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ON THE PHYSIOLOGY OF THE FORMATION OF NODULES ON LEGUME ROOTS

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It is now about 50 years since Hellriegel and Wilfarth finally elucidated the nature and importance of the nodules on the roots of *Leguminosae*, and Beijerinck obtained the nodule bacteria in pure culture. Nevertheless on the three essential points in regard to the nodules we are just as much in the dark as ever. These are:

1. How does the nodule fix nitrogen? The bacteria alone, in culture, fix no nitrogen, neither does the plant without them.

2. How do the bacteria enter the root? They pass through the cell-walls with apparent ease, yet in culture they do not attack cellulose.

3. How does the nodule develop? Morphologically, of course, its development has been described many times, but how do the bacteria bring about the proliferation and enlargement which constitute the nodule?

The present note is an attempt to answer the third of these questions, and to put forward a physiological theory of nodule formation, which is strongly supported, if not proved, by the facts.

Studies upon the plant growth hormones, or auxins, especially in relation to roots, have established the following facts (for literature up to Dec., 1934, see the author's review[1]):

a. A large number of substances, most, if not all, of which are unsaturated organic acids of various types, or their esters, have plant-growth-promoting activity. Not only the actual activity, by assay depending on cell-elongation, but also the ability of the substances to be transported in plant tissues, varies widely.[2] Indole-3-acetic acid is one of the most active.[3]

b These auxins act not only on cell elongation, but they also promote, and are necessary for, the formation of roots.[1]

c. They also inhibit the elongation of roots very strongly.[1,4]

d. In the pea at least they promote the formation of branch or lateral roots on the main root.[5]

e. They stimulate cambial activity in the stem.[6]

f. Under certain conditions they may cause swelling or enlargement of the root tissues, principally of the cortex.[7]

g. Indole-3-acetic acid, and possibly other active substances, are frequently produced by bacteria and fungi in culture.[1]

On the other hand, studies on the morphology of the nodules have shown that they arise either in the cortex or, like lateral roots, in the pericycle (for literature see[8]). They consist of greatly enlarged cells containing the bacteria, while outside the infected cells other cells are stimulated to growth and division. There is therefore strong indication that a substance causing cell enlargement and division is produced in, and diffuses out of, the bacterially infected cells.

Combining these two groups of facts we get the following picture of the series of events after the bacteria enter the root tissues; the bacteria multiply in the cells, causing breakdown of carbohydrate and protein. In their metabolism is produced, among other things, small amounts of an auxin. This substance causes enlargement of the cells in which it is produced, and also, being readily diffusible, enters the pericycle behind the cortical tissue inhabited by the bacteria, and there also stimulates growth and division, giving rise to the first stages of a lateral root initial (*d* above). However, since auxin production continues, this lateral root is prevented from elongating (*c* above). Instead its cells increase in size isodiametrically (*f* above), while certain of the uninfected cells are stimulated to division (*e* above) by auxin diffusing out of the infected area. In this way we get a shapeless mass of parenchymatous tissue, which is essentially a lateral root prevented from elongating. The differentiation of the vascular system within the nodule offers a complication which cannot be dealt with here, as nothing is yet known about the causes of differentiation.

Such a picture is in line with all the known facts. Can it be supported experimentally?

In the first place, if auxin is produced in the nodule, it must be possible to detect it by the standard Avena technique. Direct diffusion experiments were therefore made. Young, still growing nodules from roots of *Pisum* plants 3–4 weeks old were sectioned and placed on plain agar. The agar was then tested upon Avena coleoptiles, and its auxin activity was considerable. This is in marked contrast to root-tips which do not, in general, yield auxin to plain agar, but only to agar containing dextrose or other nutrient. The nodule appears to possess no marked polarity since about the same number of units diffuses into the agar when either its apical or its basal half is used, the area of contact being a cut through the

center parallel to the root in each case. The auxin is therefore not due to the meristematic tip of the nodule, but comes directly from the infected tissue. The maximum yield, 10–12 plant units per nodule in 3 hours' diffusion, was attained with nodules 2–3 mm. in diameter. As soon as the nodule turns brown and soft, auxin production ceases completely. Lateral root-tips similarly treated gave up only traces of auxin to the agar. The nodule is therefore an active auxin-producing center, its auxin production roughly paralleling its growth.

In the second place, the application of auxin, locally, to very young lateral roots (0.5 mm. long) of the same plant resulted in their complete inhibition. The root-tip remained more or less conical, but after 4 days there was swelling at its base. There was no generalized swelling of the main root. In one case two lateral root initials grew together to produce a nodule-like object. The swelling was due to increase in size of the cells of the cortex. It is hardly to be expected that application of the active substance from outside would imitate its action when applied from within by the bacteria, but at least, the complete inhibition of elongation of the lateral rootlet is confirmed.

Lastly we come to the nature and source of the auxin produced by the bacteria. The auxin produced by *Rhizopus* is indole-acetic acid formed from the tryptophane present in the peptone of the culture medium.[1] The ability of bacteria to carry out this reaction is fairly general, especially, according to Frieber,[9] if they do not produce free indole from the tryptophane. This the *Rhizobium* bacteria do not do.[10] Further, it has been observed by the author many times that the application of indole-acetic acid solution to roots results not only in inhibition, but also in a swelling which is characteristically due to *radial elongation* of the cortical cells, together with divisions in the cambium and pericycle. These two characteristic features are exactly what was shown to be produced by sterile filtrates of nodule-bacteria cultures (Molliard[11]). He found marked inhibition of elongation of the root, division in the pericycle and radial elongation in the cortex. There is no doubt, therefore, that the bacteria in culture do produce auxin in considerable amounts.

Experiments were also carried out to determine whether uninfected parts of the pea plant could convert tryptophane to indole-3-acetic acid; no indications of the ability could be found, hence the presence of the bacteria is essential. The amount would be small, being presumably limited by the cell protein, but the effects are in any case of the chronic rather than the acute type.

Taken together, the above facts, new and old, thus provide a satisfactory explanation for the growth of the nodule and the rôle of the bacteria in its formation.

Similar considerations, with some modifications, would apply to galls

and other outgrowths of the plant. The analysis of these phenomena will be dealt with at a later date. Detailed experimental data on the nodules will be published elsewhere.

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THE NATURE OF TONAL BRIGHTNESS

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It is well known that tones can be characterized as *bright* or *dull*. When intensity does not alter greatly, tonal brightness seems to vary with pitch: high tones are bright, and low tones are dull. Thus Rich[1] concluded that brightness and pitch are identical as tonal attributes. Abraham[2] concluded that the brightness of tones produced by a Seebeck siren is a function of the ratio of the size of the hole in the siren disc (H) to the size of the closed interval between holes (I). For values of H/I from about 1.0 to 0.1 he concluded that brightness varies inversely with H/I. By an analysis of what he supposed these waves forms to be he decided that brightness is not due to the presence of higher partials, for the reason that his analyses did not persistently show an increase in the higher partials for the brighter tonal complexes. He thus was led to suppose that brightness depends upon the form of the primary wave in the complex. Ogden[3] later accepted Abraham's conclusion.

Apparatus.—For stimuli we cut sirens from cardboard (0.04 in. thick). Each siren is a disc of 16-in. diam. The holes are sectors, 0.75 in. in radial dimension, with the ends cut exactly along radii and the sides cut approximately as arcs about the siren's center. In this way we avoided the ambiguity that arises with the circular holes of the Seebeck siren, where it is hard to say just what is the exact duration of the hole and of the interval be-