that they also grow well on salts of the common organic acids. It seems probable that the range of compounds from which one and the same species can obtain carbon, that is, which it can decompose, is a very wide one.

The behaviour of soil bacteria on nitrogen compounds is also characterized by this ability to utilize a wide range of compounds. The same organism may be able to ammonify, to make nitrite from the ammonia that it has itself formed, to utilize nitrite as a source of nitrogen for growth, and to reduce nitrate. The great majority can ammonify to some extent, and there are a fair number that can produce nitrite in small quantities from ammonium salts, both inorganic and organic, while a much larger number reduce nitrate to nitrite, and some carry the reduction further to ammonia, or even to nitrogen.

The question of the formation of nitrite from the various ammonia compounds is again a point on which light has been thrown by laboratory work on pure cultures. It is clear that there is a very much larger number of bacterial species than can produce nitrite in small quantities from ammonia than was previously supposed, and that they can carry out the reaction under very varied environmental conditions, but their behaviour too is variable and the factors that govern it are still obscure. One factor that influences the formation of nitrite and its removal by biological agencies is the carbon-nitrogen ratio. Thus, in cultures, a ratio higher than fifteen to one tends to be accompanied by the disappearance of nitrite without the production of nitrate or ammonia; while lower ratios lead to nitrite formation. For example, the nitrite production in a pure culture after four days' growth when the C/N ratio was half, was 3.2 grammes of nitrite nitrogen per million, while when the ratio was 2 : 1 the amount was 0.15.

From these heterotrophic bacteria the amounts of nitrite produced are small when the medium consists of simple inorganic ammonium salts, but when urine is provided, as the source of nitrogen, the quantity of nitrite formed is very much increased.

In the soil there are considerable quantities of nitrogenous organic compounds, which are probably equally available for the formation of nitrite; but even if this were not the case the numbers in which the nitrite organisms occur are sufficient to ensure the conversion of large quantities of ammonia to nitrite even though the individual contribution may seem to be extremely small.

The elucidation of the problems of soil bacteriology is further complicated by the presence of other micro-organisms; and, from the point of view of their interference with bacterial activities, the protozoa have received more attention than any other group. The soil structure provides a good environment for the life of numerous small amoebae and flagellates, and, in small numbers, ciliates such as Colpoda, are usually found.

More than 250 species of protozoa have been recorded and among these there are twenty-one that have not as yet been recorded from any other locality. There are, however, certain species that are practically ubiquitous, and no soil examined at Rothamsted has failed to yield a protozoan population, though the number of species in any one soil may be small.

Just as the bacteria fluctuate so also do the numbers of protozoa, and again there is no obvious correlation between their fluctuations and the changes taking place in their physical environment. Although apparently the highest numbers of protozoa tend to occur when the soil is both cold and damp, and further, under these conditions, there is the greatest amount of activity. There is, however, a very marked negative correlation between the numbers of bacteria of the types that will grow on nutrient agar plates, and the numbers of active amoebae present in the soil. Whether these amoebae feed indifferently upon all the bacteria that occur in soil, or whether they select among them is still unknown. It is certain that bacteria which are morphologically alike have very different nutritive values, as judged by their effect on amoebic growth, and there is a certain amount of evidence from cultural studies that the amoebae are able to select the food which they prefer. But the problem is very intricate for even when a bacterial species is itself readily eaten by an amoeba, the same bacteria when crushed in the liquid containing the products of their own growth, will inhibit the growth in a culture of the amoebae, and also appear to encourage premature encystment.

Since the majority of protozoa in the soil feed on bacteria, they will by their predactory action tend to keep the bacteria at a higher level of efficiency by actually reducing their density.

The soil then, not only because of its physico-chemical structure, but also from its population presents a very intricate problem. The various members of the community are continually acting and re-acting one with another ; the chemical compounds present in the soil are of diverse natures ; the bacterial species are equally diverse, so that at the present time it is almost impossible to disentangle the various end results of the micro-organisms' activities. Only by the laborious process of tracing out piece by piece the work done by the various groups is it possible to hope in the future to obtain a general picture of this, possibly one of the most interesting communities, and further one where the balance of the population is most delicately adjusted.

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