ROTHAMSTED RESEARCH

Report for 1939-45

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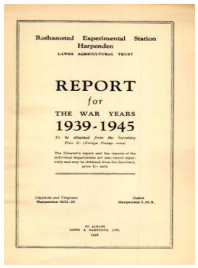

Soil Microbiology Department

H. G. Thornton

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DEPARTMENT OF SOIL MICROBIOLOGY

By H. G. Thornton

Origin of the soil microbiology department

The present department was formed during the period under review by amalgamation of the earlier Bacteriology and General Microbiology Departments, shortly after the death of Mr. D. Ward Cutler, in January, 1941. The loss that his death entailed to Rothamsted and to soil microbiology in general is well appreciated by all who are acquainted with his work and particularly by those who had the honour to be his colleagues. Under his leadership the General Microbiology Department, started in 1919 for the particular study of soil protozoa, had extended its interests, as was inevitable, to cover other component groups of the soil micro-population, so that by 1941 no logical distinction in fields of work divided it from the Bacteriology Department and fusion of the two departments was a reasonable development.

This fusion was greatly facilitated by an important new environmental factor; in that year Bacteriology moved from its old home in the James Mason Laboratory into the new laboratories which had been built for it in the New Wing and which were designed to be continuous with those of the General Microbiology Department. These new laboratories, though completed in 1939, were not in fact occupied by the Bacteriology Department until 1941, because they were taken over until then by the Department of Public Health as an emergency war-time measure. Moreover the new combined department lost the use of four of its rooms. Two of them provided temporary quarters to Professor J. B. S. Haldane, F.R.S. and the Genetical Department of London University, welcome guests whose laboratories in University College had suffered bomb damage. One room similarly helped to house the Leather Research Association workers who were in the same plight. The fourth room was occupied by Dr. J. H. Quastel, F.R.S., Head of the A.R.C. Unit of Soil Metabolism. With the return of these rooms for the use of the Department a considerable increase in the number of workers has been possible.

Staff

The following Scientific Staff have worked in the Department or in the two Departments from which it arose, during the period under review, and are still working here unless otherwise stated.

Permanent Staff

D. Ward Cutler, M.A., formerly Head of the Department of General Microbiology, died January, 1941.

H. G. Thornton, B.A., D.Sc., F.R.S., formerly Head of the Bacteriology Department, now of the combined Soil Microbiology Department.

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Lettice M. Crump, M.Sc.

Jane Meiklejohn, Ph.D. Joined the W.A.A.F. November, 1942. Demobilised, 1945.

P. S. Nutman, B.Sc., Ph.D., A.R.Sc.

Workers receiving grants from the Agricultural Research Council

Janina Kleczkowska, Eng.Agric., Ph.D., came in 1939.

Dagny Oxford, B.A., came in 1944.

B. N. Singh, M.Sc., Ph.D., came in 1938.

Janet Mollison, B.Sc., Ph.D., came in 1944.

P. C. T. Jones, B.Sc., A.R.Sc., came in 1944.

Workers receiving scholarships

H. K. Chen, B.Sc., Ph.D., came in 1937, left in 1940.

A. Vasco Garcia, Eng.Agric., B.Sc. (British Council Scholar), came in 1944.

S. Bhaduri, M.Sc. (Ghosh Scholar), came in 1944.

In addition to the above the Department is grateful for valuable help from a number of temporary and part-time workers, especially from Mrs. Kalmus and Mrs. Derry.

Scientific work

In the account which follows no attempt is made to separate the work of the combined Soil Microbiology Department from that of its two predecessors because the latter were already working on lines complementary to each other at the time of their amalgamation so that no radical change in programme was then found to be needed.

In the Soil Microbiology Department and its precursors, two main lines of research have been followed, first the study of the soil micropopulation as a whole and of the inter-relationships of its main constituent groups, and secondly investigations dealing with the nitrogen-fixing bacteria of leguminous plants. Other special investigations have been concerned with organisms that attack resinous substances incorporated in soil for making roads, with the occurrence of mycorrhizal associations in crop plants, with the assay of thiamin in potatoes, and with the toxicity to plants of 2 : 4-dichlorophenoxyacetic acid.

A. Soil micropopulation

In studying the soil micropopulation there is need for knowledge of the inter-relationships of the different groups and of the " micro-ecology " of the soil generally. Classical researches on soil bacteria have largely dealt with isolations studied in pure culture and too often assumptions have been made as to the production of chemical changes in the soil by organisms producing such changes *in vitro*, sometimes even without evidence that these organisms are active in soil. A proper understanding of biochemical changes in soil must, however, be based on further knowledge as to which organisms are in fact active in field soil and why they are able to maintain their active existence in the face of acute competition from other groups. For this reason the problem of competition between different

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groups of the soil population has been constantly borne in mind in planning research.

The development of adequate methods for counting the individuals of different groups of the soil population is an obvious preliminary to the study of soil micro-ecology so that special attention has been paid to the development of new, and improvements in existing, technique.

1. *Direct microscopic examination of soil*

The fact has long been appreciated that the enumeration of bacteria, actinomycetes and fungi in soil when based on a plating technique necessarily gave counts that represented but a small fraction of the soil population. This is because no medium will support the growth of all the physiological groups of organisms differing so much in their food requirements. Moreover, in the case of fungi and actinomycetes the difficulty of knowing whether colonies arise from hyphae or from spores invalidates such counts as a measure of active mycelium in the soil. To meet this difficulty various methods have been evolved for the direct microscopic examination of stained films of soil and for the counting of bacterial cells in them. These methods have usually involved the staining of films of soil suspension dried on a slide or cover-slip and stained with one of the eosin dyes. The only method that had given satisfactory results on statistical tests when applied to the enumeration of bacterial cells in soil was the ratio method of H. G. Thornton and P. H. H. Gray (1934, Proc. Roy. Soc. Ser. B., Vol. 115, p. 522). This is based on an estimate of the relative numbers of bacterial cells and indigo particles, a known number of the latter having been mixed with a suspension of the soil sample. The erythrosine dyes, however, give a weak stain to many organisms in soil and are unsuited to staining fungal and actinomycete mycelium, while drying of the film makes it difficult to observe all the bacterial cells embedded in small soil aggregates. A new quantitative method has now been evolved by Miss J. Mollison and Mr. P. C. T. Jones in which films of a suspension of the soil in agar are mounted and examined in euparal. The method at present used is as follows. A known quantity of soil is shaken with a known volume of melted 1.5 per cent. agar. A drop of the mixture is allowed to solidify on a haemocytometer slide of 0.1 mm. depth to produce a film of known thickness. The resulting film is transferred to a microscope slide, stained in acetic aniline blue and examined in euparal. Counts of micro-organisms are made in 20 random microscope fields on each of 4 replicate slides. The volume of the 20 fields is readily calculated and from this the number of bacteria per gram of soil can be obtained. In a test of the method, bacterial counts from duplicate samples of soil each examined independently by two workers gave a mean count of 3,334,000,000 bacterial cells per gram with a standard error of 5·9 per cent. When a previously counted suspension of bacteria was mixed with sterilised soil the numbers " recovered " were 93 per cent. of those added.

The method can also be used to estimate fungal and actinomycete

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mycelium, which stains excellently by it. Here the fragments of hyphae are counted and measured. In a test comparison of replicate soil samples, a mean length of 393 metres of mycelium was found per gram of soil, this estimate having a standard error of 10.45 per cent. Both the above estimates were made from Rothamsted allotment soil. That for bacteria is of the same order as those previously obtained from the soil by the ratio method with added indigo particles. No previous estimates of fungus mycelium in soil by direct measurement have been made. The estimated length of 393 metres represents a total volume of 0.69 mm.^{3} assuming the mycelial hyphae to have a mean diameter of 1.5μ. This compares with the 3.3 mm.^{3} per gram represented by the 3.3×10^9 bacteria found in the same soil.

2. *Plating technique*

Although estimates of total numbers of bacteria and quantity of mycelium in soil can be obtained only by direct examination, such methods do not enable the organisms to be isolated and studied. Nor can any division into physiological groups be obtained by them. For this reason the complementary information yielded by plating and dilution methods is still needed. A fresh study has therefore been made by Mr. A. Vasco Garcia of the statistical errors inherent first in taking samples from the field and secondly in the various stages of the plating technique.

3. *Survey of the micro-population of differently manured plots*

This survey has been undertaken with the object of determining what differences occur in the soil micro-population in plots given farmyard manure and artificials. Bacterial colony counts on two plating media, and estimates, based on dilutions, of cellulose-attacking organisms and of amoebae have so far been made from plots of Barnfield (mangolds) that received (*a*) no manure or fertiliser, (*b*) farmyard manure and (*c*) complete minerals and ammonium sulphate. The results indicate that whereas farmyard manure has considerably raised the level of the soil micro-population owing doubtless to its supply of energy material, the treatment with artificials has also increased numbers of both bacteria and amoebae relatively to the no-manure plot. This confirms data previously obtained by direct counts of bacterial cells, in showing that such artificial manuring, after many years of application, has increased rather than decreased the population level of the soil micro-organisms.

An attempt is being made to divide the bacteria found on platings from these plots, into physiological groups, using a modification of the methods developed in Canada by Lochhead.

4. *Characterisation of common soil bacteria*

A survey has been made by Miss L. M. Crump of the commonly occurring bacteria that appear on dilution platings of soil. Fifty-three soil samples comprising both arable and pasture were examined. Sixteen strains of bacteria were found to occur in at least ten of these samples, seven of them occurring in at least twenty samples. Different strains tended to be predominant in arable and grassland

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respectively, although a few strains were common in both situations. The soil reaction also affected the abundance of certain strains. The cultural characters of these sixteen common strains have been compared with those of a number of relatively rare strains obtained from the same soils. But the abundant could not be separated from the " rare " types by any common feature in their morphology or in their physiological behaviour.

The problem of why these particular bacteria predominate in soil in the field raises the important question of competition between groups of the micro-population. Dr. B. N. Singh has determined the multiplication rates in soil of a number of common and rare soil bacteria. There is a tendency for the former to have higher rates of increase in sterilised soil, but there are exceptions to this rule. Other factors must also be looked for and competition or inhibition by other organisms is obviously suggested. This problem is being studied with regard to several groups of organisms.

5. *Differential feeding of soil protozoa*

It was known from the work of Cutler, Crump and their colleagues that the numbers of bacteria in soil are kept down by protozoa and that their numbers fluctuate inversely to those of soil amoebae. (D. Ward Cutler, L. M. Crump and H. Sandon, **1922**. Phil. Trans. Roy. Soc. London, Ser. B., Vol. **211**, pages **317-350**). Dr. B. N. Singh has found that amoebae and holozoic flagellates obtained from soil are very differential in their bacterial food requirements (**15, 16, 17**). Some bacterial strains are readily edible by all soil protozoa tested, others are inedible to some or all of these protozoa, which will die if supplied with a pure culture of such bacteria. Bacteria producing pigments other than yellow or orange were nearly all found to be inedible to amoebae (**19**). Some bacteria are not only inedible but produce secretions that are highly toxic to amoebae (**18, 19**). The existence of bacterial strains edible, inedible and toxic to protozoa clearly means that the latter can affect both the total numbers and also the quality of the soil bacterial population by differential feeding.

6. *Estimation of protozoal numbers in soil*

This discovery of edible and inedible bacteria has enabled Dr. B. N. Singh to develop a much more reliable method for estimating numbers of protozoa in soil than the method previously used, which consisted in applying a range of soil dilutions to poured plates of " nutrient " (peptone-meat extract) agar and counting the number of plates that produced a growth of protozoa. The bacterial food was derived from such bacteria as developed from the soil suspension. An evident source of error lay in the possibility that a protozoan added to a plate might come to lie amongst bacteria inedible or toxic to it. In the present technique, plates of plain agar are poured and a pure culture of an edible bacterial strain is spread on the plate surface in the form of patches each surrounded by a glass ring. These patches are then inoculated from the soil dilutions. Eight such patches can be fitted into one petri dish thus giving a good replication with an economy of plates. By this method a protozoan if present in the inoculum is assured of a suitable bacterial

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food and will not be exposed to toxic bacterial secretions. The number of protozoa is estimated by the number of patches in which they develop at each dilution. A statistical examination has been made of the results of this method with satisfactory results.

7. *Occurrence of giant rhizopods in soil*

Certain giant multinucleate rhizopods have from time to time been observed in soil by previous workers, but were believed to be rare and of little importance in the soil economy. The use by Dr. B. N. Singh of edible bacteria for cultivating and counting soil protozoa has now realed the occurrence of such a rhizopod in the soil of our classical plots in numbers of the order of 1,000 per gram. Since this organism can attain a volume some 250,000 times that of a soil amoebae it seems likely to be of some importance in affecting the bacterial population of the soil.

8. *Distribution and behaviour of Acrasieae in soil*

This very interesting group of organisms was known to occur in soil, but was previously believed to be adventitious and derived from manure : nothing was known of its distribution. Dr. B. N. Singh's method of culture has enabled him to study the distribution of Acrasieae and roughly to estimate their numbers in soil. They occur in nearly all arable soils examined both from our own plots and elsewhere, but are much less abundant in grassland soils. Their occurrence in plots treated over a long period with artificials only shows that they are normal soil inhabitants and not dung organisms. They can pass through all the stages of their life-cycle in sterilised soil inoculated with edible bacteria, and cause a marked reduction in the numbers of the latter. Both Acrasieae and giant rhizopods are differential in bacterial food requirements, but differ in some cases from amoebae in the types of bacteria that are edible to them. The type of bacterial food so greatly affects both the shape and the colour of the sporangium produced by Acrasieae as to invalidate the present classification of these organisms, which is based on these characters.

Myxomycetes have also been found to be easily obtained from soil by Dr. B. N. Singh's method.

9. *Soil myxobacteria*

The great interest of this group of organisms owing to their morphology and life-cycle has lately aroused the active interest of a number of workers in different countries. Dr. B. N. Singh has found that certain species are readily cultivated from soil by his method and can be found in many soils. As with the Acrasieae their occurrence in plots treated only with artificials disproves the common opinion that they are derived from dung. They occur in soil in numbers of the order of 70,000 per gram. The species studied produce extremely active proteolytic and bacteriolytic enzymes which can lyse many species both of gram-positive and gram-negative Eubacteria. When grown on agar media, the myxobacteria can readily attack living bacteria including gram-negative types.

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10. *Soil actinomycetes*

The actinomycetes form a numerically important section of the soil's micro-population, but technical difficulties have so far prevented us from knowing much about their numbers, habit of life or physiological activities in the soil. On agar media they show striking differences in habit of growth and pigment formation, but in the soil itself their growth is quite different and uncharacteristic. Dr. Dagny Oxford, in a study of their habit of growth in soil and sand culture, finds that this depends chiefly on physical properties such as particle or crumb size and moisture content. In the substance of the soil they occur as straggling mycelia with occasional sporing branches, while on the soil surface tufts of aerial mycelium are formed, which are quite different in appearance from their colonies on an agar surface. Under dry conditions in soil there is an enhanced development of aerial mycelium and spores, both of which possess a waxy coat which doubtless assists survival. This waxy coat makes a dispersed suspension of spores difficult to obtain and thus affects the counting of actinomycetes by the plate method.

Dr. Oxford has also studied the nutrient requirements of actinomycetes in a synthetic " soil " composed of sand and bentonite, and found that they need only very dilute solutions of nutrient salts and can obtain nitrogen from a highly resistent organic source. Many of the soil actinomycetes secrete bacteriolytic substances. Most of these act only on gram-positive bacteria. The nature of this specificity is being investigated.

11. *Mycorrhizal associations in crop plants*

It has been claimed that common agricultural crops benefit from mycorrhizal associations. There is, however, very little known as to the frequency of occurrence or functions of mycorrhizae in agricultural crop plants. Dr. Janet Mollison has made a search for them in clover and in wheat. She found a phycomycete fungus forming such associations in nearly all clover plants examined which were obtained from widely scattered localities in Great Britain. Similar mycorrhizae have also been found in wheat roots although in this case their occurrence seems to be more seasonal.

B. THE NODULE BACTERIA (*Rhizobium*) OF LEGUMINOUS PLANTS

The work on this subject has dealt mainly with the differences between strains of nodule bacteria, particularly those infecting clover, and with the problems arising therefrom. It was known that strains of these bacteria derived from a single host species differ greatly in the benefit that they confer on the host plant. Some strains produce nodules that fix very small amounts of nitrogen and confer no visible benefit on the host when grown in a nitrogen-deficient medium. These *ineffective* strains are particularly prevalent amongst clover nodule bacteria, and our preliminary survey suggested that such ineffective strains were to be found especially in poor hill pastures. An investigation was therefore commenced with the ultimate object of improving the clover content of such pastures, either by inoculation with beneficial (i.e. effective) strains of bacteria, or by so changing the soil conditions as to encourage more beneficial strains to develop.

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1. *Distribution of strains of clover nodule bacteria in Great Britain*

The first need was a knowledge of the geographical distribution of ineffective strains of clover *Rhizobium*, in order to know in what districts inoculation might help and also what soil conditions encourage the development of beneficial and ineffective strains, respectively. A survey of the distribution of strains of clover *Rhizobium* over Great Britain has therefore been undertaken. Clover plants have been collected from districts covering most of Great Britain, and from the nodules on these plants isolations of the bacteria were made. Each strain isolated has been tested for effectiveness in fixing nitrogen on clover grown aseptically in nitrogen-deficient agar medium. So far 463 strains have been isolated and tested and 126 others are under test. From 290 isolations obtained from Scotland, Wales and from the North and West of England, 28 per cent. were ineffective, 12 per cent. were intermediate in effectiveness and 60 per cent. were effective. From the South, Centre and East of England 173 strains have been tested; of these 9.2 per cent. were ineffective, 4.6 per cent. intermediate and 86.2 per cent. effective. The ineffective strains were mostly obtained from hill pastures, but so far it has been impossible to correlate their occurrence with any particular soil type.

2. *Serological characters of nodule bacteria*

During the course of the geographical survey described above a study was made of the serological behaviour of the strains isolated. The object of this investigation was to develop an additional means of classifying and identifying the strains, and also to see whether the effectiveness of a *Rhizobium* strain in fixing nitrogen was correlated with any serological character that might then be used as a quick means of selecting effective strains. In collaboration with Dr. A. Kleczkowski (8), 161 strains of *Rhizobium* from clover and 29 strains from plants of the pea group were tested for agglutination with antisera against four strains of clover *Rhizobium* and two strains from pea.

The bacterial strains tested were found not to contain any antigen common to the whole group. Some strains reacted with none of the antisera, some with several of them, but none with all of them.

Strains entirely unrelated in antigenic structure could be found amongst those isolated either from clover or pea. On the other hand some strains from peas showed close resemblance in antigenic reactions to some derived from clover. Classification by antigenic structure thus fails to support the separation of the clover and pea nodule bacteria into different species. Nor was any necessary association found between the antigenic structure and effectiveness in fixing nitrogen or susceptibility to bacteriophage. Indeed an effective strain and a very ineffective variant from it were found to be serologically identical. The knowledge gained of the serological behaviour of clover and pea nodule bacteria has since been of much use in identifying strains introduced into a field soil and picked out again, as will be described later.

The serological relationship between clover and pea *Rhizobium* suggested that strains might be found that would infect both host

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plants. Tests of a number of strains were therefore made by Dr. P. S. Nutman and Dr. J. Kleczkowska at Rothamsted and by Dr. Bond at Glasgow (7). These tests revealed several strains capable of infecting both host plants, although in no case were the strains effective on the " wrong " host.

3. *Competition between strains*

Before there is much hope of improving the growth of clover by the inoculation of soils infected with ineffective strains, there are at least two difficulties that must be surmounted—namely (*a*) competition of the " wild " bacterial flora with the strain used to inoculate the soil and (*b*) lack of stability of effective strains in certain soils. These difficulties will be separately considered.

When a pea or clover plant is supplied with a mixture in equal numbers of two strains of *Rhizobium* both capable of infecting it, some 80 to 95 per cent. of the resulting nodules are usually produced by one of the two strains. In an investigation of this phenomenon Dr. H. Nicol and Dr. H. G. Thornton (12) showed that it resulted from a corresponding competition between the two strains in the surroundings *outside* the plant roots. No evidence of a differential resistance to infection by the plant roots could be found, but the presence of these roots stimulated the bacteria to multiply in their neighbourhood, whereupon the strain having the higher initial multiplication rate suppressed growth of the other strain. Similar competition between bacterial strains could be induced in the absence of roots by the addition of a source of energy.

This competition appears to be specially acute between strains capable of infecting the same host plant. It has an important bearing on the use of inoculation in the case of soils bearing ineffective strains. In the past, practical success with legume inoculation has most usually been obtained in cases where it was used to supply a legume crop with nodule bacteria capable of infecting it, in a soil from which these were absent often because the crop was of relatively recent introduction. Here, of course, there is no problem of competition with other strains capable of infecting the crop. The case is very different where we seek to replace a wild population of ineffective strains by a relatively small inoculum of a beneficial but otherwise very similar strain that will at once be subjected to inter-strain competition in the soil.

Fortunately when choosing strains for such inoculation under field conditions use can be made of the fact that strains differ specifically in their ability to compete with others in the soil. A strain found to be thus successful in laboratory tests has proved similarly able to maintain itself in the field. The study of the competition between two pure strains placed under laboratory conditions is relatively easy. But when a strain is used to inoculate seed, the competition to which it is exposed in the field soil is much more difficult to investigate. The main difficulty is that of identifying the individual strain inserted into the soil in the midst of a large and unknown number of wild strains most of which will be indistinguishable from it in culture. The extensive survey of serological reactions of clover bacteria outlined above has now given us a means of identification. For it is now possible to select, for

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seed inoculation, strains belonging to uncommon antigen groups and against which an antiserum has been prepared. After the clover has grown, isolations can be made from random nodules taken from inoculated and uninoculated plots and those isolations which are of the same strain as the seed inoculum can be identified by agglutination with the appropriate antiserum. In this way one can determine what percentage of the nodules in a field plot sown with inoculated seed has in fact been produced by the strain used to inoculate that seed. Experiments of this nature have been carried out at four centres in widely scattered districts with different soil types. Two strains of clover nodule bacteria were tested, both equally effective in pure culture. One of them produced an average of 59.1 per cent. of the nodules in plots sown with seed inoculated with it, the other strain produced an average of only 12.9 per cent. of the nodules, in competition with the wild strains.

Such results have an important bearing on the issue of cultures for seed inoculation for they show that, in choosing strains for this purpose, effectiveness in fixing nitrogen under laboratory or glasshouse conditions in pure culture is not an adequate criterion ; a suitable strain must also be able to survive competition with other strains in the field soil. The exact characters that give a strain this competitive superiority need investigation, but we have found that initial multiplication rate in sterile soil supplies a rough measure of a strain's competitive strength.

4. *Changes in effectiveness in pure strains*

The second difficulty confronting our attempts to improve clover by inoculation is the instability of *Rhizobium* strains in some soils. It has long been known that bacterial strains often show a marked tendency to change their characters. Our present knowledge of bacterial genetics is still primitive and such changes have the appearance of being of two kinds—slow adaptive changes and sudden changes resembling mutations. Changes in effectiveness of strains of *Rhizobium* had been observed before, and it was claimed that a slow adaptive change in effectiveness could be brought about by repeated passage of a strain through the host plant. If this were proved to be so it would have an important bearing on crop rotation. A very extensive test was made by Dr. P. S. Nutman in which an effective strain A was passed thirteen times through the clover plant, and during these passages attempts were made to change the strain by selection and isolation from large and small nodules and from plants deriving more or less benefit from the bacteria. Neither frequent plant passage nor deliberate selection had the smallest effect on the bacterial strain or on its action on the plant whether judged by its effectiveness in benefiting the plant or by the mean size of nodule—a character well correlated with effectiveness.

A similar series of plant passages likewise failed to produce any change in an ineffective strain.

The strains of *Rhizobium* used in the above extensive series of experiments were thus very thoroughly tested for purity and constancy of behaviour. Nevertheless when the effective strain A was afterwards stored for nine months in sterilised Woburn soil,

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platings made from these soil cultures produced colonies, 35 per cent. of which consisted of a highly ineffective mutant strain which retained its new character in culture for several years. Dr. J. Kleczkowska has been investigating such mutations in soil culture and finds that they occur readily in some soils, but not in others. At present we do not know what character in the soil determines whether mutants of the nodule bacteria will appear in it. Addition of NPK or changes in reaction do not alter the soil's natural tendency in this respect. The problem is one of great importance to the practice of legume-seed inoculation since it is clearly of little use to add effective nodule bacteria to a soil in which they will turn into ineffective strains.

Similar mutant forms have also appeared in old agar-slope cultures and when cultures have been stored at a low temperature. Until recently all such variations in effectiveness have been less effective than the parent strain, and by no means could the effectiveness of a strain be increased, although when passing the ineffective mutant S_2Q, obtained from soil, through the plant a very small percentage of effective mutants appeared. During the past year, however, Dr. Kleczkowska in her work with bacteriophage (see below) has obtained an interesting series of mutant types amongst the 'phage-resistant survivors left after treating the ineffective mutant strain S_2Q with bacteriophage. These mutants not only include remarkable rough and pigmented forms, but also a proportion that have regained their effectiveness towards the host plant characteristic of the original strain A from which the ineffective mutant S_2Q was obtained by storage in soil. These variants from strain A, some ineffective, some that have regained a lost effectiveness and others with aberrant types of growth on media, all resemble the parent strain in their serological reactions.

5. *The cause of ineffectiveness*

When the existence of ineffective strains of *Rhizobium* was first discovered, it was generally believed that these strains lacked the ability to utilise nitrogen gas owing to some defect in their enzymic constitution. This could not be demonstrated owing to the difficulty of getting any strain, whether effective in the plant or not, to fix nitrogen *in vitro*. It had, however, been noticed that ineffective nodules tended to remain small, and it thus seemed possible that their ineffectiveness might be related to the anatomy or physiology of the nodule as a whole. The anatomy and development of nodules produced by effective and ineffective strains were therefore investigated by Dr. H. K. Chen and Dr. H. G. Thornton (2). This investigation included nodules developed on clover, peas and soybeans.

The main differences between effective and ineffective nodules were found to concern the central tissue whose cells contain the bacteria and which is here called the " bacterial tissue."

The conclusions may be summarised as follows.

First. In effective nodules the bacterial tissue attains a much greater volume than in ineffective nodules. In soybean nodules, there is, in additions, a much larger percentage of infected host-plant cells within this tissue in effective than in ineffective nodules.

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Secondly. In effective nodules the bacterial tissue has a much longer active life. In all nodules, by whatever strain they are produced, the bacterial tissue finally disintegrates. This is due to the bacteria becoming actively parasitic and destroying the nodule tissue. The details of this remarkable phenomenon have been elucidated by earlier Rothamsted work (W. E. Brenchley and H. G. Thornton, 1925. Proc. Roy. Soc. Ser. B. Vol. 39, p. 373 : H. G. Thornton, 1929. Proc. Roy. Soc. Ser. B. Vol. 106, p. 110). In effective nodules this disintegration of the bacterial tissue begins when the nodule is three to four months old and is complete in a further one or two months. The tissue thus has an active life of perhaps four months. In ineffective nodules the small volume of bacterial tissue is completely disintegrated when the nodule is two weeks old. Effective nodules thus differ from ineffective ones both in the volume and in the duration of the active bacterial tissue. The total volume (v) of bacterial tissue in a nodulated plant can be estimated, as well as its mean duration (t) before it disintegrates. If we determine the increase in nitrogen content (n) of such a plant grown in a medium lacking combined nitrogen, it is possible to estimate the quantity of nitrogen fixed by a unit volume of bacterial tissue in a unit time $\left(\frac{v\ t}{n} = f\right)$. It was found that this quantity (f) was the same for an effective as for an ineffective strain, hence, amongst the strains studied, the ineffectiveness was due not to an inability to fix nitrogen, but to the bacteria of the ineffective strain being unable to multiply sufficiently or to last for long enough within the plant's tissues. Some evidence has been produced that this inability is related to the production in ineffective nodules of some substance in the root juice that is toxic to the nodule bacteria (3). There is another noticeable difference in fresh material that distinguishes effective from ineffective nodules. The remarkable haemaglobin pigment is as a rule easily seen in effective but not in ineffective nodules. This fact has been recently stressed by Virtanen : it seems explicable by the very short duration of the bacterial tissue in ineffective nodules, which could scarcely give time for visible amounts of pigment to accumulate.

6. *Variation in the response of the host plant to infection*

We have so far considered the changes in effectiveness due to variations in the nodule bacteria. But since the cause of ineffectiveness lies in a failure of the correct adaptive balance between the host plant and the bacterium, we should expect the plant also to exhibit variation affecting this balance. Indeed, during the extensive tests of bacterial strains on clover and especially during Dr. Nutman's investigation of strain stability on plant passage, the very large number of replicates examined and analysed brought out the fact that a considerable variance both in nodule size and effectiveness was attributable to variation in the host plant. Further tests showed that when seedlings from a commercial sample of red clover were supplied in replicate with a normally effective bacterial culture, one or two per cent. of them showed an ineffective response. On the other hand when a bacterial strain of low effec-

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tiveness was used a few of the replicate plants showed a response much above the average. With the highly ineffective strains tested, however, uniformly ineffective responses have been obtained. This variance in effectiveness attributable to the clover plant has been shown to be hereditary by Dr. P. S. Nutman (*Nature*, Vol. **147**, p. **463**) who has carried out a genetical analysis of some of the factors concerned. Recessive genes in red clover produce ineffective responses when their homozygotes are infected with the normally effective bacterial strain A. These genes are highly specific in their action towards certain bacterial strains, for the same recessive gene in the host plant will produce an ineffective response with the parent bacterial strain A, but will allow other unrelated strains and even a bacterial mutant derived from strain A to produce an effective response.

7. *Factors controlling infection and nodule numbers*

The work on *Rhizobium* already outlined was either concerned with or arose directly from the study of differences in the effectiveness with which nodules fix nitrogen. But not only is the effectiveness of nodules, when formed, determined by the joint action of factors in the bacterial strain and of genes in the host plant, but a similar joint control also determines whether the root shall be infected and the number of nodules that are produced.

On the bacterial side, in a number of cases, strains of *Rhizobium*, some effective and some ineffective, have lost their power of producing nodules on the host plant. Drs. H. K. Chen and P. S. Nutman studied some of these avirulent variants and found that they were unable to infect the root hairs, but were able to produce that characteristic deformation or curling of the root hairs which is the preliminary stage of infection. The study of the bacterial secretions producing this curling and the investigation arising from this is described in a later section.

The number of nodules produced by a given bacterial strain is subject to an upper limit. This limit is, of course, affected by the plant's physiology as will be seen, but also by some factor in the bacteria, since Dr. H. K. Chen (1) has found that the number of nodules per gram of root is a characteristic of the bacterial strain used.

On the plant side a case of the complete inhibition of nodule formation due to genes in the clover host plant has been investigated by Dr. P. S. Nutman. A plant completely resistant to infection appeared amongst some clover seedlings supplied with nodule bacteria. This was grown to maturity, and a genetical analysis made of its offspring. Resistance to infection here depends on a recessive gene which can, however, cause the resistance only if homozygous in a female plant of a particular genetic constitution. This very interesting type of inheritance, of course, results in such resistant plants being very rare in nature. Other genes in the clover plant have also been found by Dr. P. S. Nutman to influence the time at which the first nodule appears and the number of nodules produced by a given strain of bacteria. The time of first appearance of nodules is typically coincident with the opening of the first leaf as found by Dr. H. G. Thornton in lucerne, but genetical factors

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in the plant can delay their appearance considerably. Both the first appearance and number of nodules are controlled by factors in the plant that produce a blending type of inheritance suggestive of a complex genetical picture not yet analysed.

The relation between the rooting habit of the clover plant and the number of nodules formed has been studied by Dr. P. S. Nutman, using lines of clover plants selected to form differing numbers of nodules at maturity. In this material the mean number of nodules produced by the effective strain A was found to be correlated with the rate of lateral-root formation, but not with the total length of the root system. Moreover, inoculated seedlings developed fewer lateral roots than uninoculated, the nodules seeming to replace lateral roots. These results suggest that there are points in the roots capable of developing laterals or, if infected, of producing nodules, and that the number of such points is influenced by genetic factors in the plant. Further work is needed to investigate these relationships using different bacterial strains, since, as mentioned above, these also differ characteristically in their potentiality in producing few or many nodules.

Dr. P. S. Nutman has also found that in agar cultures the number of nodules and of laterals developed on clover was determined by the number of plants sharing the same root space (13). This reduction cannot wholly be accounted for by competition for nutrients. Addition of nutrient solution in which other clover plants have grown decreases nodule numbers while the addition of distilled water increases them. Moreover, if lucerne and clover seedlings are grown in the same tube, lucerne reduces the number of nodules on clover to the same degree whether or not the lucerne is also inoculated, but clover reduces the number of nodules on lucerne only if the clover is also inoculated. The further investigation of such interactions may throw light on the competitive root action between mixed crops.

8. *Bacteriophage*

The investigation of *Rhizobium* bacteriophage was begun because claims have been made that the failure of legume crops, particularly lucerne, may be due to the decay of nodules caused by bacteriophage. Moreover, the presence of 'phage in soil might well affect the competition between strains of *Rhizobium* the importance of which has been illustrated above. The investigation has been carried out by Dr. Janina Kleczkowska using a 'phage originally isolated from garden soil surrounding the roots of peas, and originally grown on pea *Rhizobium*. Quantitative studies were greatly facilitated by an improvement in the technique for producing " plaques," by using poured platings in which the diluted 'phage suspension was mixed with a young culture of the host bacterium in melted agar before pouring the plate (5). Statistical examination of this method showed that by it valid comparisons could be made between the numbers of particles in two or more 'phage suspensions.

The plaques produced by this method develop in the substance of the agar by lysis of the numerous microscopic bacterial colonies, thus enabling the action of 'phage on bacterial colonies of different ages to be studied. By use of this technique the successive

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phenomena that occur during 'phage attack on a bacterial culture were followed quantitatively (6). The secondary development of bacterial growth in a culture apparently cleared by 'phage was also investigated. It was found that this growth often consisted of susceptible bacterial cells co-existing with a high concentration of potentially active 'phage, but protected against its action by some product of the previous lysis of bacteria.

The 'phage used in this investigation attacked a small number only of the bacterial strains upon which it was tested, but these include clover as well as pea *Rhizobium*. It has been possible to separate from the original crude 'phage a number of 'phage strains more specific in their action and also differing from the original 'phage in the mean size of the plaques produced by them.

A number of interesting 'phage-resistant mutants of clover *Rhizobium* have been isolated from cultures after subjection to 'phage. Some of these, as mentioned above, differ in effectiveness towards the host plant. Others are very abnormal in growth characters, being " rough " or pigmented on agar. The addition of 'phage to a culture of *Rhizobium* in sterilised soil rapidly converts this into a 'phage-resistant population. This fact and the specificity of 'phage strains makes it improbable that wholesale destruction of nodules in a crop can be brought about by 'phage. Moreover, attempts to induce 'phage to enter and attack growing nodules were unsuccessful. But 'phage is a very useful laboratory tool, as the specific strains can be used to identify susceptible strains of *Rhizobium* isolated from a mixture, and 'phage provides a rapid method of obtaining interesting and perhaps useful mutant forms of the bacteria.

C. Special lines of work

1. *The use of the fungus* Phycomyces Blakesleeanus *in the estimation of thiamin in potatoes*

The Phycomyces method for assaying thiamin was found to be valid for this use in potato tubers and was applied to study the increase in thiamin content during growth of the tubers and the loss that occurs during storage in winter and spring. This loss was especially great during spring storage, when it was apparently related to sprouting (10). Experiments showed that after heating in a steamer for 45 minutes on three successive days the thiamin was not destroyed, but that the bulk of it was washed out into the liquid in which the pieces of potato were immersed (9).

2. *The relation of organisms to decay of resins added to soil*

This piece of research has been undertaken at the request of the Road Research Board (D.S.I.R.) and concerns a problem involved in processes for making temporary roads by incorporating various resinous powders in the soil in order to waterproof the road base. Failure of such roads occurs in circumstances suggestive of attack by micro-organisms, but nothing was known about soil organisms that could attack such substances. In preliminary tests, however, Miss L. M. Crump isolated a variety of soil bacteria that could attack the resins and use them as a source of energy.

Mr. P. C. T. Jones is investigating the matter. He has isolated

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a number of resin-attacking organisms, both bacteria and fungi, and has shown that their presence decreases the waterproofing action of the incorporated resin in experimental blocks made up with it, and has obtained evidence of the increase in numbers of such organisms in an actual road made with resins, that was beginning to fail. The incorporation of antiseptics in the soil with the resin is being tested, so far with encouraging results.

3. *The discovery of the use of dichlorophenoxy-acetic acid in controlling plant growth*

This work began as a result of the previous study by Dr. H. K. Chen of the deformation of legume root hairs by secretions of *Rhizobium*. This suggested that the active substance in the secretions was β-indolyl-acetic acid, since the addition of tryptophane, the precursor of β-indolyl-acetic acid, to media caused an enhanced production by the bacteria of plant-growth substance. The action of β-indolyl-acetic acid itself was therefore tested on clover seedlings under aseptic conditions, and it was shown that the pure compound would, in fact, produce root-hair deformation similar to that produced by bacterial secretions. In these experiments it was also noticed that both this compound and the related plant-growth substance α-naphthyl-acetic acid were exceedingly toxic to clover seedlings grown aseptically in agar, toxicity showing at a concentration of 1 to 0.1 part per million of the solution. The two compounds were tested in fresh soil, but were found to be without toxic effect, no doubt owing to their rapid destruction by soil micro-organisms. A search was threfore made for some molecule known to be physiologically active towards plant growth that would be less likely to be attacked by micro-organisms. A chlorinated molecule seemed likely to meet this requirement since such organic molecules are usually resistant to bacterial attack. A likely compound answering these requirements was 2:4—dichlorophenoxy-acetic acid. This and several other compounds were therefore tested and the former was found to have a toxicity towards clover seedlings grown aseptically that was as high as β-indolyl-acetic acid, and to maintain a high toxicity even in unsterilised soil. Further tests on other plants showed that 2 : 4 — dichlorophenoxy-acetic acid was differentially toxic. Its toxicity was very high towards sugar beet and clover, but ten or a hundred times the concentration was needed to damage wheat. This investigation was carried out conjointly by Dr. H. G. Thornton, Dr. P. S. Nutman and Dr. J. H. Quastel (Head of the A.R.C. Unit of Soil Metabolism) (14). At this point the results were reported to the A.R.C. and further work was carried out in collaboration with Imperial Chemical Industries mostly at Jealott's Hill. In this work a large number of compounds were tested on a wide variety of plants, and field trials were carried out. 2 : 4 —dichlorophenoxy-acetic acid and a related methylated derivative, found in the above tests to be similarly effective, were also included in an extensive series of weed-control experiments carried out by Dr. G. E. Blackman of Imperial College. As a result of these further investigations, both compounds are now being successfully used in practice for the differential control of weeds in cereal crops.

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PUBLICATIONS

1. CHEN, H. K. 1941. *The limited numbers of nodules produced on legumes by different strains of* Rhizobium. J. Agric. Sci., **31**, 479-487.

Pot experiments were made with red clover and with soy beans to determine how far the number of nodules developed was a specific character of the strain of *Rhizobium* supplied.

The number of nodules per gram of root was found to reach a limit specific to each strain. This limiting equilibrium was attained regardless of the size of the root system or the age of plant at which the culture was first supplied, provided enough time were allowed for the limit to be reached.

When two different strains were applied to the root surroundings in succession, the final number of nodules was determined by the limit specific to the strain in contact with the roots while these were making most of their growth. In clover this was the second and in soy beans the first applied strain.

2. CHEN, H. K. and THORNTON, H. G. 1940. *The structure of " ineffective " nodules and its influence on nitrogen fixation*. Proc. Roy. Soc. B., **129**, 208-229.

In clover the mean volume of active bacterial tissue is about three times as great in " effective " as in " ineffective " nodules owing to an early arrest of growth in those produced by ineffective strains. In all nodules the active bacterial tissue eventually disintegrates, but in effective clover nodules it remains without disintegration for about six times as long as in effective nodules.

In clover inoculated with an effective and an ineffective strain the difference in the amounts of nitrogen fixed could be accounted for by the differences in volume and in duration of the active bacterial tissue.

In peas, nodules produced by an effective strain were nearly twice the length of those produced by an ineffective strain, and their bacterial tissue remained without disintegration for about twice as long. In soy beans the mean volume of bacterial tissue was 4·75 times as great in effective as in ineffective nodules and the percentage of that volume composed of infected cells was twice as great. In effective soy-bean nodules disintegration of the bacterial tissue began when the plant was 4 weeks old and was practically complete by the 12th week, at which time no disintegration could be found in effective nodules.

The difference in amount of nitrogen fixed by soy bean plants bearing each type of nodule could be accounted for wholly by the factors mentioned above. Thus in both clover and soy bean nodules the volume and duration of the active infected tissue are the main, if not the only, factors determining differences in nitrogen fixation amongst the strains tested.

3. CHEN, H. K., NICOL, H. and THORNTON, H. G. 1940. *The growth of nodule bacteria in the expressed juices from legume roots bearing effective and ineffective nodules*. Proc. Roy. Soc. B., **129**, 475-491.

Strains of pea and soy-bean nodule bacteria, differing in their effectiveness in benefiting the host legume, were grown in media containing the unheated root juices from uninoculated host plants and from host plants bearing effective and " ineffective " nodules, and their growth was measured.

The growth of the different bacterial strains on root juice from uninoculated plants was not correlated with their effectiveness.

The juice from roots with effective nodules produced significantly better growth of the bacteria than juice from roots with ineffective nodules in twenty-seven comparisons out of forty-four, the differences in the remaining comparisons being insignificant.

The juice from roots with effective nodules produced significantly better growth than the juice from uninoculated roots in ten comparisons out of twenty-five, and significantly poorer growth in three comparisons.

The juice from roots with ineffective nodules produced significantly poorer growth than the juice from uninoculated plants in eleven comparisons out of twenty-five, and better growth in only one comparison.

The production, as a result of infection, of soluble substances affecting growth of the bacteria, affords an explanation of those differences in nodule growth that determine the effectiveness or ineffectiveness of the different strains of bacteria as regards nitrogen fixation within the host.

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4. DIXON, A. 1939. *The Protozoa of some East Greenland soils.* J. Anim. Ecol., **8**, 162-67.

The protozoan species occurring in twelve soils from East Green land were determined, a large protozoan population was present in soils frozen for nine months in the year, and there was an unusually large number of species of testaceous Rhizopods. The largest numbers of species occurred in the soils supporting the richest vegetation.

5. KLECZKOWSKA, J. 1945. *The production of plaques by* Rhizobium *bacteriophage in poured plates and its value as a counting method.* J. Bact., **50**, 71-79.

A statistical examination of plaque counts, using a technique of poured plates, showed that this method can be so standardised as to give reliable estimates by which 'phage suspensions can be compared. The 'phage studied was derived from garden soil surrounding the roots of peas and first grown on pea nodule bacteria.

Plaques produced by this method developed in the substance of the agar by the lysis of the minute bacterial colonies developing therein. These colonies ceased to be susceptible to 'phage attack after about 24 hours of incubation at 25°C., thus limiting the plaque size to the volume filled by 'phage diffusion within this time.

Both the number and size of the plaques were affected by the concentration of agar, the composition of the nutrients in the medium, the temperature of incubation, and the age of the bacterial suspension used for plating.

6. KLECZKOWSKA, J. 1945. *A quantitative study of the interaction of Bacteriophage with* Rhizobium *using the technique of poured plates.* J. Bact., **50**, 81-94.

Using a bacteriophage derived from a strain of *Rhizobium* producing nodules on *Pisum* it was found that when suspensions of 'phage and of live susceptible bacteria are mixed, a constant percentage of the 'phage particles become attached to the bacteria. Dead bacteria and live bacteria that are resistant to attack by the 'phage attach to themselves a percentage of the 'phage particles which decreases with increasing 'phage concentration. When added to a young liquid culture of bacteria the 'phage begins to multiply within 45 minutes and does so until the culture is cleared.

The final concentration of phage particles is independent of the initial dose, but depends on the initial supply of bacteria ; it is also greatly affected by the age of the bacterial culture when infected with 'phage.

When a cleared culture of 'phage and susceptible bacteria is kept for some 5 to 6 days, growth of the bacteria may recommence. This secondary growth consists of susceptible bacteria or of new dissociant strains resistant to the 'phage.

The repopulation of the previously lysed culture by susceptible bacteria, growing in the presence of 'phage, is made possible by the appearance, during the process of lysis, of some substance that protects the bacteria against 'phage attack.

The resistant dissociant strains of bacteria resembled the parent forms antigenically and were as effective in nitrogen fixation within the host legume. They maintained their 'phage resistance after cultivation in the laboratory up to 2 years. They were readily produced in soil cultures of bacteria treated with 'phage.

When clover, grown aseptically, was infected with a pure culture of a *Rhizobium* strain susceptible to the 'phage, resistant variants appeared in the nodules in the absence of the 'phage.

Among the naturally occurring strains of *Rhizobium* that were tested, only a small proportion of strains of pea or clover nodule bacteria (10 to 15 per cent.) were found to be susceptible to the strain of 'phage studied.

7. KLECZKOWSKA, J., NUTMAN, P. S. and BOND, G. 1944. *Note on the ability of certain strains of* Rhizobium *from peas and clover to infect each other's host plants.* J. Bact., **48**, 673-675.

The ability of certain strains of *Rhizobium* from clover to produce nodules on peas and of certain strains from peas to infect red clover has been confirmed by independent cross inoculation tests made at Rothamsted and at Glasgow. In these tests, strains were cross-inoculated two or three times and

after reisolation were found to agree in serological behaviour with the original cultures.

The formation of nodules on such cross inoculates, however, took place only to a limited extent, and, at least in the case of pea strains on clover, after an unusually long interval. In no case did the host plant derive visible benefit from the strain belonging to the other inoculation group. The fact that only certain strains will cross-inoculate may explain the disagreement in the findings of other workers.

8. KLECZKOWSKI, A. and THORNTON, H. G. 1944. *A serological study of root nodule bacteria from pea and clover inoculation groups.* J. Bact., **48**, 661-672.

Twenty-nine strains of nodule bacteria derived from plants of the pea inoculation group, and 161 strains derived from clover nodules, were tested for agglutination with antisera against six strains of nodule bacteria, four derived from clover and two from pea.

No antigen or set of antigens of either O or H type was found to be common to the whole group. Some strains reacted with none of the antisera, some with only one, and others with several antisera, but none with all of them.

Strains belonging to both inoculation groups were found to give agglutination with five of the antisera ; one antiserum reacted only with a small number of strains all from clover.

Neither ability to cross-inoculate between clover and pea host plants, nor effectiveness in fixing nitrogen within the host plant, nor susceptibility to a bacteriophage was necessarily associated with the presence or absence of any one antigen or group of antigens. Indeed, an effective strain and a very ineffective variant derived from it were found to be serologically identical.

There was, however, a partial correlation between effectiveness and the H antigenic constitution.

9. MEIKLEJOHN, J. 1940. *Aerobic denitrification.* Ann. Appl. Biol., **27**, 558-573.

Two species of *Pseudomonas* are described, which reduce nitrate to nitrite and nitrogen gas in simple synthetic media. An adequate supply of a suitable organic compound is necessary for denitrification. Both species will denitrify in aerated, and in undisturbed aerobic cultures, as well as under anaerobic conditions.

At C/N ratio 10, the bacteria grow to higher numbers in aerobic than in anaerobic cultures. The amount of precipitable nitrogen retained in a culture is directly proportional to the bacterial numbers, and therefore the smaller loss of nitrogen from aerobic compared with anaerobic cultures is a consequence of the greater growth of the bacteria.

At pH 6·9 nitrite has a poisonous effect, proportional to its concentration, on both species ; but at pH 8·0 it is harmless and freely reduced.

10. MEIKLEJOHN, J. 1943. *The vitamin-B content of potatoes.* Biochem. J., **37**, 349-354.

Using the *Phycomyces* method of estimation it was found that the vitamin B_1 content of Majestic tubers at the time of lifting (September) is about 140ug/100g.

During the growing season the vitamin B_1 content of tubers increases continuously up to the time of lifting of maincrop potatoes ; in stored tubers it falls during the winter and spring ; the greater part of the loss takes place in spring, and is apparently due rather to sprouting than to storage, especially as white sprouts have a higher vitamin B_1 content than the tuber which produced them. The content of young potato leaves is very high.

An adjuvant factor of undetermined nature is present in the centres of tubers between April and August, and always present in the skin layer.

Green sprouts completely inhibit the growth of the fungus.

11. MEIKLEJOHN, J. 1943. *Loss of thiamin from cooked potato.* Nature, **151**, 81.

In potato samples sterilised in liquid media at a pH of 6.4 by steaming for 45 minutes on three successive days it was found that from 2/3 to 3/4 of the thiamin present in the tubers was washed out into the liquid in the course of steaming.

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12. NICOL, H. and THORNTON, H. G. 1941. *Competition between related strains of nodule bacteria and its influence on infection of the legume host.* Proc. Roy. Soc. B., **130**, 32-59.

Where two strains of nodule bacteria are both present in the surroundings of their host's root system, active competition between them may cause the strain having the higher initial growth rate almost completely to check multiplication of the other strain outside the plant. This dominant strain will then be responsible for nearly all the nodules.

In peas and soy beans, where growth of the root system is rapid and of comparatively short duration, the nodule-producing capacity of the plant may be partially or wholly satisfied by the nodules produced within the first few weeks, so that further infection, whether by the same or by a different strain, is checked or inhibited.

In clover, whose root system continues to grow over a long period, the first-formed nodules do not stop further nodules from being formed either by the same or by a different strain.

There are large differences in the rates of appearance and final numbers of nodules produced by different strains supplied in pure culture, particularly with clover.

The relative numbers of nodules produced by the two strains simultaneously applied to the roots is conditioned by the specific infectivity peculiar to each strain, unless some other factor, such as competition outside the plant, masks this effect.

13. NUTMAN, P. S. 1945. *A factor in clover nodule formation associated with the volume of the medium occupied by the roots.* Nature, **156**, 20.

The number of nodules formed on a clover plant growing on a limited volume of agar depends upon the presence of other plants in the same tube and the volume of the medium. The results of an experiment in which both the rate of sowing and the volume of the medium were varied show that the number of nodules per plant is inversely proportional to the number of plants sharing the same root space and directly proportional to the volume of the medium ; reduction of the volume of the medium to half being equivalent to doubling the sowing rate in effects on individual plant nodule number.

The simplest explanation that this specific volume relationship is due to direct competition for nutrients appears to be inadequate and it is suggested that reduction in nodule number may be due to the production of a nodule-inhibiting root secretion, the concentration of which varies with the plant number and volume of root medium.

14. NUTMAN, P. S., THORNTON, H. G. and QUASTEL, J. H. 1945. *Inhibition of plant growth by 2 : 4-dichlorophenoxyacetic acid and other plant-growth substances.* Nature, **155**, 497.

The toxic activity of β-indolylacetic and α-naphthylacetic acids on red clover grown under sterile conditions on agar is shown to be considerable, germination and growth being markedly affected by concentrations higher than 1 ppm. Under similar conditions and at higher concentrations tryptophane is also toxic ; its toxicity being increased by inoculation with nodule bacteria indicating that synthesis of indolylacetic acid from tryptophane occurs in the presence of nodule bacteria.

In sterile soil the toxicity of the above compounds is maintained but it is lost in unsterilised soil probably owing to microbial decomposition. Other growth promoting substances of the phenoxyacetic acid group, in particular 2 : 4-dichlorophenoxyacetic acid, are equal in toxicity to heteroauxin and also retain activity in soil for several weeks.

The activity of 2:4-dichlorophenoxyacetic acid is less in heavy Rothamsted soil than in light Woburn soil. The compound is water leached from soil with difficulty.

In soil wide differences in susceptibility appear between the plants tested (clover, wheat and sugar beet) suggesting that dichlorophenoxyacetic acid in minute amounts may be employed in weed control.

15. SINGH, B. N. 1941. *Selectivity in bacterial food by soil amoebae in pure mixed culture and in sterilised soil.* Ann. Appl. Biol., **28**, 52-64.

By using a plate culture method it has been shown that certain limax amoebae can select among five strains of *Aerobacter* the ones most readily

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eaten, although the strains differ only in very minor respects. A wide range of bacterial species were also tested, of these some were completely uneaten, others readily eaten and others eaten to some extent. No distinction was shown between gram positive and gram negative forms. In sterilised soil inoculated with edible and non-edible species of bacteria the amoebae reduce the numbers of the edible species.

16. SINGH, B. N. 1941. *The influence of different bacterial food supplies on the rate of reproduction in* Colpoda steinii, *and the factors influencing encystation.* Ann. Appl. Biol., **28**, 65-73.

The rate of reproduction in *Colpoda* varies considerably with the bacterial food supply, the age of the culture, the size of inoculum, and the metabolic products. The metabolic products of bacteria and Protozoa have a slight retarding effect on the rate of reproduction. There is no evidence of allelocatalysis in *Colpoda* either in isolated individuals or in mass cultures.

The resistant or " dauer " cysts are formed only when there is practically no bacterial food present in the culture solution or in the presence of unfavourable food supply. The metabolic products of bacteria and Protozoa have no influence on the formation of resistant cysts in *Colpoda*.

Excystation takes place even in dried cysts of more than 3 months in the presence of bacteria. No excystation takes place in such dried cysts when they are moistened with only soil extract without the presence of bacteria.

17. SINGH, B. N. 1942. *Selection of bacterial food by soil flagellates and amoebae.* Ann. Appl. Biol., **29**, 18-22.

The food preferences of two soil amoebae and a soil flagellate, *Cercomonas crassicauda*, were compared as regards forty-eight strains of bacteria, which included a miscellaneous group mostly from soil, a group of *Rhizobium* strains and a group of plant pathogens. The amoebae were able to eat about half of the strains belonging to the miscellaneous group and most of the plant pathogens. Nearly all the strains of *Rhizobium* were inedible. The *Cercomonas* ate a rather larger number of strains than did the amoebae and differed from the large amoeba in its food preference as regards eleven out of the forty-eight bacterial strains. The plant pathogens that were inedible by amoebae produced an exo-toxin harmful to amoebae, but without apparent effect on *Cercomonas*, which could eat all these strains partly or completely. Slime produced by certain strains of the bacteria did not inhibit the feeding of the protozoa.

18. SINGH, B. N. 1942. *Toxic effects of certain bacterial metabolic products on soil protozoa.* Nature, **149**, 168.

19. SINGH, B. N. 1945. *The selection of bacterial food by soil amoebae, and the toxic effects of bacterial pigments and other products on soil protozoa.* Brit. J. Exp. Path, **26**, 316, 325.

Bacteria may be edible, inedible but harmless, or toxic to soil amoebae. In a survey of 103 bacterial strains it was found that those producing red, violet, blue, green or fluorescent pigments were not eaten by protozoa. The pigments of *Bacillus prodigiosus* and *Chromobacterium violaceum* are toxic to protozoa, as are the metabolic products of *Bacterium pyocyaneum*. The calcium salt of penicillin is not toxic to protozoa even in strong concentrations, while penicillic acid and citrinin seem to be toxic up to a concentration of 1/1,500.

20. THORNTON, H. G. 1945. *Effective and ineffective strains of legume nodule bacteria.* Nature, **156**, 654.

A summary of work at Rothamsted as it touches upon the results obtained in Professor Virtanen's laboratory at Helsinki.

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