ROTHAMSTED RESEARCH

Report for 1968 - Part 1

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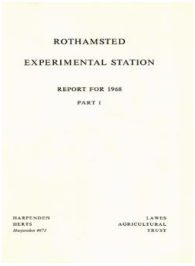

Soil Microbiology Department

P. S. Nutman

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SOIL MICROBIOLOGY DEPARTMENT

P. S. NUTMAN

The department studies some of the soil-inhabiting microbes (other than plant pathogens) that are important agriculturally, either by their direct influence on crop growth or indirectly by the chemical changes they mediate in soil. Work continued on various aspects of the symbiosis between legumes and root nodule bacteria, on vesicular-arbuscular mycorrhiza, on some of the stages of the nitrogen cycle, and on the breakdown of cellulose (anaerobically) and pesticides and herbicides. As a contribution to the International Biological Programme (IBP), work was increased on the influence of the environment on nitrogen fixation in legumes.

Research on the production of plant-growth substances by microbes was extended to organisms of the soil and rhizosphere other than *Azotobacter*. Related to rhizosphere studies is new work on the extracellular polysaccharide of *Rhizobium*. Work also started on the genetics of the symbiosis of *Trifolium glomeratum*, chosen for its short life cycle, and on the use of acetylene reduction to study nitrogen fixation.

Collaboration continued with other departments and with Imperial College, Harwell and Wye College.

Nitrification. From nine out of ten soil samples from Spitzbergen provided by Dr. A. H. Neilson (Sussex University), active enrichments of ammonia-oxidising nitrifiers were obtained. Of these, one yielded a pure culture of a *Nitrosomonas* and five others are being purified.

Deoxyribonucleic acid (DNA) was prepared from *Nitrosomonas europaea*, *Nitrosococcus* and a nitrifier strain isolated in pure culture from Park Grass and not yet fully identified. The molar % guanine + cystosine in the DNA, determined by Professor De Ley in Ghent, suggests that *Nitrosomonas* sp. and *Nitrosococcus* sp. are distinct and that the Park Grass strain is more closely related to *Nitrosococcus* than to *Nitrosomonas*. (Walker)

Fine structure of *Nitrosomonas* and *Nitrosococcus*. These nitrifying bacteria have a basically similar organisation of peripheral membranes. The structure of the cell wall is that characteristic of gram-negative bacteria, with an amorphous layer (after fixation or negative staining) covering the cell wall membrane and its associated electron dense rigid layer. The plasma membrane has connections with the 'thylakoid' membranes, which are regularly arranged in stacks of 3–4 units around the cell periphery. Each thylakoid seems to contain two apposed membranes. Before the cells divide, the membrane stack cleaves. *Nitrosococcus* has less regularly organised thylakoids than *Nitrosomonas*.

A nitrifier isolated from Park Grass soil (organism 25) has a structure differing from any previously described, which seems to resemble that of

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mycoplasma more than the other nitrifiers. The cell has an irregularly scalloped profile with cytoplasm contained in several rounded structures. A single cell-wall membrane encloses these units, which are often bounded only by a single plasma membrane and abut directly on to each other with no space between them. (Dart and Walker)

Microbial degradation of herbicides. *Thermoactinomyces vulgaris* and *Aspergillus fumigatus* both caused some decomposition of atrazine when cultures were growing in the presence of an additional carbon source.

Two fresh strains of soil bacteria, probably pseudomonads, grew with 1-naphthol as carbon source and decomposed it. Work is in progress to see whether chloro-anilines, which are components in a number of substituted urea herbicides, are decomposed by microbes. (Walker)

Metabolism of aromatic acids by *Azotobacter*. Some *Azotobacter* strains can grow on benzoate as their sole carbon source. Salicylic acid has been reported as a likely intermediate in benzoate metabolism by *A. vinelandii*, and was detected in cultures of an unspecified *Azotobacter* strain by T. L. Wang and Y. T. Tchan (*Ann. Inst. Pasteur* (1948), *74*, 423).

The report by J. P. Voets (*Naturwissenschaften* (1968), *45*, 386) that *A. vinelandii* grown on benzoate can immediately oxidise salicylate but benzoate-grown *A. chroococcum* organisms do not, was confirmed. *A. vinelandii* does not grow on salicylate. A strain of *A. chroococcum*, isolated from soil after enrichment with benzoate, grew on benzoate or salicylate as sole carbon source. When grown on salicylate, it produced catechol.

There is a moderate oxygen uptake by washed benzoate-grown *A. vinelandii* organisms in the presence of either *m*- or *p*-bromobenzoate. These bromo-benzoates also competitively inhibited oxygen uptake in the presence of benzoate. (Walker)

***Auxin production by* Azotobacter.** Work on auxins in *Azotobacter chroococcum* cultures ended. Indolyl-3-acetic acid accumulated in cultures with and without added L-tryptophane. The presence of indolyl-3-acetic acid in senescent cultures was confirmed by chromatography and by bioassay; cultures grown on agar medium for 9–10 days contained about 2–3 mg/l. (Brown and Walker)

Growth of micro-organisms in soil and rhizosphere. Bacteria isolated from root-free soil, from the rhizosphere and from the surface of roots of wheat seedlings were examined for their ability to produce plant-growth regulating substances related to gibberellins and indolyl-3-acetic acid. Of the bacteria examined, half of those from the rhizosphere produced one or other or both of the growth substances, whereas fewer of the isolates from soil did. Cultures of many produced substances inhibiting the growth of pea stems in the bioassay for gibberellins.

The growth of these bacteria in soil is being studied by two techniques to see whether bacteriostasis restricts their growth, as a fungistatic factor restricts fungal growth. In one, slides are coated with suspensions of bacteria, buried in soil and seeds planted so that the roots grow in con-

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tact with the slides. Bacteria from root-free soil usually develop within a few days into small colonies all over the slides and sometimes into large colonies near the seeds, whereas those from the rhizosphere and root surface usually grow only within 1–2 mm of the root and near the seeds. In the other technique inoculated agar discs are placed over soil and various substances added to the discs to see whether any affect growth. Sugars and amino acids sometimes stimulate growth when without these the soil could inhibit it. (Brown)

The growth of *Ophiobolus graminis* in soil is also being studied by the buried-slide technique. New mycelial growth was stimulated by germinating wheat seeds; hyaline hyphae ramified among the root hairs, clumps of large cells were formed, root hairs were infected and hyaline hyphae ran along the root surface and penetrated the root. Dark brown hyphae were produced within 7 days. The clumps of cells and infected root hairs finally lysed; so too did microconidia which formed, often abundantly, except near the root or seed.

Plant species of families other than Graminae also stimulated hyaline hyphal growth and clump formation.

Wheat seedlings stimulated growth of hyaline mycelium resembling that of *O. graminis* from infected debris sieved from soil, but its identity was not certain at this stage. The dark brown hyphae formed late were almost certainly *O. graminis*; these ramified among the root hairs, produced small clumps of cells but not microconidia and infected root hairs. Debris mixed with sand produced mycelium but not clumps of cells or microconidia. (Brown and Hornby, Plant Pathology Department)

Ecology of *Endogone*. The relationship between vesicular-arbuscular mycorrhizal infection of wheat roots, and the number of *Endogone* spores in the soil, was studied through the year in soils treated with formalin and fertilised differently. Soil collected monthly from plots carrying winter wheat on Little Knott field (*Rothamsted Report for 1967*, pp. 55 and 136) contained *Endogone* spores of only the laminate type. Numbers were similar from December to June, increased greatly during July, and began to decrease in September. Formalin applied to the soil in 1967 made the spores fewer in plots not previously treated with formalin than in plots treated in 1964 and 1965. Plots without nitrogen fertiliser consistently contained more spores than plots with (maxima of 97–125 and 18–50, respectively, per 50 g air-dried soil). The plots without nitrogen showed the effect of formalin only during the first half of the year, and after July spore numbers were similar. Few roots were infected with vesicular-arbuscular mycorrhiza in May (0–20%), but the proportion increased during June to give a late summer peak (15–62%). The length of infected root also increased during this period to a maximum of 32 cm/100 cm of root in the plot without nitrogen or formalin. Mycorrhizal infection, which correlated with spore numbers, was more abundant in the plots without nitrogen and increased rapidly during July.

The proportion of young spores each month ranged from 10 to 50%, suggesting that new spores were formed throughout the year. However, spore age is not easily assessed because of possible differences between

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strains of *Endogone*. The occurrence of take-all complicates the assessment of the effect of spore numbers and mycorrhizal infection on growth and yield of wheat. However, spore numbers and mycorrhizal infection did not increase until after the period of rapid root and shoot development in the spring, and would be expected to have little effect on the wheat until after the flowering stage.

Soil was also collected in February and September from plots 3 (no manure), 7 (N_2PKNaMg), 10 (N_2) and 11 (N_2P) on Broadbalk. Reticulate spores were most abundant in plot 3, and fewest in plot 7; all 4 plots had similar numbers of yellow vacuolate and laminate spores. In February total spore numbers per 50 g air-dried soil were 95, 58, 85 and 96 in plots 3, 7, 10 and 11, respectively, and in September were 189, 119, 130 and 119. These results from both fields agree with the general observation of Mosse (*Trans. Br. mycol. Soc.* (1968) *51*, 485–492) that *Endogone* tends to be less frequent in the most fertile soils. (Hayman)

Effect of *Endogone* on plant growth. The undefined conditions in which plants benefit from infection by *Endogone* (*Rothamsted Report for 1967*, p. 88) are being studied by inoculating seedlings of *Coprosma robusta* grown in soil, with sporocarps of a single strain of *Endogone*. Eighteen soils covering a range of types, some known to respond to phosphate, were irradiated to free them from natural infection and then given the five following treatments: (i) control (no addition); (ii) with sporocarps; (iii) with sporocarp leachings; (iv) with phosphate; (v) with sporocarps and phosphate. All plants were given nitrogen and potassium throughout the experiment. Plants in only two inoculated soils (pH 7 and pH 5·3) became infected; one set showed a positive growth response (more than 30-fold increase in dry weight) to mycorrhizal infection and to phosphate, the other did not. Soils in which the plants did not become infected ranged from pH 3·4–4·6. Plants grew well in some of these acid soils and in six they responded to phosphate. After lime was added to ten of the acid soils, plants in six became infected within six weeks whether or not freshly inoculated with *Endogone*. Clearly acidity affected the establishment of the mycorrhizal association rather than the survival of the 'inoculum'. (Mosse and Hayman)

Life history of *Endogone* spores. Single strain cultures were established of five *Endogone* spore types (yellow vacuolate, laminate, honey-coloured sessile, bulbous reticulate and white recticulate) on *Coprosma robusta* growing in pots. The first three produce spores abundantly, the others only sparsely. All form arbuscules and vesicles in the host; vesicles of the honey-coloured spore are small and many are divided into two segments.

Detailed studies of the honey-coloured sessile spores showed that the resting spore develops as a bud on the stalk of the previously formed mother spore. It reaches full size (circa 150 μ) in 3–4 days by rapid migration of the mother spore contents. These contain three unusual organelles in large numbers: pigment granules, rectangular crystals, $1 \times \frac{1}{2}$ μ (probably protein), and an unidentified self-duplicating organelle (possibly a rather small gram positive bacterium living within the fungus). The first

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two organelles seem to be specifically associated with spore formation. Migration of the contents from the mother spore is associated with the production of many membranes in myelin-like figures. When fully-formed the resting spore lays down a coloured outer wall, becomes almost transparent and the cytoplasm loses all recognisable structural elements including nuclei. The resting spore contains only large lipid vacuoles and islands of undifferentiated ground cytoplasm. Before it germinates two dense polar areas develop, corresponding to regions of regenerated cytoplasm with many dividing nuclei. A split develops between the layers of the complex wall and cytoplasmic contents migrate into it, laying down new radial and tangential walls. Germ tubes arise from the small compartments thus formed within the split wall. (Mosse)

Factors affecting the growth of *Rhizobium* in batch culture. Although cultures of rhizobia are grown commercially for inoculating soils, little work has been published about the optimal conditions for the batch liquid culture used. Hence these were examined using the strain TA1 of *Rhizobium trifolii* grown in 1 litre batches of a yeast extract–sucrose medium that could be aerated and stirred in a small fermenter.

Supplying cultures kept at pH 7·0 and 28° C with air at 2, 1 or 0·5 litres/minute increased bacterial populations by as much as 65 times in 24 hours giving maximum populations of total and viable cells of 5·2 and $3{\cdot}4 \times 10^9$/ml respectively. Populations increased up to $1{\cdot}4 \times 10^{10}$ (total) and $4{\cdot}2 \times 10^9$ (viable) cells/ml of culture after incubation for 48 hours. Numbers of cells in cultures stirred but not aerated increased by only 5 times in 24 hours. Populations of total and viable bacteria were then $4{\cdot}9 \times 10^8$ and $2{\cdot}1 \times 10^8$/ml of culture respectively, and increased very little on further incubation.

Generation times in the aerated cultures ranged from 2·8–3·7 hours and were unaffected by the rate of aeration; the smallest rate used, of 0·5 litre/minute, apparently provided enough oxygen. Without aeration, the generation time was never less than 4 hours, and this was maintained only during an 8-hour period of logarithmic growth. During the 2–3 day period of each experiment pH remained constant to within $\pm 0{\cdot}2$ of a unit.

The concentration of yeast extract in a mannitol-salts medium affected the character of the bacteria, and their numbers and viability. After 5 days' growth, with 0·1% yeast extract the cells were short rods of usual appearance, with fewer than 1% distorted. With 0·35% yeast extract, 72% of the cells were swollen, branched or otherwise distorted, and with 0·5% and 1% yeast extract all the cells were abnormal.

The larger concentrations of yeast extract slowed division and hastened cell death. Thus, after 12 days the total populations with yeast extract increasing from 0·1% to 1%, were $2{\cdot}28 \times 10^9$, $1{\cdot}43 \times 10^9$, $1{\cdot}01 \times 10^9$ and $2{\cdot}4 \times 10^8$/ml of culture respectively, with percentage viabilities of 53, 33, 32 and 10%. Increasing the concentration of casein hydrolysate, in medium containing only a trace of yeast extract (0·01%) to supply essential growth factors, similarly affected the growth of *R. trifolii*. These results confirmed those obtained by Jordan and Coulter (*Canad. J. Microbiol.* (1965) *11*, 709), with *R. leguminosarum*. (Skinner and Roughley)

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Phage mutation and establishment of lysogeny in *Rhizobium trifolii*. Strains of *Rhizobium* susceptible to a virulent phage can produce resistant mutants some of which coexist with the phage indefinitely (*Rothamsted Report for 1967*, p. 84). Further work showed that some bacteria from such cultures were carrying phages, i.e. they were lysogenic. The virulent phage seems to mutate to a temperate form and establish the lysogenic condition in the bacterium, but to establish this it was necessary to exclude the possibility of contamination. Therefore phage-carrying strains were grown in liquid media containing more than enough antiserum to the phage to inactivate any adsorbed on the surface of the bacteria. After 48 hours the bacteria were centrifuged from the medium, resuspended in saline, inoculated on agar slopes and later tested for phage. The supernatant fluid from all centrifuged cultures contained circa 10^7 phage particles/ml. That these are the same phage was shown by the fact that they were all inactivated by antiserum to the virulent phage as readily as was the original virulent parent. The mutant phages became lysogenic in indicator bacterial strain whereas the original virulent phage did not. (Kleczkowska)

These results bear on the origin of virulent phage in soil (or in nodules) where temperature phage and bacteria may coexist for long periods of time. Abundant phage was isolated from potting soil and substrates (mixtures of vermiculite, sand and quartz) in which clover plants inoculated with strain 0403 had been growing for a year or longer. The phages, of which there were several distinct types, were isolated using bacteria cultured from nodules on plants growing in these soils. Phage was never isolated from samples of soil or potting mixture not planted with clover. Attempts to isolate phage from nodulated plants growing in sterile conditions in tubes also failed, but the plants did not live for as long as a year. (Kleczkowska and Subba Rao)

Extracellular polysaccharides of *R. trifolii*. The polysaccharide slimes produced by nodule bacteria are of interest immunologically and may be important in some phases of the symbiosis. Those produced by different strains differ chemically and the one produced by *R. trifolii* (strain 0401) is being studied in detail. The polysaccharide isolated by adding ethanol to shake cultures has a molecular weight, estimated by gel filtration, greater than 300 000. After removing protein, the purified material precipitated by a cationic detergent contained about 15% uronic acid. The uronic acid is not yet identified. The major sugars of the purified polysaccharide are glucose and galactose; colour tests gave no indication of pentoses or heptoses. (Hepper)

Effect of day length and light intensity on nodulation. How day length and light intensity influence symbiosis in cowpea (*Vigna sinensis* var. Poona), soyabean (*Glycine max* var. Lincoln), Golden Tares Vetch (*Vicia sativa*) and dwarf bean (*Phaseolus vulgaris* var. Canadian Wonder) was studied at controlled temperature and humidity. Nodules in all species increased in number and size with increase in day length. Day length also affected the distribution of nodules between the primary and secondary roots.

Plants of Golden Tares Vetch given 3000-ft candles produced several

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nodules on the primary root with a 16-hour photoperiod whereas with a 12-hour photoperiod only an occasional plant produced even a single nodule and none was formed by plants with a photoperiod of 8 hours. Short days also delayed nodulation, producing fewer and smaller nodules which were farther away from the crown of the plant. Nodules were smaller and less numerous with decreased light intensities. With a photoperiod of 16 hours, cowpea and soyabean did not nodulate on the primary root when the light intensity was 2250 and 1500-ft candles and secondary root nodulation was much slower than at 3000-ft candles.

Phaseolus vulgaris is much less affected than the tropical species by light intensity and nodulated well at 1500-ft candles but it did not produce primary root nodules in any of the conditions tested. (Day and Dart)

Cluster clover (*Trifolium glomeratum*) flowers readily and sets its small seeds when grown on agar in a test tube. To aid genetic work it was grown at 15° or 20° C and with photoperiods ranging from 8 to 24 hours to find the conditions in which nodulated plants flower and fruit soonest. Plants inoculated with *Rhizobium trifolii* strain 1 were grown either in test tubes on agar or in small pots of soil, and kept in controlled-environment cabinets at the department of horticulture, Wye College, by the courtesy of Professor W. Schwabe.

Plants in continuous light flowered soonest, viz. after 38 days in test tubes and 27 days in soil. Although flowering was a few days later with a 16-hour day, seed matured faster than in continuous light. Plants grown in short days remained vegetative.

All plants nodulated well, but nodule initiation was two days later with 16- and 24-hour photoperiods than with shorter ones. Nodules became fewer and larger as day length increased. (Subba Rao, Nutman and Mr. S. George, Wye College)

The effect of root temperature and nodulation on subterranean clover. Several cultivars and sparsely and abundantly nodulating selections of subterranean clover were grown in cabinets under 2000-ft candles of light (16-hour day), an air temperature of 25° C day and 15° C night, and root temperatures of either 7°, 11°, 15° or 19° C. They were inoculated with *Rhizobium trifolii* using either strain TA1, SU297 or 0403, and later examined to assess infections of their root hairs, time of initial nodulation, and nodule numbers and structure.

At 19° C root hairs began to be infected when the seedlings were three days old, whereas at 7° C infection did not start until 13 days, and then increased more slowly. When seedlings were transferred from 7° to 19° C, infection began within a day and proceeded faster than on plants grown continuously at 19° C, but only for a short time and the final number of infections was not significantly increased. In contrast to plants grown at 19° C, the infection rate at 7° C was not slowed by the formation of nodules. At 19° C the interval between infection and nodulation was 3–5 days, whereas at 7° C and in plants transferred from 7° to 19° C, it was only one day.

As in other clover species, infection began at a few well-defined zones which increased in number and tended to amalgamate as the plants grew.

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The oldest portion of the root hair zone was not always the first infected, and at 7° C it often remained uninfected. At 19° C the length of the younger uninfected zone, extending from the root tip, increased with plant age.

The cultivar Yarloop sustained many fewer hair infections than the other varieties, but the sparsely and abundantly nodulating selections had similar numbers. Also, similar numbers of hairs were infected by the three bacterial strains used. (Roughley, Dart, Barreuco and Nutman).

Root temperature, bacterial strain and host all affected the time required to initiate nodules. Increasing the temperature from 7° to 19° C shortened the time by at least 1 week. Strain differences were greatest at 7° C and ranged from 14·7 days with strain TA1 to 18 days with strain 0403. The host selections differed in nodulation time only at 7° C, where the sparse line nodulated sooner.

All plants transferred from 7° to 19° C 9 days after inoculation, nodulated within 24 hours, suggesting that some requirements for nodulation, possibly the development of meristematic foci, were fulfilled during the period at 7° C. Root temperature affected the number of nodules, the rate they formed and their distribution. At 7° C nodules were many fewer than at 11° to 19° C, and increased little during 30–40 days. Nodules had not formed by 40 days at 7° C on secondary roots, whereas at 11° C secondary roots nodulated rapidly after 30 days. Nodule formation at 19° C was almost complete in 20 days, when nodules on secondary roots were 60% of the total.

After 9 days at 7° plants nodulated very fast when transferred to 19° C. Genetic characters controlling nodule number had different effects at different temperatures. Within the range 15°–19° C (approximately the temperature at which the original selections were made), the sparsely nodulating selections formed fewer nodules than those selected for abundant nodule formation where as at 11° C there was no difference.

At different stages of growth the aggregate amounts per plant of the component tissues of nodules (bacteroid zone, cortex meristem, etc.) were estimated by measuring areas in median longitudinal sections. Cold delayed nodule formation and the completion of the symbiosis by prolonging the time for the bacteroid tissue to differentiate. The delay depended on host line and bacterial strain. The relative ability of strains to fix nitrogen differed at different temperatures. Thus, TA1 and SU297 both fixed nitrogen equally well at 19° C, whereas TA1 fixed 20 times as much as SU297 at 11° C. Cold decreased both the amount of bacterial tissue formed by SU297 and its efficiency in fixing nitrogen. It prolonged the life of the nodules, which seemed not to degenerate until a given amount of nitrogen was fixed.

A self-regulating mechanism that controls fixation was also shown by plants inoculated with strain TA1, which was best suited to cold and formed more nodules and more bacterial tissue at 11° C than at 19° C. (Roughley and Dart)

Nodule and *Rhizobium* fine structure. Nodules were examined on the following species, all susceptible to *Rhizobium* for Lotus: *Lotus corniculatus*, *Lotus uliginosus*, *Lotus hispida*, *Astragalus glycophyllus* and

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Ornithopus sativus. Each membrane envelope within the infected nodule cell in all the hosts encloses several bacteroids, whether the inoculum was originally isolated from the same species examined, from other members of the group or from *Lupinus* nodules. Bacteroids in *Lotus* nodules are less pleomorphic than those in clover nodules; they retain the rod-shape of *Rhizobium* cells in pure culture, but are larger. *Astragalus* bacteroids are larger and more pleomorphic. Both types of bacteroids have a round electron-dense inclusion associated with the nucleoid. Rhizobia enter nodule cells through infection threads. (Dart and Professor H. L. Jensen, Copenhagen)

A tube (100–150 Å diameter) often occurs between the membrane envelope and the enclosed bacteroids in nodules of the above species and of soyabean and *Phaseolus vulgaris*. Nodules of all the species and of *Medicago* and *Trifolium* show small vesicles, containing electron-dense material, which are associated with the development of membrane envelopes. The vesicles seem to fuse with the envelope and release their contents into the space between bacteroids and envelope. They may be formed from the extensive rough endoplasmic reticulum in these host cells. Microtubules are associated with bacteroids while bacteroids are enlarging and dispersing in *Medicago truncatula* nodules, and with the apposition of vesicle contained material into the developing infection thread. Plastids in invaded nodule cells have extensive crystalline arrays of phytoferritin. (Dart)

Sections were cut of pure cultures of *Rhizobium trifolii* strain TA1, in peat as used for commercial inoculants for clover, and examined by electron-microscopy. Peat has a lamellar structure with prominent 'membrane' profiles. Almost all the bacteria are in the large gaps between the peat particles, with few embedded in the peat matrix. (Dart and Roughley)

The chemistry of nodule leghaemoglobin. Leghaemoglobin from soyabean nodules can be separated into two main fractions that differ electrophoretically and in their amino acid composition. Continuous flow electrophoresis showed the faster component to be twice as abundant as the slower, and electron spin resonance (ESR) measurements gave different 'g' values for the two proteins.

Leghaemoglobin from cowpea nodules separated on DEAE-cellulose columns also seems to consist of major and minor components but these differ electrophoretically and in amino acid composition from those from soyabean fractions, although the major ones from cowpea and soyabean give similar 'g' values in ESR.

Leghaemoglobin from soyabean enriched with ^{57}Fe and its cyanide and fluoride derivatives, give Mossbauer spectra clearly resembling that of rat haemoglobin. Bacteroids that are active in reducing acetylene to ethylene (and thus active in N fixation) have a characteristic Mossbauer spectra for the nitrogenase enzyme Fe. (Dart, with Dr. G. Lang and Dr. A. Thompson of Atomic Energy Research Establishment, Harwell, Dr. J. Gibson of Imperial College, University of London, and Marjorie Byers, Biochemistry Department)

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Peroxidase activity in soyabean root nodules. Peroxidase activity was localised in nodule slices incubated in a mixture of hydrogen peroxide and 3,3′-diamino-benzidine tetra hydrochloride and could be detected by electronmicroscopy of thin sections after the method of Karnorsky (*J. Cell Biol.* (1965), **27**, 37A). Peroxidase was detected in the plant cell walls, especially in the middle lamella near intercellular spaces. The infection thread wall and adjacent cell walls also gave an intense reaction. The cristae of mitochondria reacted positively but variably, and the large inclusion granules associated with poly-β-hydroxybutyrate in the bacteroids showed peroxidase activity. There was no reaction in the space between bacteroid and enclosing membrane.

Leghaemoglobin has peroxidase activity, so these results suggest that the protein may be outside the membrane envelopes, contrary to the suggestions of F. J. Bergersen (*Bact. Rev.* (1960), **24**, 246) and M. J. Dilworth and D. K. Kidby (*Expl Cell Res.* (1968), **49**, 148). This suggestion was supported by light microscopy using essentially monochromatic light ($\lambda = 4047$ Å), which showed absorption by the bacteroids and cytoplasm outside the envelopes. (Dart)

Estimating the nitrogen fixed in lucerne (IBP). Field trials started in 1967 continued and two further ones were begun at Rothamsted and at Woburn, of identical design but with higher rates of application of 'Nitro-Chalk', viz. 0, 30, 60 and 90 lb N/acre at drilling and after each cut. These larger amounts produced larger responses and increased the nitrogen contents of the lucerne and rye grass, though the highest rate had less effect than the others except on rye grass. Otherwise responses to fertiliser and inoculation with *Rhizobium* resembled those in 1967. Maximum lucerne yields in the new experiments (3 cuts each) from plots given lime, P, K, but not N, were 58 cwt/acre dry matter at Rothamsted and 35 cwt/acre dry matter at Woburn. With nitrogen they were 67 cwt/acre dry matter and 50 cwt/acre dry matter. Maximum grass yields with nitrogen were 85 cwt/acre dry matter and 89 cwt/acre dry matter.

Comparison with the unfertilised grass and the ineffectively inoculated plots, showed that inoculation with the effective strain led to 100 lb N/acre being fixed at Rothamsted and 90 lb N/acre at Woburn. Plants infected with the bacteria naturally present in the soil fixed 20 lb N/acre at Rothamsted and 27 lb N/acre at Woburn.

The experiments sown in 1967 yielded more: maximum grass yields were 114 cwt/acre dry matter from 4 cuts at Rothamstead and 70 cwt/acre dry matter from 2 cuts at Woburn. Maximum lucerne yields were 99 cwt/acre dry matter at Rothamsted and 59 cwt/acre dry matter at Woburn. Maximum nitrogen fixation was 250 lb N/acre at Rothamsted and 180 lb N/acre at Woburn. Large differences showed between plots of lucerne during spring but later these plots became nearly uniform, except those inoculated with ineffective rhizobia, where the initially poor stand of lucerne was overgrown by weeds and volunteer clover (which was effectively nodulated by naturally occurring bacteria). These changes in appearance corresponded to the spread and multiplication of effective bacteria. (Bell)

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Staff and visiting workers

Christine Hepper was appointed in January and D. Hayman in August.

Visiting workers included Mr. H. Glaeser, Institute of Microbiology, Göttingen University, Germany; Dr. Roderiguez-Barrueco, Salamanca University, Spain; Dr. N. S. Subba Rao, Indian Agricultural Research Institute, Delhi (Nuffield Fellow); Dr. Angelica van Hofsten, University of Uppsala, Sweden; Dr. Hana Mareckova, Central Research Institute for Plant Production, Prague, Czechoslovakia; Dr. Therese Stelz, University of Rouen, France.

Assisted by the Royal Society European Programme Fund, N. Walker spent three weeks working with Professor J. De Ley at the State University of Ghent, Belgium. P. S. Nutman attended the International Soils Congress and International Biological Programme meeting at Adelaide, Australia.

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