ROTHAMSTED RESEARCH

Report for 1934

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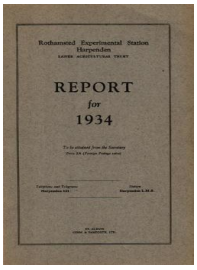

Review of the Work of the Bacteriology Department

Rothamsted Research

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General

Much time has necessarily been devoted to the improvement and standardisation of analytical methods.(19)

The Department has taken an active part in the cooperative investigations on soil analysis by Committees of the Agricultural Education Association(20), and of the International Society of Soil Science (Mechanical Analysis and Soil Reaction Committees for the Second Congress in Leningrad in 1930, and Organic Carbon and Soil Reaction Committees for the Third Congress in Oxford in 1935.)

Since 1921 the Head of the Chemistry Department has prepared an annual report on the progress of investigations on soils and fertilisers for the Society of Chemical Industry's "Reports on the Progress of Applied Chemistry."

REVIEW OF WORK OF THE BACTERIOLOGY DEPARTMENT

H. G. Thornton

The work that has been carried out in this department can be divided into three sections :—

The quantitative study of the bacterial population of the soil; investigation of various specific bacterial activities that occur in soil; investigations concerning the nodule organism and its relation to leguminous crop plants.

The Quantitative Study of the Bacterial Flora

The study of ecology of the soil micro-population at Rothamsted was initiated by the work of Russell and Hutchinson on partially sterilised soil, which led to the conclusion that an antagonism existed between certain groups of the population, particularly between soil bacteria and protozoa. Some results obtained by Russell and Appleyard at the same time indicated that the numbers of bacteria in soil were far from constant. It was therefore decided further to investigate these fluctuations in numbers, particularly to discover whether they were related in any way with the numbers of soil protozoa. Before this could be done, however, it was necessary to improve the existing technique of plate counting, which was clearly

(19) T. Eden—"A Note on the Colorimetric Estimation of Humic Matter in Mineral Soils." Journ. Agri. Sci., 1924, Vol. XIV, pp. 469-472; H. J. Page—"On the Perchlorate Method for the Estimation of Potassium in Soils, Fertilisers, etc." Journ. Agri. Sci., 1924, Vol. XIV, pp. 133-138; E. M. Crowther and W. S. Martin—"The Volumetric Determination of Total Carbonic Acid in Dilute Solutions of Calcium Bicarbonate." Journ. Chem. Soc., 1924, Vol. CXXV, pp. 1937-1939; E. M. Crowther—"The Determination of Hydrogen Ion Concentration." The Chemists' Year Book, 1928, pp. 610-629; E. M. Crowther and J. K. Basu—"Note on a Simple Two-compartment Electrodialysis Cell for the Determination of Exchangeable Bases." Trans. 2nd. Comm. Inter. Soc. Soil Sci., Budapest, 1929, Pt. A., pp. 100-102; V. Subrahmanyan—"Determination of Soluble Carbohydrates, Lactic Acid and Volatile Fatty Acids in Soils and Biological Media." Journ. Agri. Sci., 1929, Vol. XIX, Pt. IV, pp. 649-655; V. Subrahmanyan—"An Improved Method for the Determination of Dissolved Oxygen in Water." Journ. Agri. Sci., 1927, Vol. XVII, Pt. IV, pp. 468-476; R. G. Warren and A. J. Pugh—"The Colorimetric Determination of Phosphoric Acid in Hydrochloric Acid and Citric Acid Extracts of Soils." Journ. Agri. Sci., 1930, Vol. XX, pp. 532-540; J. K. Basu—"Studies on Soil Reaction VII. An Electrodialysis Apparatus for the Determination of Replaceable Bases in Soils." Journ. Agri. Sci., 1931, Vol. XXI, pp. 484-492; E. Troell—"The Use of Sodium Hypobromite for the Oxidation of Organic Matter in the Mechanical Analysis of Soils." Journ. Agri. Sci., 1931, Vol. XXI, pp. 476-483; E. M. Crowther and K. Troell—"Oxidation of Organic Matter in the Pretreatment of Soils for Mechanical Analysis." Proc. 2nd. (1930) Intern. Congr. Soil Sci., Comm. I, 1932, pp. 48-51, pp. 253-255. A. Walkley and I. Armstrong Black—"An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method." Soil Sci., 1934, Vol. XXXVII, pp. 29-38.

(20) E. M. Crowther (with R. Stewart)—"Report of the Analysis of Soils Sub-Committee of the Agricultural Education Association." Agric. Prog., 1934, Vol. XI, pp.1 06-114.

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subject to large errors, for the estimation of which no adequate statistical method existed.

1. *Improvement and Testing of the Plate Technique.*

The earlier plate counts of soil bacteria had been made either upon the gelatine medium which gave inaccurate results owing to liquefaction, or else on agar media containing a high proportion of organic matter. On such agar media great difficulty was experienced owing to the development of spreading colonies on the plates. The investigation of such spreading colonies showed that the spreading was due to active motility of certain bacteria over the surface of the agar plate and that it could largely be checked by employing a synthetic medium of such a composition that growth of these organisms was delayed during the early stages of incubation.(1)

A satisfactory synthetic medium having been developed, a series of tests were made with it to determine the degree of accuracy obtainable and to find out at which stages in the plating technique errors occurred.

It was always recognised that the plate method can give results of only comparative value and that counts on a single plating medium cannot give any idea of the total content of bacteria, since these belong to diverse physiological groups. For such comparative purposes it was shown that, with a carefully standardised technique, the errors due to shaking, diluting the soil and pouring the plates were negligible when compared with those shown between parallel plates of the final dilutions. Such errors are due to two causes: (a) the random distribution of bacteria in the suspension, equal volumes of which are added to each plate, and (b) irregularity in development of colonies in the plate. It was found that in the great majority of counts the whole detectable error was due to random distribution but that in a few cases the irregular development of colonies increased the error. Fortunately it was found possible to detect such departures from random sampling by testing the variance between parallel plates. A simple statistical test was developed for this purpose by the Statistical Department working in collaboration with this department.(2) Thus when parallel plates agree satisfactorily, the error depends directly on the number of colonies counted and any greater variance, causing the results to be questionable, is readily detected by a simple test. The plating technique was thus placed on a sound statistical basis before further quantitative work was attempted.

2. *Short-period Fluctuations in Bacterial Numbers as found by plating.*

The problem was then taken up by workers in the Microbiology Department whose work is elsewhere described. Using the plate method, they found that bacterial numbers in a field soil showed striking fluctuations from day to day. Fluctuations in protozoal numbers were also found: those of active amoebae showing an inverse relationship to the bacteria in about 80 per cent. of cases.

The problem was then taken up in the Bacteriology Department

(1) H. G. Thornton—"On the Development of a Standardised Agar Medium for Counting Soil Bacteria, with especial regard to the Repression of Spreading Colonies." Annals Appl. Biol. **9**, 1922, 241.

(2) R. A. Fisher, H. G. Thornton, and W. A. Mackenzie—"The Accuracy of the Plating Method of Estimating the Density of Bacterial Suspensions." Annals Appl. Biol., **9**, 1922, 325.

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where it was shown that large fluctuations in bacterial numbers occurred between samples taken at two-hourly intervals during the day and night. These fluctuations usually bore no relation to either moisture or temperature of the soil.(3)

3. *Development of Microscope Count Method for Counting Bacterial Cells in Soil.*

The work described above was carried out by means of the plate method, but it was recognised that this method enabled only a small fraction of the bacterial flora to be measured and did not enable one to determine whether fluctuations affected the whole bacterial flora or were confined to a fraction comprising organisms adapted to grow on the medium used for plating. The only means of measuring the total bacterial flora is that of direct microscopic examination of soil films. A suitable staining technique for enabling bacteria to be distinguished in such films was developed by H. J. Conn in America and further improved by S. Winogradsky in France and by the Bacteriology Department at Rothamsted. Two very serious difficulties had, however, prevented this staining technique from being developed into an exact quantitative method. These were (a) the difficulty of estimating with any accuracy the minute mass of soil examined in a thin film with an oil immersion objective and (b) the fact that the bacteria were not distributed at random over that film from which microscope fields had to be taken as samples.

These difficulties were eventually overcome by means of a ratio method, the principle of which is as follows.(4) A suspension of indigo particles averaging one micron in diameter is made up, and the number of particles per cubic millilitre is determined by means of a haemocytometer count. A known mass of soil is shaken up in a known volume of this counted suspension. Films of the resulting mixture are prepared and stained. The bacteria and indigo particles are counted in a suitable number of random microscope fields and the ratio of bacteria to indigo is determined. Since the absolute number of indigo particles added per gram of soil is known, the numbers of bacterial cells is calculable from the ratio. The calculation is, of course, independent of the quantity of soil in the film and since the indigo and bacteria are similarly disturbed by the surface tension forces during drying, it is found that the ratios of bacteria to indigo show a random distribution over the film.

The method was subjected to the following tests. (a) Bacterial numbers found in four portions of a single soil sample agreed with a standard error of 3.3 per cent. (b) The results of different workers counting and preparing the films agreed as random samples. (c) Counted suspensions of pure cultures of bacteria added to sterilised soil were estimated with a standard error of 3.5 per cent.

The numbers of bacteria found in soil by this method are of the order of one hundredfold those found by plate counts, and range from 1,000 to 4,000 million per gram. An exploration of some of the plots on Hoos field indicated a connection between the numbers found by the microscope method and the yield of the plots.(4)

(3) H. G. Thornton and P. H. H. Gray—"The Fluctuations of Bacterial Numbers and Nitrate Content of Field Soils." Proc. Roy. Soc. London, Ser. B, **106**, 1930, 399.

(4) H. G. Thornton and P. H. H. Gray—"The Numbers of Bacterial Cells in Field Soils, as estimated by the Ratio Method." Appendix by R. A. Fisher. Proc. Roy. Soc. London, Ser B, **115**, 1934, 522.

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4. *Fluctuations in the Numbers of Bacterial Cells in Soil.*

The above-described method was then applied to the study of the fluctuations in bacterial numbers in soil. It has been found that the total numbers of bacterial cells showed marked fluctuations between samples taken at daily and also at two-hourly intervals ([4,5]). These fluctuations did not as a rule agree with those found by plate counts from the same samples.

There was usually no correlation between the fluctuations and either temperature or moisture. Significant fluctuations have been found in soil stored in an incubator at constant temperature and moisture. Soil sterilised and supplied with a mixed culture of soil bacteria also showed significant fluctuations in bacterial numbers. This proves that protozoa are not the only cause of fluctuations although in a single experiment in which two-hourly counts of amoebae and bacteria were made, an inverse relationship between them was found.

It seems likely that a somewhat delicate equilibrium exists in the soil both between different groups of bacteria and between bacteria and other forms, and that if a disturbing factor upsets this equilibrium, a series of fluctuations is set up.([5])

Investigations Concerning Special Groups of Bacteria

1. *Cellulose-Decomposing Organisms.*

The study of cellulose-decomposing bacteria began with Hutchinson and Clayton's discovery of the remarkable *Spirochaeta cytophaga*. Since then a considerable number of aerobic cellulose-decomposing bacteria have been isolated and described by workers in the Department. They include one of the few known agar-liquefying bacteria, *Microspira agar-liquefaciens* ([6]) and some interesting bacteria producing reducing sugars from cellulose ([7]). An investigation was made to discover what types of organisms were active in decomposing cellulose in natural soil and it was concluded that the nature of the cellulose decomposing flora was determined by the soil's reaction. In acid soils the cellulose was attacked by fungi, in very slightly acid soils principally by *Spirochaeta cytophaga* and in neutral and alkaline soil by a variety of non-sporing bacteria.([8])

Related to this study was an investigation of the bacteriology of farmyard manure decomposition in soil. This showed that the early stages of decomposition are accompanied by a rise in bacterial numbers and by a delay in the production of nitrate or even by an assimilation of soil nitrate. This period probably represents the decomposition of carbon compounds by bacteria which are assimilating nitrogen compounds. There is then a fall in bacterial numbers and a large increase in nitrate which is probably derived from the decom-

(5) C. B. Taylor—Unpublished Thesis. Univ. London, 1935.

(6) P. H. H. Gray and C. H. Chalmers—" On the Stimulating Action of Certain Organic Compounds on Cellulose Decomposition by means of a New Aerobic Micro-organism that attacks both Cellulose and Agar." Ann. Appl. Biol., **11**, 1924, 324.

(7) A. Kalniņš—" Aerobic Soil Bacteria that decompose Cellulose." Acta Univ. Latviensis, Lauksaim. Fakult., Ser. I. **11**, 1931, 221.

(8) H. L. Jensen—" The Microbiology of Farmyard Manure Decomposition in Soil. II. Decomposition of Cellulose." Journ. Agric. Sci., **21**, 1931, 81.

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position of bacterial cells.(9) It was found that bacterial protoplasm is, in fact, readily nitrifiable in soil. (10)

2. *Bacterial Decomposition of Aromatic Antiseptics.*

This work arose from a difficulty experienced by growers of glass-house crops who used such compounds as cresylic acid and naphthalene to destroy soil pests, but found that these compounds rapidly disappeared from soil. The problem was taken up in the Bacteriology Department where a large number of bacteria were isolated which could decompose phenol, *m*-cresol, *p*-cresol, *o*-cresol, toluene, and naphthalene, and could utilise these compounds as their only source of energy.(11)

3. *Bacterial Production of Indigotin.*

In the course of the work with aromatic compounds, two organisms were isolated which could oxidise indol to indigotin.(12) These are of some interest to soil bacteriologists as they explain the fate of indol, of which appreciable amounts are added to soil in manure.

Investigations Concerning the Nitrogen-Fixing Bacteria from the Nodules of Leguminous Plants

A large part of the work of the Department has been devoted to this subject. It began with a study, in soil, of the life cycle of the nodule organism which had been investigated in artificial culture by Bewley and Hutchinson.

1. *Life Cycle of the Nodule bacteria in Soil and the Development of Lucerne seed inoculation.*

The nodule organism from lucerne (*Medicago sativa*) when grown in sterilised soil was shown to pass through the same life cycle already found to exist in artificial media. This cycle contains a stage during which the organisms develop flagellae and are actively motile. It was found that during this motile stage the organisms were capable of migrating through Rothamsted soil at a rate of one inch in 24 hours. The addition of minute amounts of calcium di-hydrogen phosphate to the soil along with the bacteria stimulated the production of the motile stage and hastened the migration through soil.(13)

This result was seen to have a bearing on current methods of legume seed inoculation. These in general consisted in wetting the seed before sowing with a suspension of the appropriate nodule bacteria in some liquid. When inoculated seed is sown the bacteria migrate from the seed into the soil and reach the roots, which they infect to form nodules. It seemed clear that the addition of the acid calcium phosphate, by causing an increased motility of the bacteria in the soil, should increase the chances of root infection. Experiment confirmed this expectation.

(9) H. L. Jensen—" The Microbiology of Farmyard Manure Decomposition in Soil. I. Changes in the Microflora, and their Relation to Nitrification. Journ. Agric. Sci., **21**, 1931, 38.

(10) H. L. Jensen—" The Microbiology of Farmyard Manure Decomposition in Soil. III. Decomposition of the Cells of Micro-organisms." Journ. Agric. Sci., **22**, 1932, 1.

(11)—P. H. H. Gray and H. G. Thornton—" Soil Bacteria that decompose certain Aromatic Compounds." Centrbl. f. Bakt. II, **73**. 1928, 74.

(12) P. H. H. Gray—" The Formation of Indigotin from Indol by Soil Bacteria." Proc. Roy. Soc., London. Ser. B, **102**, 1928. 263.

(13) H. G. Thornton and N. Gangulee—" The Life Cycle of the Nodule Organism. *Bacillus radicicola* Beij., in Soil and its Relation to the Infection of the Host Plant." Proc. Roy. Soc. London. Ser. B. **99**, 1926, 427.

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In pot experiments with lucerne the addition of calcium dihydrogen phosphate to the inoculating fluid approximately doubled the number of nodules that were produced. (13)

The new method of seed inoculation thus developed was applied on a field scale to the inoculation of lucerne. Previous attempts at inoculating the lucerne crop in this country had met with indifferent success, although there was reason to suspect a deficiency of lucerne nodule bacteria in the soil over the greater parts of the country. Field experiments, financed by the Royal Agricultural Society and carried out by numerous experimenters, showed a large benefit from the new method of inoculation at most centres outside the South Eastern Counties and proved that good crops could be obtained in most parts of England from inoculated seed. (14) The inoculation of lucerne has now been placed on a commercial basis and some 4,000 acres of inoculated lucerne are sown annually.

2. *Clover Inoculation and the Problem of Strain Competition.*

Recently the more difficult problem of clover inoculation has been studied. This problem was set to us by the Welsh Plant Breeding Station in connection with their attempt to improve the feeding value of upland pastures. On these pastures clover usually does badly.

Examination of the soil from such localities revealed the existence of a strain of nodule bacteria which, though forming large numbers of small nodules, produce little or no benefit to the plant. Similar strains had already been described in America. The presence of these strains in the soils gives rise to a peculiar difficulty affecting seed inoculation with beneficial strains. They tend to prevent the entry of beneficial strains into the plant. This unfortunate characteristic was shown to be possessed by the Welsh inefficient strain.

The problem, therefore, is to find an efficient strain of clover nodule bacteria of sufficient infective virulence to compete against the presence of the inefficient strain. A good strain has been found which is only slightly affected by the presence of the Welsh strain.

The competition effect of the inefficient Welsh strain and the action of this virulent good strain (here called strain A) was illustrated by a sand culture experiment with Alsike Clover in which all the pots were supplied with a mixed flora of local Rothamsted races of clover nodule bacteria while half of them received in addition a suspension of the Welsh inefficient strain.

Where the local nodule bacteria were alone present the nitrogen fixed by the clover averaged 275 milligrams per pot. Where these had to compete against the Welsh strain, nitrogen fixed by the clover added to the sand was reduced to 34 milligrams. Where seed was "inoculated" with Strain A, however, the harmful effect of the Welsh strain was partly overcome and the nitrogen fixed amounted to 112 milligrams. Beneficial results have been obtained in field trials in Wales with Strain A. These results are shown in the following table :

(14) H. G. Thornton—"The 'Inoculation' of Lucerne (*Medicago sativa* L. in Great Britain." Journ. Agric. Sci. **19**, 1929, 48.

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Effect of Welsh strain and of Strain A on milligrams of nitrogen fixed by Alsike Clover grown in sand.

	Seed not Inoculated	Seed Inoculated Strain A
Sand with only local Nodule Bacteria	275.2	318.0
Sand with local Nodule Bacteria plus Welsh Strain	33.8	111.6

3. *The Infection of the Legume Root.*

Nodules are rarely found on seedlings of lucerne and clover in the cotyledon stage but their appearance is closely associated with the development of the true leaves. Evidence was found that at this stage the roots of the plant secrete some substance stimulatory to the growth of the bacteria. Nodules can indeed be induced to form on seedlings in the cotyledon stage if these are watered with the solution from around the roots of older plants.(15)

The actual mechanism of infection has been investigated. Infection takes place through the root-hairs and is preceded by a characteristic deformation of these hairs. Numerous observations have shown that this preliminary deformation of the root-hairs is necessary to enable infection to take place. It was shown that the deformation was caused by a bacterial secretion and that it could be produced by a cell-free filtrate of the bacterial secretions. It is an interesting fact that the action of the secretions from a given strain of nodule bacteria is not specific to the particular legume species which that strain is capable of infecting. Thus although lucerne nodule bacteria cannot infect clover roots, the secretions of lucerne bacteria can deform clover root-hairs.(16) The nature of the active substance is being investigated. It is thermostable and from the secretions a gum can be precipitated with acetone. In its crude form this gum is also active, but it seems likely that the active substance is some other compound associated with the gum.

4. *The Action of Nitrates on Nodule Formation.*

The study of the root-hair curling has provided an explanation of a phenomenon which had for a long time remained a problem. If inoculated legumes are grown in media rich in nitrates or ammonium salts, nodule formation is harmfully affected and may be stopped entirely.

An experiment in which inoculated lucerne was grown in sand supplied with a range of doses of sodium nitrate, showed that both the number and the size of the nodules were progressively reduced with increasing dosage of nitrate. The nitrate thus produced a twofold effect ; firstly upon the infection by the bacteria and, secondly, upon the growth of such nodules as are formed. These two effects were separately investigated.

(15) H. G. Thornton—" The Role of the Young Lucerne Plant in Determining the Infection of the Root by the Nodule forming Bacteria." Proc. Roy. Soc. London. Ser. B, **104**, 1929, 481.

(16) E. F. McCoy—" Infection by *Bact. radicicola* in Relation to the Microchemistry of the Host's Cell Walls." Proc. Roy. Soc. London. Ser. B, **110**, 1932, 514.

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(*a*) *Effect of Nitrate on Root-hair Infection.*

When lucerne was grown in agar containing 0.1 per cent. $NaNO_3$, not only was nodule formation stopped but no infection of the root-hairs took place. This was accounted for by the fact that the nitrate inhibited the deformation of the root-hairs by the bacteria, which is a necessary prelude to infection.

Sodium nitrate at that concentration did not harm the bacteria or prevent them from producing their root-hair curling secretions. On the other hand the bacterial secretions separated from the bacteria by filtration were largely prevented from deforming the root-hairs when these were grown in the presence of sodium nitrate of the above concentration. The results are shown in the following table :

Influence of sodium nitrate upon the deformation of lucerne root hairs by nodule bacteria and by their secretions.

	Percentage of Deformed Root Hairs.
A. Plants grown in Agar without Nitrate.	
1. Plus Nodule Bacteria	80.3
2. Plus Bacterial Secretions ..	71.8
B. Plants grown in Agar with Nitrate.	
1. Plus Nodule Bacteria	14.1
2. Plus Bacterial Secretions ..	12.8

(*b*) *Effect of Nitrate upon Nodule Growth.*

When lucerne plants bearing young nodules were transplanted into a medium containing 0.1 per cent. $NaNO_3$ the growth of the nodules was slowed down or entirely checked. Microtome sections of such nodules showed that the growing cap at the end of the nodule was often walled off by the formation of a layer of cells with thickened walls.

5. *Effect of Nitrate on a Mixed Crop.*

There are so many occasions when legumes and non-legumes are grown in association that the influence of nitrate manuring on such a mixture is of great agricultural importance. A sand culture experiment was made in which three doses of sodium nitrate were applied to (a) lucerne grown alone and (b) lucerne growing with Italian Rye grass.

It was found that the yield and nitrogen content of the lucerne when grown alone were unaffected by the nitrate dose, but where lucerne and grass were grown together, the yield and nitrogen content of the lucerne and even of the combined crop were inversely related to the dosage of nitrate applied because the growth of the lucerne was adversely affected by competition with the more rapidly growing grass.(17)

6. *Uptake of Nitrogen by Grass Associated with Lucerne.*

In the above experiment and in a confirmatory trial, grass growing

(17) H. G. Thornton and Hugh Nicol—" The Effect of Sodium Nitrate on the Growth and Nitrogen Content of a Lucerne and Grass Mixture." Journ. Agric. Sci., **24**, 1934, 269.

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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.

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