

The Relation between the Development, Structure and Functioning of the Nodules on Vicia faba, as influenced by the Presence or Absence of Boron in the Nutrient Medium.

By WINIFRED E. BRENCHLEY, D.Sc., F.L.S., and H. G. THORNTON, B.A.

(Rothamsted Experimental Station.)

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[PLATES 27-30.]

The problems connected with the nodules that are produced by *Bacillus radicicola* on leguminous plants fall naturally into four divisions :

- (a) The life of the organism in the soil and the causes leading to its arrival at the usual point of infection—the root hair.
- (b) The entry of the organisms into the tissues of the plant.
- (c) The causes leading to the formation and development of the nodules after the entry of the organism into the plant.
- (d) The physiological processes within the nodule, especially those leading to the fixation of elemental nitrogen.

The life of the organism outside the plant and the causes leading to its initial entry into the tissues do not concern the present paper, which deals with the relation between the organisms within the nodule, and the anatomy and physiology of the tissues of the host plant.

The general course of formation of a nodule is well known and has been carefully described by other workers (6, 20, 29, 31). The entry of the bacteria results in a complicated response on the part of the plant, whereby, under normal conditions, there is produced an outgrowth having, in its centre, a mass of swollen cells containing large numbers of the organisms. In the developed nodule almost the whole of the organisms are to be found in these swollen cells. Since nitrogen fixation has been shown to be associated with the presence of the bacteria (12), it is most probable that this swollen cell tissue with its large numbers of contained organisms is the seat of this process. The central problems in the physiology of the nodule, therefore, are the causes leading to the formation of this swollen cell tissue and the nutrition of the bacteria within it.

With regard to the nutrition of the bacteria, it is an important point that

they require a free supply of energy-giving material. Numerous experiments with other nitrogen-fixing bacteria have shown that a large supply of easily oxidisable organic material is necessary for nitrogen fixation (3, 13, 34), and carbohydrates and related substances have proved to be the most efficient sources of energy for this purpose (15). Both on theoretical grounds and by analogy with organisms fixing nitrogen in culture, therefore, one may suppose that the bacteria within the nodule require an abundant supply of carbohydrate or similar easily available compounds of high energy value.

Under normal conditions vascular strands develop which run up the sides of the nodule.* A consideration of the requirements of the bacteria for nitrogen fixation suggests that these strands have the function of supplying them with carbohydrate as a source of energy and of removing the products of their metabolism. On this view the vascular supply forms, physiologically, the connecting link between the plant and the bacteria within the nodule. A study of the exact relation between the strands and the development and functioning of the nodule thus becomes a first essential to any understanding of the physiology of the organisms within it.

Hitherto it has not been possible to study the rôle of this vascular supply by comparing the growth and metabolism of normal vascular nodules with that of nodules where the vascular supply was deficient or absent. The abnormal growth produced by growing the broad-bean (*Vicia faba*) in water culture from which boron has been excluded and, in particular, the derangement of the vascular development in the root produced in this way, provide a means whereby this problem may be attacked.

The important rôle of boron in the nutrition of the broad-bean has recently been clearly demonstrated by Warington (32), though the physiological function of the element still remains obscure. Further experiments have entirely confirmed her results, and have also revealed a definite relationship between the presence of boron and the structure and development of nodules on the roots.

A.—*Origin of the Work.*

The investigation arose as a result of some observations made on sand cultures of *Vicia faba* grown in the presence and absence of boron. With boron 13, 38, 65 and 146 nodules were developed on the individual plants; whereas of the plants in the boron-free environment, one had nine nodules and the remainder none. In this experiment the nodules were due to chance infection, no bacterial inoculum being intentionally added. The result, how-

* These were observed by Tréviranus in 1853 (25).

ever, stimulated an investigation into the effect of a total absence of boron on nodule formation, cultures in nutrient solution being employed as giving better controlled conditions.

B.—*Smaller Number of Visible Nodules developed in a Boron-free Medium.*

Experiment I.—A series of broad beans was grown in a mineral salt solution* of which the reaction was adjusted to give a pH value of 6·2, this being favourable to the activity of *Bacillus radicicola*. Half the plants received boron at the rate of 1 : 500,000 boric acid, and all were inoculated by introducing into each bottle at every change of solution a few c.c. of a culture of *B. radicicola* grown in Prucha's "medium 335" (21) with the agar omitted, the liquid medium being more convenient to handle. On plants supplied with boron, nodule formation became evident after about a month, and numbers of typical, good-sized nodules were produced. In the absence of boron, the first visible signs of nodule formation were delayed for another month and the nodules remained small and undeveloped throughout.

The number of nodules visible on individual plants† in the two series were as follows :—

Table I.

<i>With Boron.</i>	<i>Without Boron.</i>
234	116
248	147
351	201
420	241
512	288
—	—
Mean 353	199

Experiment II.—Tests were made to determine how nodule formation was affected by the *absence* of boron in solutions of varying acidity and also by the absence of nitrate, as many authors (22, 27, 28, 33) have shown that *excess*

* Potassium nitrate, 1 gram.; magnesium sulphate, 0·5 gram.; sodium chloride, 0·5 gram.; calcium sulphate, 0·5 gram.; potassium di-hydrogen phosphate, 0·3 gram.; potassium mono-hydrogen phosphate, 0·27 gram.; ferric chloride, 0·04 gram.; distilled water to make up 1 litre.

† In the experiments here described, the number of nodules given refers, of course, to those attaining macroscopic size. As mentioned below, there is reason to think that, on roots grown without boron, a number of nodules are formed, but cease to grow before they become visible.

of nitrate reduces nodule production. In this case the first inoculation was delayed until the plants were well started.

The results of this experiment are shown in Tables II and III.

Table II.—Inoculated Broad Beans. July 18–August 28, 1923. Average of 5 plants. With Nitrate in Solution.

Reaction of Medium.	No Boron.					With Boron.				
	Shoot.	Root.	Total.	Average number of Nodules	Range.	Shoot.	Root.	Total.	Average number of Nodules	Range.
	Grm.	Grm.	Grm.	—	—	Grm.	Grm.	Grm.		
pH 3.8	2.452	0.300	2.752	—	—	4.544	0.744	5.288	98	10–206
pH 5.0	2.426	0.360	2.786	—	—	4.022	0.632	4.654	113	2–367
pH 6.2	2.332	0.318	2.650	17	86	4.882	0.724	5.606	210	17–402
					(on one plant only).					

Table III.—Inoculated Broad Beans. July 18–September 20, 1923. Average of 5 plants. Without Nitrate in solution.

Reaction of Medium.	No Boron.				With Boron.			
	Shoot.	Root.	Total.	Number of Nodules.	Shoot.	Root.	Total.	Number of Nodules.
	Grm.	Grm.	Grm.		Grm.	Grm.	Grm.	
pH 3.8	1.195	0.211	1.406	—	1.365	0.465	1.830	—
pH 5.0	1.252	0.305	1.557	—	1.360	0.465	1.825	—
pH 6.2	2.498	0.525	3.033	71	2.046	1.213	5.259	673
				(range 8–205)				(range 334–990)

It will be seen that in cultures supplied with nitrate, the presence of boron enabled nodules to develop even at a H-ion concentration of 3.8, while in the absence of boron no visible nodules were produced at the higher acidities. Thus the unfavourable effects of acidity on nodule formation were increased by the absence of boron. In the absence of nitrate no nodules were visible at the higher acidities either in the presence or absence of boron. At the more favourable reaction of pH 6.2, nodules were developed both with and without nitrate, though in the latter case more nodules were seen. At this reaction the decreased number of visible nodules in the absence of boron, noted in Experiment I, is again striking.

C.—Effect of Boron on *Bacillus Radicicola* in Pure Culture.

The last two experiments having shown that the number of visible nodules was reduced in the absence of boron from the culture solution, the question arose as to whether the presence of traces of boron was necessary for the proper growth and activity of the nodule organism in this solution. In order to investigate this point, the growth of the organism in culture solution in the presence and absence of boron was studied. For this purpose, Prucha's "medium 335" (21), made up without agar,* was used. Both the components and the solution itself were tested for traces of boron with negative results. The same strain of organisms as was used for inoculation throughout the work was grown in this solution, without boron and also with one part of boric acid to 500,000 of solution. The cultures were grown in test-tubes each containing 10 c.c. of solution, inoculated with a suspension of bacteria from a two days old culture. The number of organisms in this suspension was counted on a hæmocytometer and each tube was inoculated with 0.1 c.c. of the suspension, so diluted that this volume contained approximately 60,000 bacterial cells. The tubes were incubated at 25° C. At intervals, counts of the number of organisms in each medium were made as follows:—A standard loopful of the

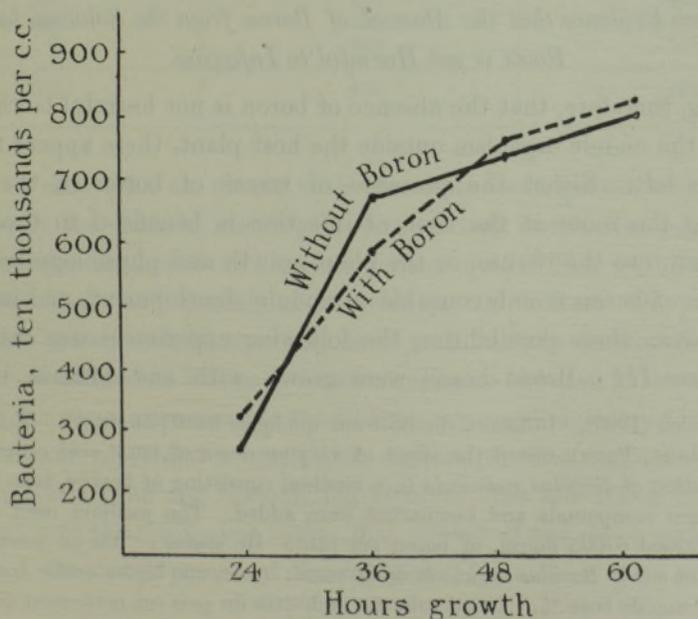


FIG. 1.—Growth of *Bacillus radicicola* from *Vicia faba*.

* K_2HPO_4 , 0.2 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $CaSO_4$, 0.2 gm.; $CaCO_3$, 0.2 gm.; saccharose, 20 grms.; tap water, 1 litre.

suspension from a tube was placed on a Thoma hæmocytometer, the organisms were killed with osmic-acid vapour and the number of cells on 30-50 squares was counted. The process was repeated with four separate loopfuls, and from the mean of the counts obtained the number of organisms per cubic centimetre was calculated. On each occasion the mean count from three parallel tubes was taken. The rate of multiplication of the organisms with and without boron is shown in fig. 1, where the number of organisms in the cultures is plotted against the time of incubation. It will be seen that the presence of boron in no way affects the multiplication of the bacteria.

The nodule organism passes through very definite changes in morphology, and to ascertain whether the absence of boron produced any effect on this cycle of changes, preparations stained with carbol fuchsine were made and examined at each time of counting. It was found that the changes through which the organism passed were similar in the presence and in the absence of boron.* The evidence from growth in pure culture, therefore, provides no reason for supposing that the absence of boron from culture solution surrounding the bean roots has any harmful effects on the nodule organism suspended therein.

D.—*Cultural Evidence that the Absence of Boron from the Solution bathing the Roots is not Harmful to Infection.*

Assuming, therefore, that the absence of boron is not harmful to the normal activity of the nodule organism outside the host plant, there appear to be two possibilities left. Either the presence of traces of boron in the solution surrounding the roots at the time of infection is beneficial to the entry of the organism into the tissues, or the plant growth and physiology induced by the absence of boron is unfavourable to nodule development, and in order to decide between these possibilities, the following experiment was made:—

Experiment III.—Broad beans were grown with and without boron for

* Juliu Voicu (1923), 'Influence du Bore sur quelques bactéries du sol' (Edition de la vie Universitaire, Paris), tested the effect of varying doses of boric acid on growth and nitrogen fixation of *Bacillus radicolica* in a medium consisting of haricot-bean extract to which nitrogen compounds and saccharose were added. The medium used as control already contained 0.005 mgrm. of boron per cent. He states: "On ne constate aucun effet stimulant sur le *Bacillus radicolica* de la vesce; mais, une légère action toxique pour la dose de 10 mg. de bore %. Le microbe des nodosités du pois est nettement favorisé par les doses fortes de 5 à 10 mg. de bore % (0.3 à 0.6 gr. d'acide borique par litre)." At these comparatively strong concentrations a stronger growth and fixation of nitrogen was found to occur. At concentrations of boric acid of the order employed by the present authors, Voicu found no effect on the organisms.

17 days. Three methods of inoculation with *B. radicum* were then adopted; first, plants grown without boron and having abnormal roots were inoculated in a solution containing boron; secondly, plants grown in a solution containing boron and having normal roots were inoculated in the absence of boron; and, lastly, plants were both grown and inoculated in the presence of boron. After eight hours' contact with the organisms the roots were well washed in running water* and replaced in fresh supplies of solutions similar to those in which they were first grown.

The number of nodules that developed are shown in Table IV.

Table IV.—Relative Effect of Root-Type and Inoculating Solution on Nodule Formation.

Numbers of Visible Nodules on Individual Plants.

Series A. Grown <i>without</i> boron. Inoculated in the <i>presence</i> of boron.	Series B. Grown <i>with</i> boron. Inoculated in <i>absence</i> of boron.	Series C. Grown <i>with</i> boron. Inoculated in the <i>presence</i> of boron.
252	649	543
122	638	498
105	576	488
26	334	449
6	294	384
Mean 102	498	422

It will be seen that on plants with abnormal roots grown without boron comparatively few nodules developed, even though boron was present when the plants were immersed in the inoculating fluid. The nodules on these plants were small, and tended to be sunk in the tissues. On plants with normal roots grown with boron many more nodules were developed, and their numbers were unaffected by the presence or absence of boron in the inoculating solution. On these normal roots the nodules were well developed, in contrast to those on the abnormal roots. This experiment thus indicated that the absence of boron from the surrounding medium does not affect the entry of the bacteria into the tissue, but rather that growth of the plant without boron has affected the development of the nodules.

* It is probable that some organisms escaped being washed off the roots, but that the bulk of the infection occurred during the immersion in the inoculating suspension.

E.—*Nitrogen Fixation in the Absence of Boron.*

Experiment IV.—The above and similar experiments suggested the question as to whether the nodules that do form are normal, and able to fix nitrogen effectively. Cultures of broad beans were, therefore, set up in an inoculated solution containing no nitrate with the addition of—

- (a) No boron.
- (b) 1 : 2,500,000 boric acid.
- (c) 1 : 500,000 „ „
- (d) 1 : 100,000 „ „

The seeds were graded in weight, and a sample analysed for nitrogen, the actual nitrogen content* varying from 0.05432 to 0.05626 gm. N, averaging 0.05529 gm. N per seed. The initial growth was good, but less rapid than in a parallel series receiving nitrate. Nodules appeared first within 17 days on plants with boron, but were a few days later in its absence. From this time onwards the difference between the plants with and without boron became increasingly marked.

No Boron.—These plants, on which the nodules presented a curious burst appearance, rapidly fell behind those receiving boron, and after a few weeks showed signs of nitrogen starvation as well as a lack of boron, as the leaves began to turn yellow from the bottom upwards, at the same time that the typical death of the growing point, upper leaves and flower buds was occurring.

With Boron.—The beans grew well, remaining green to the end, and producing good roots with fine laterals, covered with numerous well-developed nodules, the average numbers per plant being 675, 695, and 784 in the three series. Although they grew more slowly than similar ones receiving nitrate, they were apparently not suffering from nitrogen starvation. It appeared that nitrogen derived from the air dissolved in the food solution had been made available to the plant through the activity of the nodule organisms, which were, therefore, functioning normally.

Without boron the yellowing of the leaves indicated acute nitrogen starvation after the initial supply stored up in the seed had been utilised, in spite of the fact that a considerable number of nodules had been produced. The inference was that in the absence of boron the nodules were unable to function properly, either because the organism was inhibited from making use of the atmospheric nitrogen or because the nitrogen compounds formed by the organisms were in

* We are indebted to Mr. G. C. Sawyer for the nitrogen determinations in this experiment.

some way prevented from gaining access to the plant. The truth of this hypothesis was proved by determination of the nitrogen content of each individual plant, the actual amount of nitrogen fixed by the nodules being obtained by subtracting the average amount of nitrogen initially present in an average seed from the total quantity in the grown plant. The total nitrogen fixed per plant was ten or twelve times as great with boron as without, proving clearly the greater efficiency of the nodule when supplied with boron. The increased efficiency is not simply due to an increase in number, but to a definitely greater activity of the individual nodules, as is seen by comparing the amounts of nitrogen fixed per nodule with the different treatments.

Table V.—Nitrogen Fixation. Average of 5 Plants.

A. Treatment.	B. Percentage N in Dry		C. Total N fixed per Plant.*	D. Number of Nodules.	E. N fixed per Nodule.†
	Shoot.	Root.			
			Grm.		Grm.
No boron, including green plant‡	2·46	2·60	0·026	491	0·0000536
No boron, excluding green plant	2·32	2·59	0·016	455	0·0000343
1 : 2,500,000 boric acid	3·43	3·06	0·182	675	0·000269
1 : 500,000 „ „	3·35	2·91	0·165	695	0·000238
1 : 100,000 „ „	3·27	2·78	0·148	784	0·000188

* In addition to the average 0·05529 gm. N supplied by the seed.

† Obtained by dividing column C by D.

‡ This plant remained greener and more healthy than the rest, possibly owing to a higher boron content in the seed, which delayed the appearance of the characteristic phenomena due to boron deficiency.

The greater efficiency in the presence of boron is still more clearly shown when the comparative activity of the nodules on each individual plant is compared. This may be determined by dividing the average amount of nitrogen fixed per nodule on each plant by the smallest average amount fixed per nodule on any plant in the series.

For example :—

$$\begin{aligned}
 \text{Smallest average amount of N fixed by any nodule} & \dots\dots\dots = a \\
 \text{Actual average amount of N fixed per nodule on plant under} \\
 \text{consideration} & \dots\dots\dots = b \\
 \text{Comparative activity of nodules} & \dots\dots\dots = b/a = x.
 \end{aligned}$$

The comparative activity (x) of the nodules with no boron ranged from 1.0 to 18.83, with an average of 8.38. If the one exceptional plant which remained green is excluded, the range is 1.0 to 10.63, the average falling to 5.77. Where boron was present, the range was from 21.30 to 69.97, the averages being 48.04, 41.60, 34.82, with increasing concentrations of boric acid.

Table VI.—Comparative Activity of Nodules in Nitrogen Fixation.

Plant No.	No Boron.	1 : 2,500,000 Boric Acid.	1 : 500,000 Boric Acid.	1 : 100,000 Boric Acid.
1	3.13	44.00	36.52	26.36
2	18.83	69.97	59.62	21.30
3	8.33	49.87	31.00	36.58
4	10.63	30.65	44.45	61.62
5	1.00	45.72	36.43	28.26
Average	8.38	48.04	41.60	34.82
„ excluding 2	5.77	—	—	—

From the above table it is evident that in the absence of boron the functioning of the nodule is seriously interfered with and the activity of the organisms impaired.

F.—Effect of Boron on Structure of Nodule.

The results already detailed led directly to an examination of the effect of boron on the structure of the nodule. Nodules were obtained from the plants grown in Experiments III and IV, providing material subjected to considerable variation of treatment both as regards boron supply and general nutrition. The material was fixed in acetic acid and corrosive sublimate, embedded by Dowson's (8) rapid method of paraffin infiltration and stained with gentian violet and vesuvian brown.

A fully developed nodule, under normal conditions, consists mainly of a mass of large, thin-walled, nucleated cells, more or less completely filled with bacteria.* Towards the apex, on the side farthest removed from the middle of the root, may be a number of fairly large, rather empty cells, in which the bacteria have not as yet multiplied to any great extent, and beyond this again is a small-celled meristem in a state of active division. The nodule is surrounded by an endodermis, which is continuous across the base, but which appears

* For convenience this tissue is hereafter termed the "bacteroidal tissue," many of the bacteria contained in it being in the form of bacteroids. The term "tissue bactéroïdien" is due to Tschirch (26).

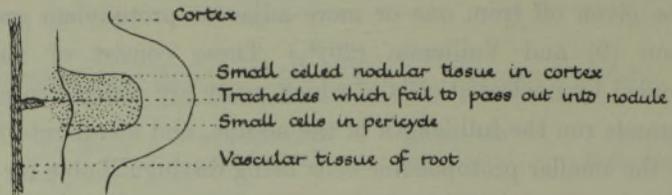
to be interrupted across the meristematic tip. Between the endodermis and the bacteroidal tissue are several layers of tissue which are traversed by vascular strands given off from one or more points opposite the protoxylem in the vascular cylinder of the root. Very frequently two strands pass off from the same protoxylem group at different levels, and in large nodules additional strands may be given off from one or more adjacent protoxylem groups. (See also Eriksson (9) and Vuillemin (29)*.) These consist of vascular elements surrounded by nucleated cells of which many are rather densely protoplasmic. The strands run the full length of the nodule, and merge into the meristematic tissue, the smaller protoplasmic cells being distinguishable for a rather greater distance than are the lignified elements. The strands follow a sinuous course and often branch, and in cross section as many as ten may appear, some of which in reality represent the same strand cut through more than once owing to the curvature. No evidence of anastomosis has been obtained, even in large nodules with an abundant vascular supply. Each strand is surrounded by an endodermis which is continuous with that of the root, and which is not evident quite so far as the point at which the tissues merge into the meristem (9, 29). Nodules of this type are characteristic of every case in which boron has been supplied to the plant, though the number and degree of branching of the vascular strands appear to depend to a great extent upon the size and vigour of the nodule. On inoculated plants supplied with a nutrient solution containing an adequate supply of nitrate the nodules are comparatively large, and often show more than two strands, with a considerable degree of branching. On similar plants not supplied with nitrate it rarely happens that more than one or two strands are produced, and these do not branch so freely; but in both cases the strands develop until they extend the full length of the nodule, and an abundance of healthy bacteroidal tissue is found. (See text-fig. 2.)

In the absence of boron such nodules are very rare, being replaced by incompletely developed nodules of two main types (see text-fig. 2). In the extreme case the nodule is abortive or undeveloped (Type I). Cell proliferation occurs in the inner layers of the cortex, but no obvious boundary layer is formed round the groups of tissue. On the inner side the cells in the region of the endodermis often suffer some changes or degeneration, developing into a more or less protuberant layer strongly marked by a mass of deeply staining, disorganised substance, which may arise from the breaking down of cells.

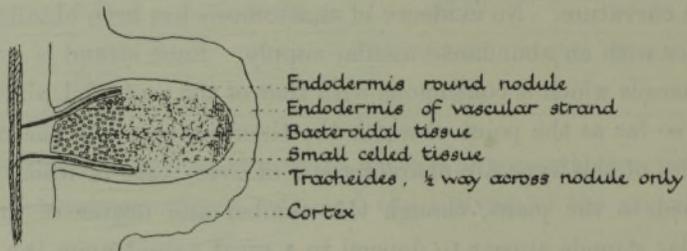
* The origin and structure of vascular strands in the normal nodule have been carefully described by Vuillemin (29). The observations of the present authors agree in general with his description.

Internal to this a certain amount of cell proliferation occurs in the pericycle, causing a slight swelling, and a very few tracheides may or may not be present, but they do not penetrate through into the incipient nodular tissue in the cortex

No Boron. Type I. Abortive or undeveloped nodule



Type II Incompletely developed nodule



With Boron. Fully developed nodule

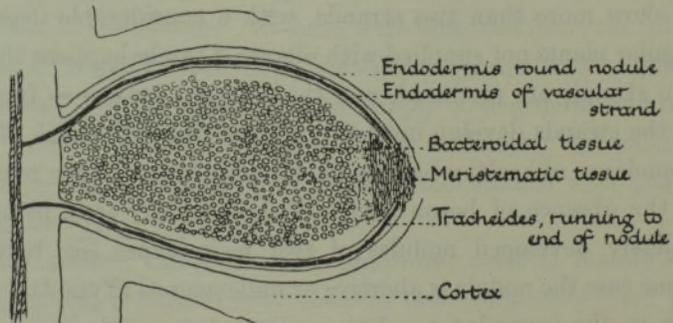


FIG. 2.—Diagrams showing influence of boron on development of nodules of *Vicia faba*. (Diagrams are compounded from observations of numerous series of sections, the sinuous vascular strands being projected into one plane.)

(text-fig. 3). It is very easy to overlook nodules of this type when examining roots macroscopically, as they are entirely buried in the cortex and make but little external swelling, rendering it almost impossible to distinguish them from incipient lateral roots, even if they are detected. This may account for

the small number of nodules usually counted on roots grown without boron. It is likely that in these cases a considerable number of nodules fail to reach macroscopic size, and that reduced nodule growth rather than diminished infection of the root is the explanation of the low nodule counts.

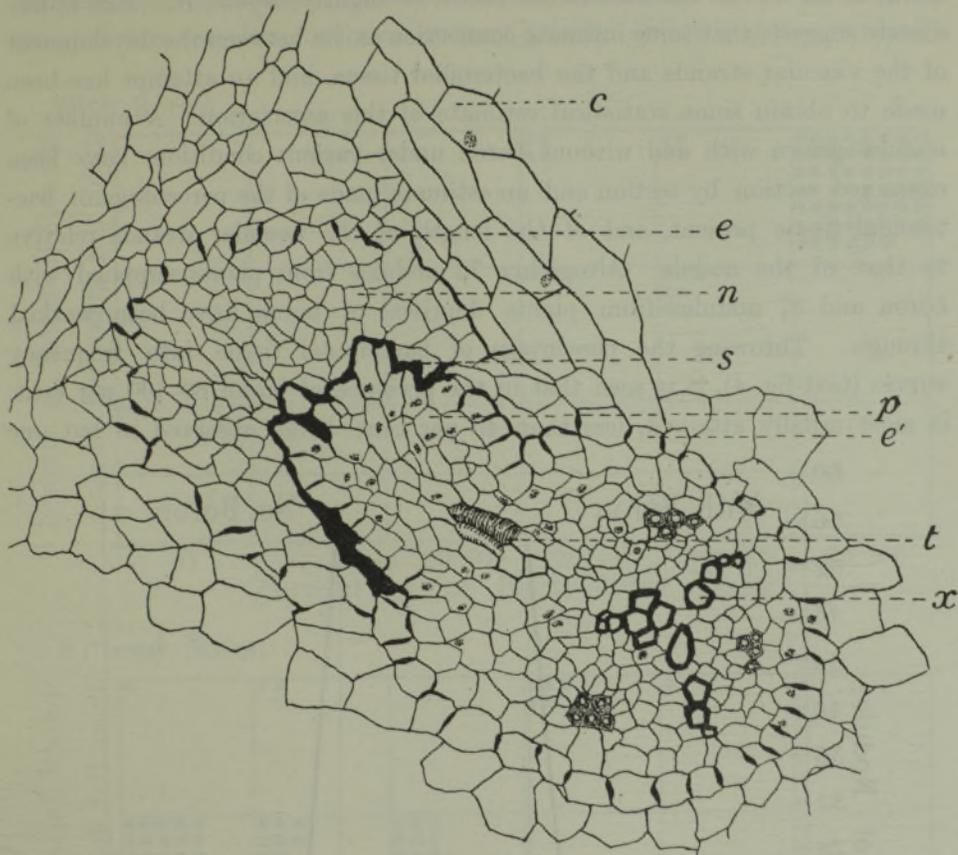


FIG. 3.—Transverse section of root of *Vicia faba* grown without boron, showing abortive nodule with no vascular strands or bacteroidal tissue.

e, endodermis of nodule. *e'*, endodermis of root. *n*, undifferentiated nodular tissue in cortex. *p*, proliferated cells in pericycle. *s*, deeply staining sheath between pericycle and nodular tissue. *t*, tracheides, not passing out into nodule. *c*, cortex. *x*, xylem of root.

The other type of nodule formed in the absence of boron develops further but incompletely (text-fig. 2, Type II). The bacteroidal tissue, instead of occupying the bulk of the nodule, is confined to a more or less constricted space at the inner end, the rest of the nodule being filled with rather smaller cells which would apparently have developed into bacteroidal tissue under more favourable conditions. Furthermore, the bacteroidal tissue is frequently

abnormal in character, being either undeveloped or in a state of apparent disintegration as regards the cell contents. The vascular strands are rarely more than one or two in number, they branch very little, and, more significant than all, they seldom run the full length of the nodule, but usually extend only about as far out as the bacteroidal tissue, or slightly beyond it. This immediately suggests that some intimate connection exists between the development of the vascular strands and the bacteroidal tissue, and an attempt has been made to obtain some statistical estimate of this association. A number of nodules grown with and without boron under various conditions have been examined section by section and an estimate made of the percentage of bacteroidal tissue present, and of the length of the vascular strands relative to that of the nodule. Altogether 72 nodules from plants supplied with boron and 87 nodules from plants deprived of boron have been worked through. Throwing the percentage of bacteroidal tissue into frequency curves (text-fig. 4), it is seen that in the presence of boron 80–100 per cent. is most usually attained, less than 40 per cent. being observed in but one

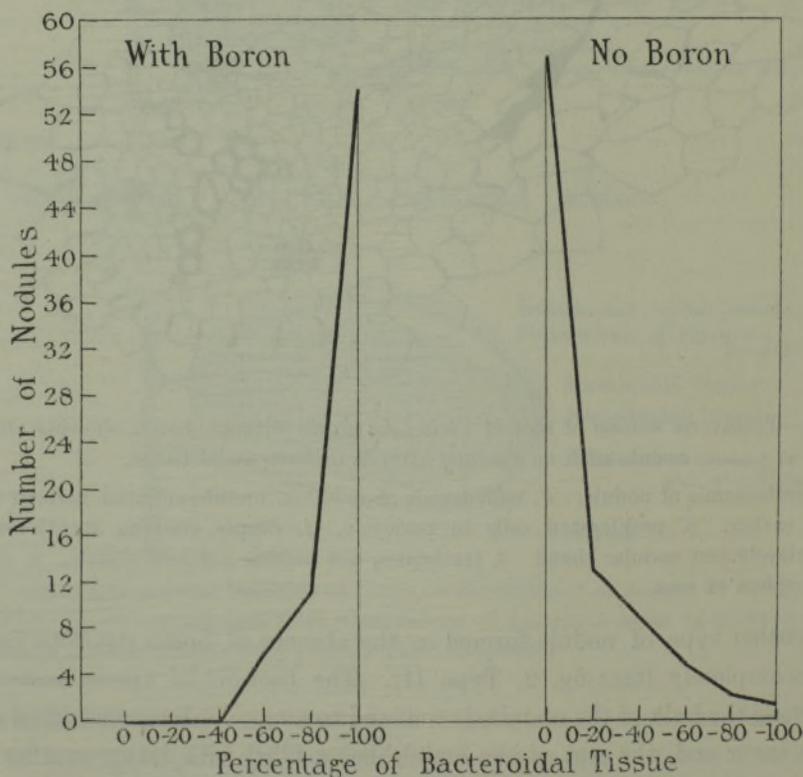


FIG. 4.—Curves showing the relative proportions of nodules with low and high percentages of bacteroidal tissue in the presence and absence of boron in the nutrient medium.

instance. In the absence of boron, on the other hand, an entire absence of bacteroidal tissue is most usual, and only in very exceptional cases does the percentage rise above 60. Even these few cases of high percentage may be explained by the fact that most of them occurred on plants which were not supplied with any nitrate, so that growth was slow and the boron contained in the seed was, therefore, not used up very quickly, and it is quite conceivable

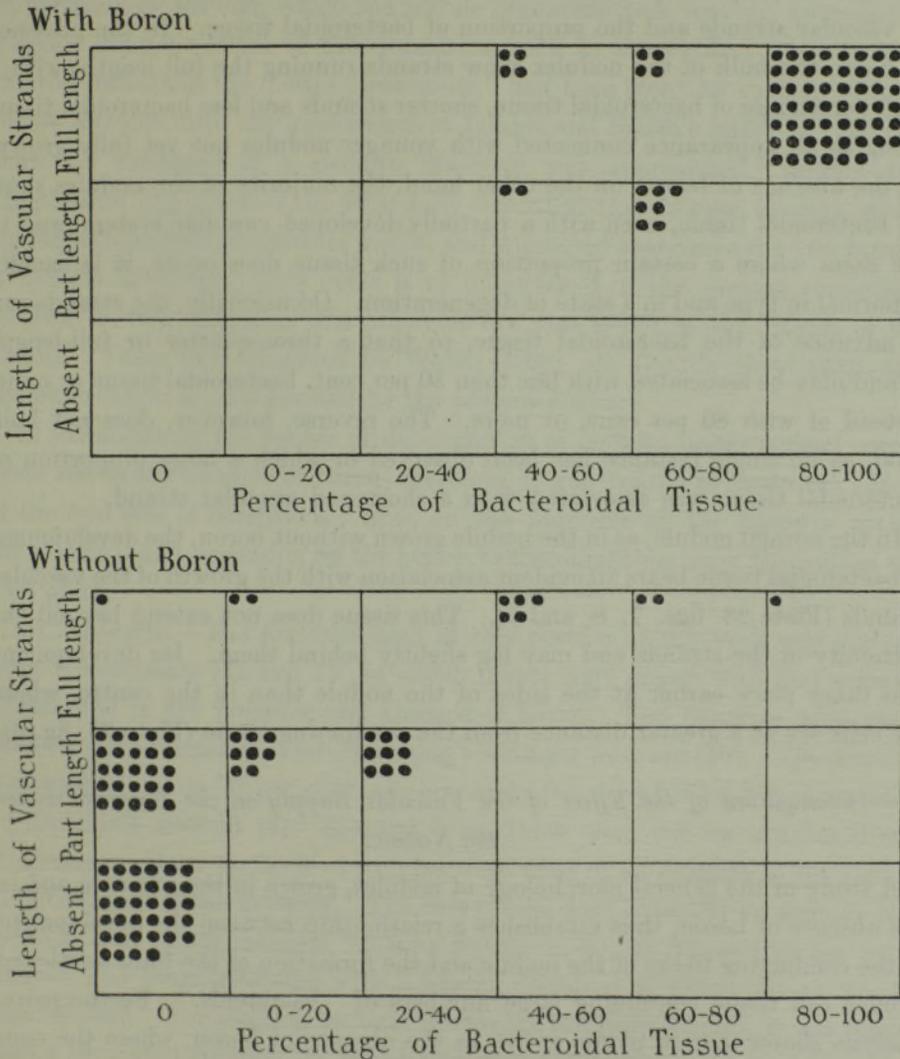


FIG. 5.—Showing relation between length of vascular strands in regard to the nodule and proportion of bacteroidal tissue developed. Each dot represents one nodule observed.

Total number of nodules with boron = 71.

Total number of nodules without boron = 87.

that part of this reserve boron supply aided in the better development of some of the earlier formed nodules on these plants.

Further analyses of the figures indicate that the production of bacteroidal tissue follows closely in the train of the development of the vascular strands, and that where the latter are arrested in their progress from any cause, the bacteroidal tissue suffers a corresponding diminution. The two charts (text-fig. 5) show graphically how close is the association between the development of vascular strands and the proportion of bacteroidal tissue. In the presence of boron the bulk of the nodules show strands running the full length, with a high percentage of bacteroidal tissue, shorter strands and less bacteroidal tissue being to all appearance connected with younger nodules not yet fully grown. In the absence of boron, on the other hand, the majority of the nodules show no bacteroidal tissue, even with a partially developed vascular system, and in the cases where a certain proportion of such tissue does occur, it is usually abnormal in type and in a state of degeneration. Occasionally, the strands run in advance of the bacteroidal tissue, so that a three-quarter or full-length strand may be associated with less than 30 per cent. bacteroidal tissue or none, instead of with 80 per cent. or more. The reverse, however, does not hold good, as no single instance has been observed in which a large proportion of bacteroidal tissue was associated with a shortened vascular strand.

In the normal nodule, as in the nodule grown without boron, the development of bacteroidal tissue bears an evident association with the growth of the vascular strands (Plate 28, figs. 7, 8, and 9). This tissue does not extend beyond the extremity of the strands and may lag slightly behind them. Its development also takes place earlier at the sides of the nodule than in the centre, where the cells are at a greater distance from the conducting tissue (Plate 28, fig. 8).

G.—*Investigation of the Effect of the Vascular Supply on the Bacteria within the Nodule.*

A study of the general morphology of nodules, grown in the presence and in the absence of boron, thus establishes a relationship between the development of the conducting tissue of the nodule and the formation of the fully developed swollen cell tissue containing large numbers of "bacteroids." Furthermore, analysis shows that in plants grown in the absence of boron, where the conducting tissue of the nodules is absent or deficient, there is little or no fixation of nitrogen. The bacteria in these nodules are thus not acting normally with regard to their nitrogen metabolism, nor do the tissues of the plant react normally to the stimuli produced by infection. It seemed probable, therefore,

that a careful study of the progress of infection and of the behaviour of the bacteria within these abnormal nodules would lead to a better understanding of the factors at work in the normal nodule.

Our present knowledge of the development and structure of nodules on leguminous plants is mainly derived from researches conducted during the nineteenth century. At the time of these investigations the cycle of change in morphology through which the nodule organisms pass was not appreciated. Various different forms of the organisms, such as the swollen "bacteroids," the rod forms and the motile "swarmers," had been seen* by various authors, but it was not until the appearance of Löhnis and Smith's paper in 1916 (16) that the existence of a definite life-cycle was appreciated. Bewley and Hutchinson (2), however, in 1920 fully described the changes through which the organisms pass in artificial media, and suggested that these changes formed a definite life-cycle. This cycle is of importance in the present connection because certain stages in this life-cycle are also found in the tissues of the nodule. These were observed by Wallin [(30); see also de Rossi (23)], and the observation is corroborated by the present authors.

In investigating the course of development of nodules under normal conditions and in the absence of boron, therefore, it was desired to study the effects of the two sets of conditions: first, on the life-cycle of the bacteria within the nodule; and, secondly, on the action of the organisms upon the cells of the host plant.

(a) *Technique.*—The material used in this portion of the work was derived from Experiments III and IV, in which plants were grown with and without nitrate and in the presence and absence of boron. Two fixatives were used, Bouin's picro-formol (4) and Flemming's stronger mixture (10). The fixative was washed out in 70 per cent. alcohol, and after dehydration was embedded by Dowson's method (8). Sections 5 m. thick were cut on a Leitz-Minot microtome. Many methods of staining were tried in an attempt to get a good differentiation of the bacteria. The best results were obtained by staining the sections with carbol fuchsine, washing rapidly in 30, 50, 70, and 90 per cent. alcohol, and counterstaining with lichtgrün dissolved to saturation in 90 per cent. alcohol. For staining bacteria within the infection threads, it was found that a saturated solution of bengal rose in 5 per cent. phenol, replacing the carbol fuchsine in the above method, gave some very fine preparations, in which the bacteria were stained red and the matrix of the infection thread green. This method, however, was very uncertain in its results, the bengal

* Some references to these earlier observations are given by Löhnis, 1921 (14).

rose being easily washed out in alcohol. Iron hæmatoxylin, followed by lichtgrün, was used where the cytology of the tissues was being specially studied.

(b) *Development of the Normal Nodule.*—The course of development of the normal nodule, in the presence of boron, was first followed, in order to have a basis for comparison with that of the abnormal types having defective vascular strands. In the normal root, the piercing of the cortex by the infection thread is followed by a multiplication of the cortical cells immediately adjoining the endodermis. This cell proliferation is limited to the inner tissue; the more external cells of the cortex do not divide. The multiplication of cells produces a mass of small-celled tissue which may be described as the “primary nodule” (Plate 28, fig. 5).

In this the infection thread ramifies, entering the cells in all directions. Within the cell the infection thread sometimes passes along the primordial utricle and sometimes crosses the middle of the cell. Strands containing only a single row of bacterial rods can frequently be seen crossing from the cell wall to the neighbourhood of the nucleus (Plate 27, fig. 1), and it seems probable that in such cases the bacteria have made use of the cytoplasmic strands of the host cell as a path of infection. In any case, the frequency with which the infection threads run up to the nucleus, as described by previous authors, was very noticeable in our preparations.

The bacteria in the infection thread are always in the form of minute rods whose protoplasm stains uniformly with carbol fuchsine or bengal rose (*cf.* Maria Dawson (6)). They are enclosed in clear spaces resembling capsules, which do not take up any of the stains used. These lie in a matrix which stains readily with lichtgrün. In several cases the infection thread has been seen running into cells with nuclei in various stages of mitosis, but this is rare, and it is clear that infection of a cell as a rule arrests its division.

After the formation by cell division of the primary nodule, further development is associated with the outgrowth of the vascular system from the stele. Young nodules, in which the strands had not yet grown into the nodule, were found showing an incipient swelling of the cells close to the stele. The swelling of the cells thus begins at the base of the nodule, and as the vascular strands grow it proceeds in an outward direction. By this time cell division in the mass of the primary nodule has been arrested by the infection of the majority of cells with the ramifying bacterial threads. At the distal extremity of the nodule tissue, however, the cells remain meristematic, and by rapid division produce an apical growing point (Plate 28, fig. 6). The bacterial strands

now grow outward towards the newly formed tissue thus produced, continually infecting fresh cells. In this way the secondary growth in length of the nodule is produced, and, in such nodules, all the stages in the infection and swelling of the cells can be seen in different parts of a longitudinal section. Close behind the apical meristem are young cells that are protoplasmic, having only small vacuoles. The infection threads can be seen entering these (Plate 30, fig. 20). The cells close behind this show the early results of infection on the cells. The uninfected cells in this region swell and become vacuolated, so that they resemble normal parenchyma cells. The infected cells also become enlarged and vacuolated and at first do not differ largely from the uninfected cells.

Some of the bacteria now pass out from the infection thread into the protoplasmic lining of the cell wall. The bacteria, after their release, lie singly in the cytoplasm of the host. They consist of evenly staining rods which could not be shown to possess capsules by any staining method tried (Plate 27, fig. 2). They very quickly multiply, become longer and exhibit a segregation of the staining material into bands crossing the rods. Increase in the bacterial numbers is accompanied by a further increase in size of the host cell and by a thickening of its protoplasmic lining. The nucleus increases in size, usually remaining in its normal position in the cell, and only in very old infected cells does it appear to be degenerating. When the infected cells are fully developed, the bacteria lying in the thickened protoplasmic lining have increased enormously in number and show a striking change in morphology. They become swollen and vacuolated, and stain faintly with fuchsine. These swollen forms, the "bacteroids" of Brunchorst (5) are, as is well known, characteristic of the "bacteroidal" tissue of the well-developed normal nodule. In this tissue one frequently finds remains of the infection threads crossing the swollen cells. These threads still contain bacteria. It is a point of some interest that the bacteria in these infection threads always remain in the form of minute uniformly staining rods, as described above (Plate 27, fig. 3). The factors in the environment that so greatly modify the bacteria lying free in the cytoplasm of the host do not effect any change in the appearance of the bacteria lying within the infection thread in the same cell.

(c) *Infection and Nodule Development in the Absence of Boron.*—In plants grown in the absence of boron the early development of the nodule is normal. The organisms enter the root hair (Plate 29, fig. 10), the infection thread, containing short rod forms, penetrates the cortex (Plate 29, fig. 11) and cell division takes place in the tissue adjacent to the endodermis and results in the

formation of the "primary nodule" (Plate 29, fig. 12). The course of events from this point differs according to the presence or absence of vascular strands in the nodule, and the extent of their growth where present.

In a great number of nodules produced in the absence of boron no vascular strands are developed at all (Type I, p. 384). A mass of cells is formed by multiplication of the tissue adjoining the endodermis, but from this stage development of the nodule is abnormal, no secondary growth being made. The conditions in such nodules are very interesting. The infection thread, which contains rods surrounded by clear spaces as in normal nodules (Plate 27, fig. 4), ramifies through the cells and, probably owing to its action in stopping cell division, the nodule soon reaches a condition in which no dividing cells can be found (Plate 29, figs. 13, 14), because the meristem cap is either not formed or, if produced, soon becomes invaded with bacteria, as described below. The cells in the centre of this arrested "primary nodule" swell somewhat in size and become vacuolated. The infection threads in this tissue undergo a remarkable development. Expansions and lobate processes appear on them (Plate 30, fig. 20). Swellings can be seen on infection threads in normal nodules and have been described and figured by previous authors (20, 24), but in such cases they are comparatively small. In the present case they develop to so great an extent as finally to fill up the whole cell, crushing the nucleus into one corner, and by further growth they form masses of bacterial zoogloea, which break up the nodule tissue. In Plate 29, fig. 15, a nodule is shown having a considerable portion of its centre destroyed and filled up with bacterial mass. The bacteria in these nodules never become swollen in the normal manner, but exist as rods or cocci. In most cases they seem to remain embedded in a zoogloal mass, but this is not always so, for they are sometimes seen lying free in the cells. It is an interesting point that the apical meristem of the nodule, if formed, is usually attacked by the bacteria, the small protoplasmic cells becoming filled with masses of rods and cocci. This is shown in Plate 30, fig. 19, which should be compared with the section of meristem in a normal nodule (Plate 30, fig. 20).

Where there is a partial development of the vascular strands (Type II, p. 384) the swelling of the infected cells takes place, and bacteria are usually released from the infection threads into the cytoplasm of the host cell. The thickening of the cytoplasmic lining and the associated rapid multiplication of the bacteria to fill most of the cells takes place to a more limited extent than in the normal nodule, and, as indicated above (p. 388), there is a definite relationship between the size of the region in which this occurs and the strand development. The

bacteria which thus multiply in the enlarged cells exhibit a swelling and a loss of staining power, but it is noticeable that they remain less swollen and more rod-like than do the bacteria in the corresponding tissue of the normal nodules. It would appear, therefore, that the swelling of the bacteria, as well as their rapid multiplication almost to fill the enlarged cells, is closely related to an efficient vascular supply. The meristem at the distal extremity develops normally, at any rate for some time, although the vascular strands have not grown out as far as that region of the nodule.

These nodules with defective vascular strands do not as a rule attain full size, degeneration of the nodule soon taking place (Plate 30, fig. 17). This degeneration first occurs in the swollen cells of the older tissue, but soon extends into the protoplasmic cells of the meristem cap, many of which can be seen to be filled with masses of bacteria. The bacteria attack the contents of the host cell (Plate 30, fig. 18). In a degenerating nodule of this type, the swollen cells may often be seen to contain neither nucleus nor any indication of cytoplasm, but appear to be filled merely with masses of bacteria (Plate 30, fig. 18). The bacteria are usually in the form of short rods or cocci, the "preswarmers" of Bewley and Hutchinson.

The nodules possessing a weak development of vascular strands thus exhibit four points of difference from the normal nodule. First, the region of the "bacteroidal tissue" is limited in proportion to the development of the strands; secondly, the swelling of the bacteria is less marked than in the normal nodule; thirdly, the bacteria soon become actively parasitic and destroy the protoplasm of the host cell, and, fourthly, the meristem cells often become filled with bacteria.

H.—*Discussion.*

The investigation of the nodules developing on plants grown in the absence of boron thus throws light on several points in connection with the normal functions of the vascular strands in the nodule.

With regard to the growth of the nodule tissue, the first point of interest concerns the relation of the vascular strands to the formation and growth of the meristem cap of the nodule. In nodules having a weak development of strands attaining only one-third or one-half the length of the nodule, the meristem cap at first appears normal and shows active cell division. Thus it can behave in an apparently normal manner although ill supplied with vascular strands and though separated by a considerable distance from the extremity of these. In these abnormal nodules, if the meristem is formed, it is, as a rule, infected during the degeneration of the nodule and many of the protoplasmic cells are

filled with masses of bacteria. In this connection an interesting theory is advanced by Pierce (19), who considers that the freedom from infection of the meristem in normal nodules is due to its rapidity of division, which results in a layer of new cells being continually produced in front of the advancing bacterial threads. In the normal nodules here considered, it seems reasonable to suppose that the deficiency of vascular supply delays the growth of the meristem so that the bacterial strands can reach it. The initial swelling and vacuolation of the cells in the primary nodule and in the region behind the meristem in the secondary nodule growth is apparently independent of the strand development, since it occurs in nodules having no strands and, where weak strands are present, it occurs beyond their extremities.

With regard to the effect of vascular supply on the bacteria within the nodule, it is clear that the branching of the infection thread through the tissues is independent of the presence of strands. The release of bacteria from the infection thread is the next point of interest. This question was discussed by Mazé (17), who thought that there was a causal connection between the development of the vascular strands and the release of bacteria into the cytoplasm of the host. A connection between the presence of vascular strands and the release of bacteria from the threads seems to be indicated by the fact that in nodules having no strands the bacteria are usually embedded in threads and masses of zooglœa. They are not always so embedded, however, even where no vascular strands are formed, and, where short strands are present, the bacteria are released into the cytoplasm in cells lying at a considerable distance beyond their extremities. Thus, while the release of the bacteria into the cytoplasm may be stimulated by the diffusion of substances from the strands, as Mazé supposed, there is no intimate connection between their release and the growth of the strands.

The case is different when we consider the final stage in the development of the infected cells, namely, the vast multiplication of the bacteria in the cytoplasm and their swelling up to form faintly staining sausage-shaped rods and "bacteroids." There is a close connection between this change and the growth of the vascular strands. The formation of the swollen-rod forms and of the typical "bacteroidal" tissue does not occur in the complete absence of vascular strands, and, furthermore, where strands grow into the nodule, the development of this swollen "bacteroidal" tissue is closely proportional to their growth, not extending beyond their extremities (*see* p. 388). Thus not merely the presence, but the proximity of the vascular supply is necessary to the formation of the swollen forms of bacteria typical of the developed nodule.

Equally striking is the change in physiology of the abnormal nodules. As described above, analysis shows that such nodules, on plants grown without boron, were fixing very little nitrogen compared with those on normal plants, where considerable fixation occurred. Thus, in the abnormal nodules, a defective vascular supply is found to be associated with a greatly decreased ability to fix nitrogen.

The development of the vessels in the nodules is thus connected on the one hand with the rapid multiplication and change in morphology of the bacteria in the cytoplasm, and, on the other hand, with the nitrogen metabolism.* It is reasonable to connect these effects with the supply of carbohydrate material brought into the nodule by the vascular strands. There is also another function of the strands that may, perhaps, be of importance—namely, the efficient removal of the products of metabolism of the bacteria. This function was discussed by Golding (11), who found that in cultures of *Bacillus radicum* greater nitrogen fixation could be obtained where the metabolic products were removed by means of a porous filter. The investigation of nodules on plants grown without boron, while it emphasises the great importance of the vascular supply in the nodule, has not at present enabled us to distinguish between these two functions.

Finally, the present investigation throws an interesting light on the relationship of the bacteria to the host plant. The failure of the vascular supply in the nodule results in the bacteria attacking and destroying the protoplasm of the host. It is maintained by Pierce that in normal nodules the bacteria are parasitic in the sense that they produce degeneration of the nuclei of the infected cells. In our material this degeneration does not occur until very late in the life of the host cell, some time after it has attained its full size and development. The state of affairs in the abnormal nodules, however, shows that under some conditions the bacteria are capable of decomposing the protoplasm of the host cell. Now, in the normal nodule cell very much larger numbers of bacteria are produced by multiplication. Why have these attained such large numbers without destroying the protoplasm of the cell in which they lie? The well-developed vascular strands have in this case introduced a factor that has altered the metabolism of the bacteria so that they do not destroy the protoplasm of the plant—at any rate, till the nodule

* Nobbé and Hiltner (18) concluded that the bacteria in the nodule that do not reach the "bacteroid" stage are harmful rather than beneficial to the host plant, and that these unchanged bacteria have no connection with nitrogen fixation, which commences with the formation of bacteroids.

decays after its period of functional activity. The nature of this factor seems to be indicated if one considers the change of energy supply in the environment of the bacteria brought about by the vascular strands.

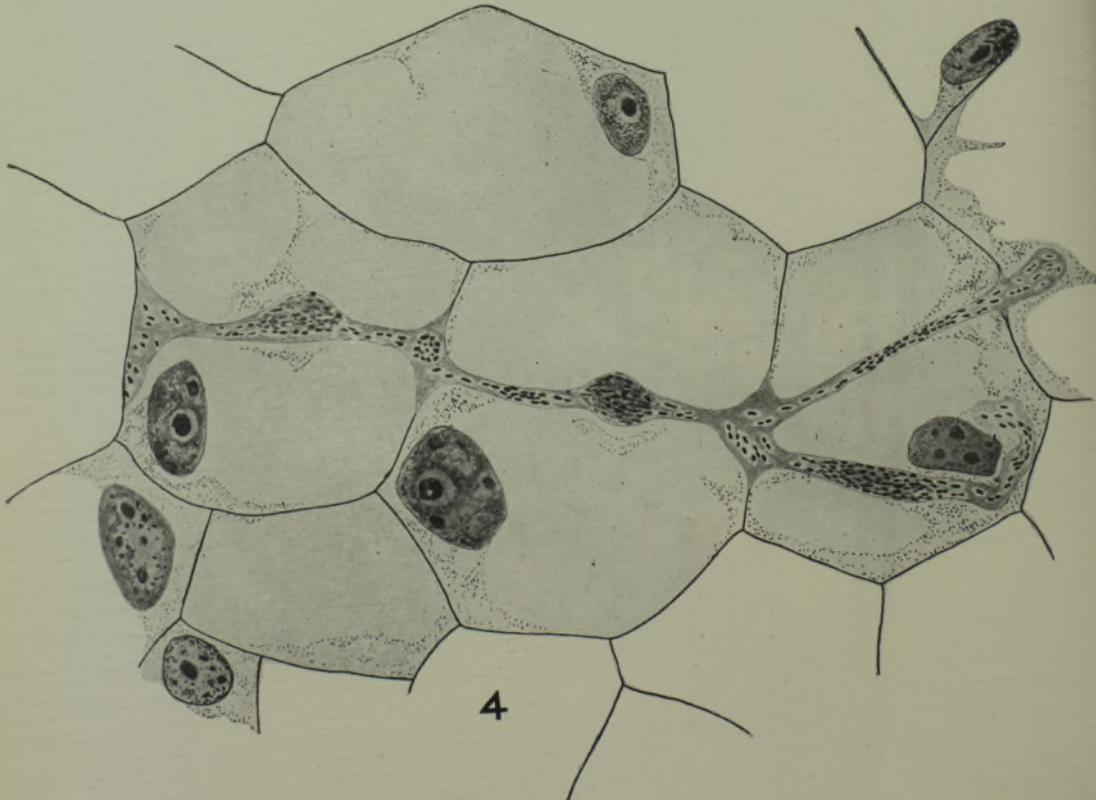
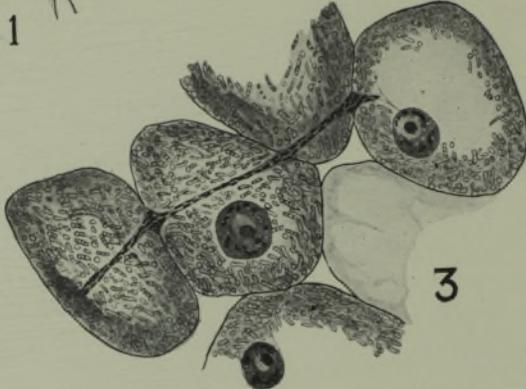
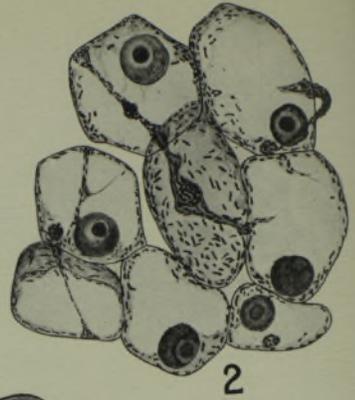
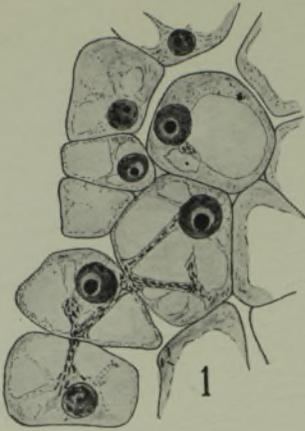
It is well established that the nitrogen metabolism of bacteria can be altered fundamentally by the nature of the energy material available to them. Thus Doryland (7), in studying the production of ammonia from organic nitrogen compounds by soil bacteria, found that this could be reduced or entirely inhibited by the addition of sugar to the medium. Similarly, the work of Berman and Rettger (1) shows that the decomposition of proteins by bacteria in culture is reduced by the addition of carbohydrate. In such examples the lessened decomposition of the organic nitrogen compounds is due to the fact that the carbohydrate added is utilised by the bacteria in preference as a source of energy. In the normal nodule there is a closely analogous state of affairs. The vascular strands supply the bacteria with carbohydrates, which they utilise as a source of energy in preference to the protoplasm of the host cell. In the nodules on plants grown without boron, in which the vascular strands are absent or defective, this supply of carbohydrate is cut off, and the bacteria therefore utilise the host's protoplasm as a source of energy. This may also explain why in the abnormal nodules the bacteria multiply so rapidly in the very protoplasmic cells of the meristem region, as described above. These cells are often found to be filled with masses of bacteria, while the vacuolated cells in the interior of the nodule are comparatively free (Plate 30, fig. 19).

It seems possible that in the nodules having no vascular supply we have reproduced a condition of affairs reminiscent of the primitive relations existing between the nodule organism and its host plant, and that, in the course of evolutionary history, the development of vascular strands running into the nodule has so altered the metabolism of the parasite as to make it harmless and eventually beneficial to its host. Finally, it is suggested that the ultimate degeneration of a normal nodule requires detailed investigations in the light of the above results.

I.—*Summary.*

(1) The work described deals with the growth and functioning of nodules on *Vicia faba*, comparing those grown in culture media from which boron has been excluded with those supplied with boron.

(2) In the absence of boron the vascular supply of the nodule is defective. The strands are often entirely absent, or, where present, are weakly developed, running only a short distance into the nodule.



(3) The nodules having no vascular strands remain minute, and are usually buried in the cortical tissues. In plants grown without boron, the number of nodules that attain macroscopic size is much reduced as compared with normal plants.

(4) In the nodules without vascular strands, the bacteria do not swell out to form the so-called "bacteroids." When weakly developed strands enter the nodule, the amount of tissue containing bacteroids is closely correlated with the extent of the strands.

(5) In the plants bearing these abnormal nodules very little nitrogen is fixed, the quantity fixed per nodule being, in one experiment, less than one-tenth of that fixed in normal plants. The defective vascular supply is thus accompanied, on the one hand, by a reduced development of "bacteroid" forms, and, on the other hand, by reduced nitrogen fixation.

(6) In the absence or weak development of vascular strands in the nodule, the bacteria tend to become parasitic, attacking the protoplasm of the host cell. This attack is chiefly directed towards the more densely protoplasmic cells of the nodule. It is suggested that this change in the relations between the micro-organism and its host is connected with the loss or reduced supply of the carbohydrate energy material normally brought into the nodule by the vascular strands, the bacteria thus being reduced to making use of the protoplasm of the host as a source of energy.

DESCRIPTION OF PLATES.

PLATE 27.

FIG. 1.—Cells from the young tissue of a nodule, close behind the apical meristem, showing their infection with bacterial zooglœa. In the middle cell, a strand containing a single row of bacterial rods can be seen extending to the nucleus.

FIG. 2.—Cells from slightly older nodule tissue, showing swelling and vacuolation. Many of the bacteria have escaped from the infecting zooglœa and lie free in the cytoplasm. They still consist of thin rods and are not yet swollen into bacteroids.

FIG. 3.—Cells of the bacteroidal tissue from the older parts of the nodule. The cytoplasmic lining of the wall has become thickened and is filled with bacteria in the swollen "bacteroid" condition. Crossing the cells can be seen the remains of the infecting zooglœa still containing bacteria in the thin rod stage.

Figs. 1 to 3 are drawn from a section of a normal nodule grown in the presence of boron.

FIG. 4.—Passage of the infecting thread of bacterial zooglœa through three cells, showing the contained rods surrounded by a clear zone. Cells from a nodule grown without boron.

PLATE 28.

FIG. 5.—Proliferation of cells (*a*) in the cortex of the root to form the primary nodule, (*b*) portion of stele.

- FIG. 6.—Young nodule showing the beginning of secondary lengthening and formation of swollen vacuolated cells in the proximal region. (a) cortex of nodule, (b) meristem, (c) swollen vacuolated cells.
- FIG. 7.—Nodule in which bacteroidal tissue has begun to form, showing its close association with a vascular strand. (a) nodule cortex, (b) meristem, (c) swollen vacuolated cells, (d) bacteroidal tissue, (e) vascular strand.
- FIG. 8.—Longitudinal section of root and nodule showing a further growth of the bacteroidal tissue associated with an extension of the vascular strands. (a) nodule cortex, (b) meristem, (c) swollen vacuolated cells, (d) bacteroidal tissue, (e) vascular strand, (f) stele.
- FIG. 9.—Longitudinal section of a large nodule. The strands extend as far as the meristem cap and the bacteroidal tissue almost fills the interior of the nodule. (a) meristem cap, (b) swollen vacuolated cells, (c) bacteroidal tissue, (d) vascular strands.

PLATE 29.

Infection and abnormal nodule development in *Vicia faba* grown in absence of boron.
Transverse sections of the roots.

- FIG. 10.—A portion of the piliferous layer of the cortex showing an infection thread of bacterial zooglæa passing down it.
- FIG. 11.—Part of a section showing at (a) an infection thread passing through the cells of the cortex (b).
- FIG. 12.—Part of a section, showing the early multiplication of cells in the deeper layer of the cortex to form the young nodule. (a) the root hair, (b) a portion of the infection thread, (c) enlarged cortical cells, (d) normal cells of the cortex, (e) the young nodule.
- FIG. 13.—Young nodule developing without the extrusion of vascular strands from the stele. (a) endodermis separating the stele (b) from the nodule (c).
- FIG. 14.—A larger nodule without vascular strands, showing the absence of bacteroidal tissue and of definite meristem. (a) a portion of the infection thread entering at the side of the nodule.
- FIG. 15.—A nodule without strands, in which the bacteria are attacking the tissues. At (a) masses of bacteria have broken the cells of the host and turgid uninfected cells can be seen projecting into the disintegrating tissue.

PLATE 30.

- FIG. 16.—Transverse section of root of *Vicia faba* grown without boron showing a nodule having a vascular strand projecting but a short distance into the nodule. (a) degenerating nodule tissue, (b) short vascular strand, (c) cortex, (d) endodermis of nodule, (e) endodermis of stele.
- FIG. 17.—Transverse section of a root of *Vicia faba* grown without boron, showing a nodule along which the vascular strands extend half-way. At (a) a vascular strand is cut across near its base. Some bacteroidal tissue has formed at (b), but the bacteria have attacked and destroyed the cell contents. At (c) is the infecting strand of bacterial zooglæa passing through the cortex.
- FIG. 18.—A further enlarged portion of a nodule in similar condition to that shown in fig. 17. The degeneration of the bacteroidal tissue can be seen. The infected cells are filled with masses of bacteria (a) that have destroyed the nucleus and cytoplasm. At (b) a branch of the infecting strand still persists.

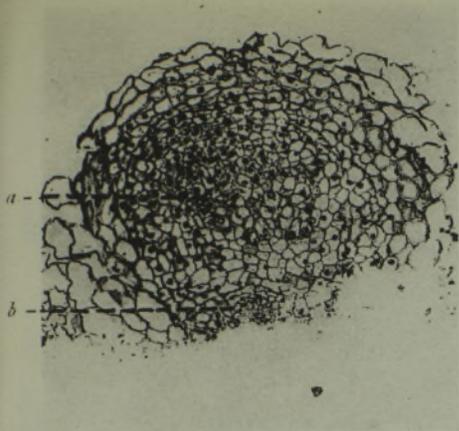


FIG. 5

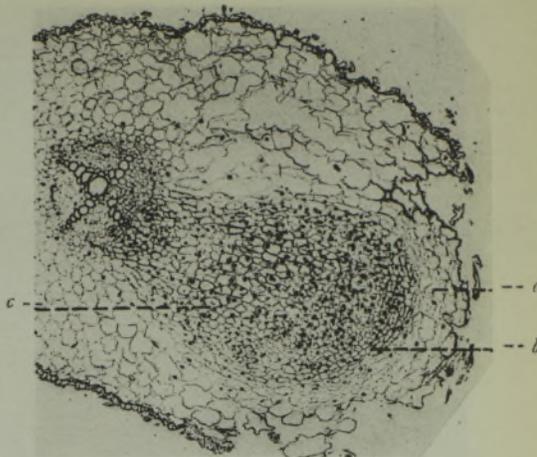


FIG. 6

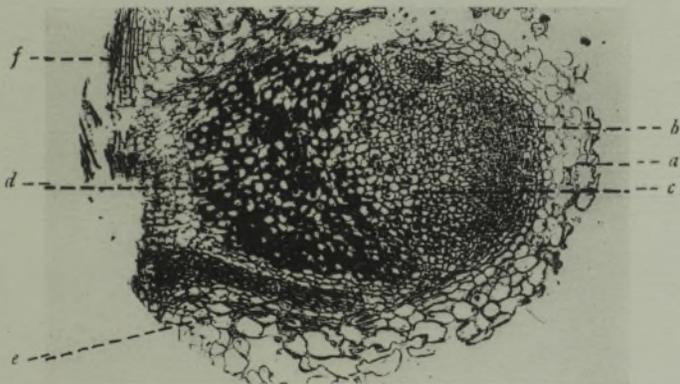


FIG. 8

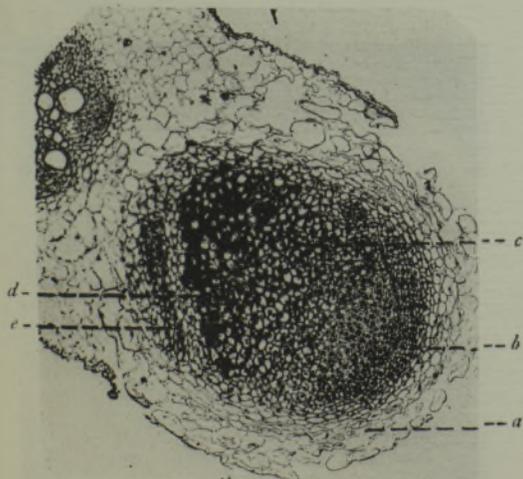


FIG. 7

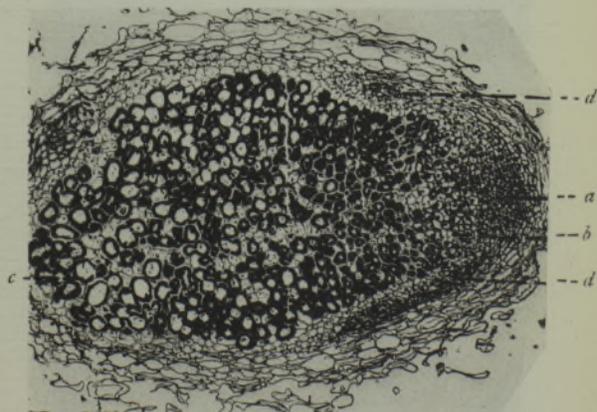


FIG. 9



FIG. 10

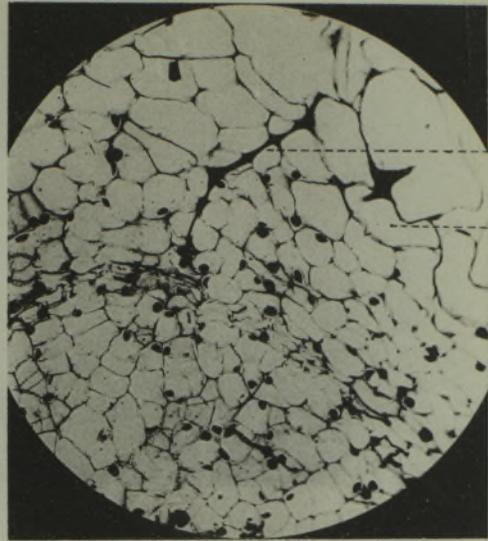


FIG. 11

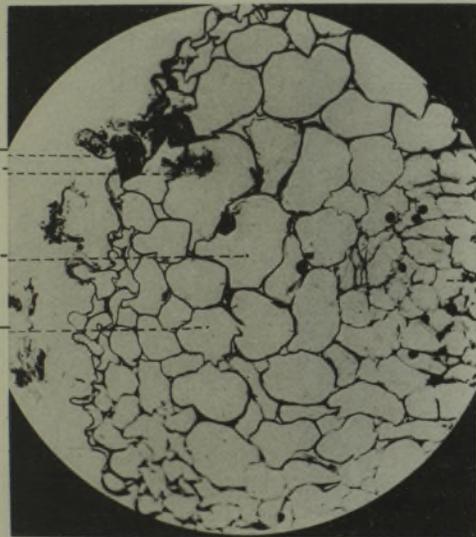


FIG. 12

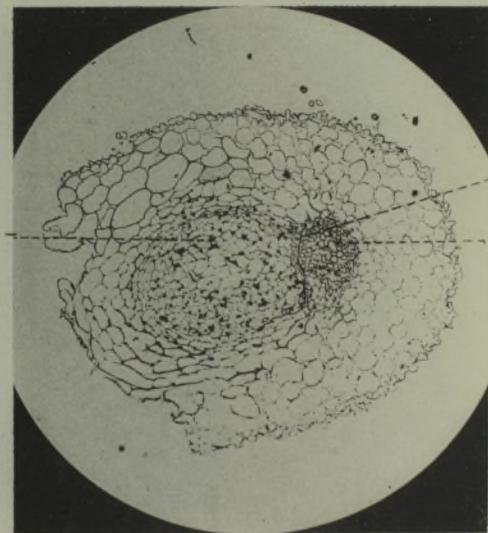


FIG. 13

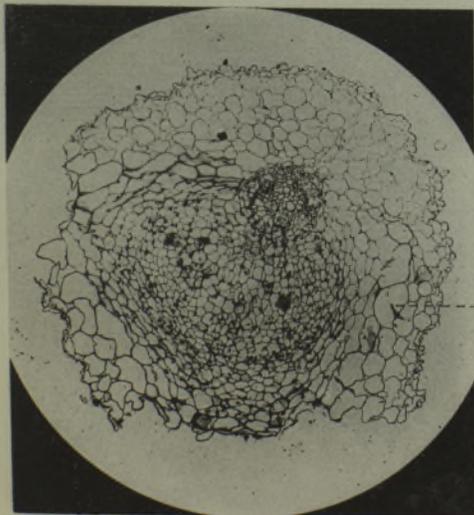


FIG. 14

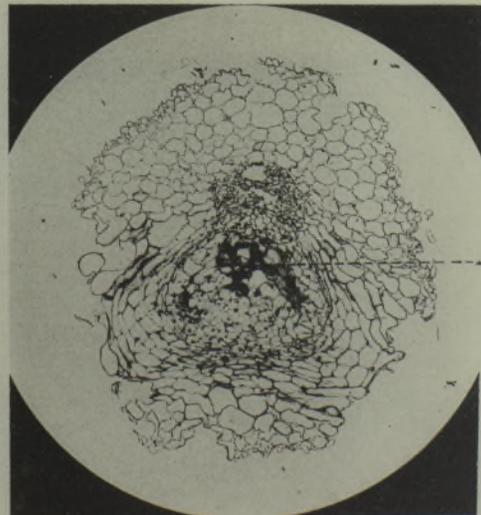


FIG. 15

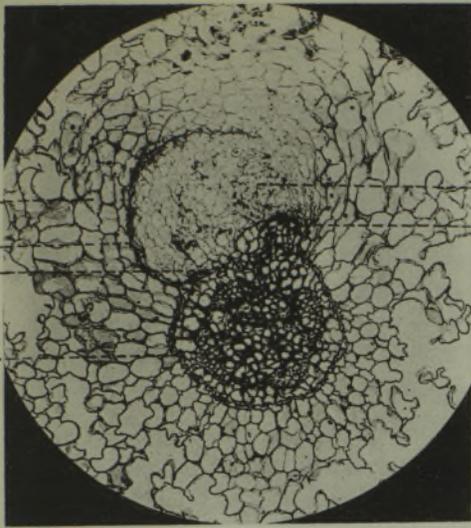


FIG. 16

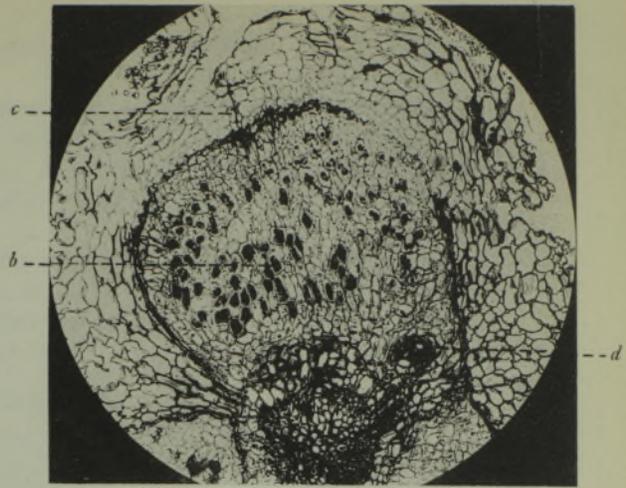


FIG. 17

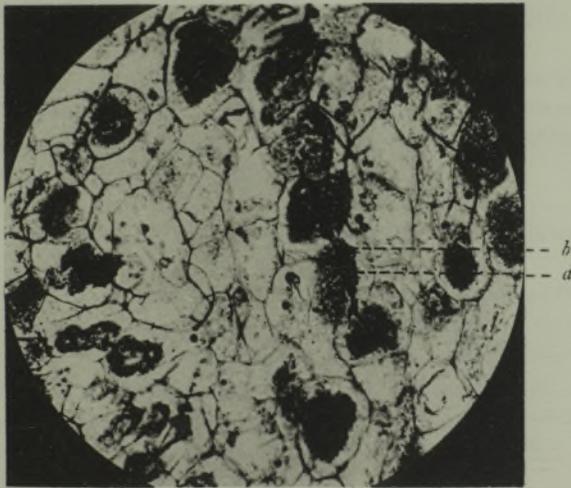


FIG. 18



FIG. 19

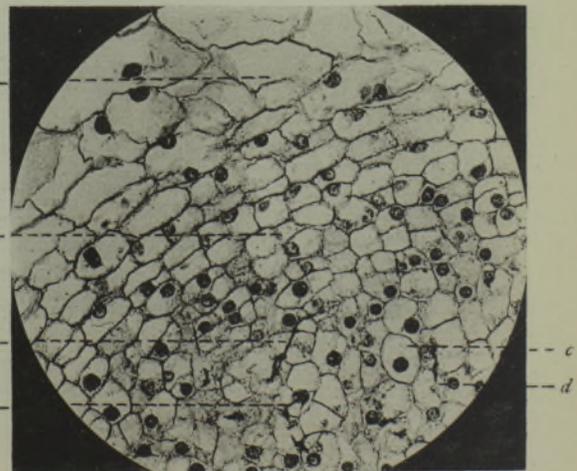


FIG. 20

FIG. 19.—Section of a nodule grown in the absence of boron and into which no vascular strands have entered. At (a) can be seen an infection thread of bacterial zoogloea that expands at (b) to form lobate processes. At (c) the nodule meristem is seen, the cells being filled with masses of bacteria. (d) cortex of nodule, (e) enlarged cell tissue in the centre of the nodule.

FIG. 20.—Section showing the distal portion of a normal nodule grown in the presence of boron showing at (a) the nodule cortex, at (b) the meristem free from bacterial infection, at (c) the swelling of the cells behind the meristem which are being invaded by the bacterial infection threads visible at (d).

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