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A discussion on symbiosis involving micro-organisms

UNDER THE LEADERSHIP OF H. G. THORNTON, F.R.S.

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[Plates 11 to 13]

CONTENTS

Introduction. The symbiosis between *Rhizobium* and leguminous plants and the influence on this of the bacterial strain

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[Plate 11]

The term symbiosis has been used with different meanings, and the question of its correct meaning and even of the desirability of its use at all has been debated. The term, indeed, raises the question as to how far it is possible to distinguish a definitely beneficial association between two or more organisms from certain states of parasitism on the one hand and from complex ecological associations on the other. For the purpose of the present discussion, it seems desirable to define the term symbiosis as implying some evidence that the partners in the association each receive some benefit from it. We can then see how far the examples that will now be described satisfy this criterion. I propose to begin by considering an association in which there is clear evidence of such mutual benefit, that of the nodule-forming bacteria of the genus *Rhizobium* with their leguminous host plants.

This association provides exceptional facilities for studying symbiotic adaptation, first because the bacteria can readily be grown in laboratory media, and in some cases the host plant can easily be grown on agar, either aseptically or supplied only with a pure culture of a bacterial strain. Thus a study can be made of the effect on the symbiosis not only of different naturally occurring strains of *Rhizobium* on different species and varieties of legume, but also of genetically determined changes both in the bacterium and in its host plant. Secondly, because the environmental conditions of the host plant can readily be varied and their effects on the symbiosis can be studied, and thirdly because we have, in the number of nodules, a measure of infection and in the quantity of nitrogen fixed, a measure of the effectiveness of the symbiosis.

The nodule bacteria, although placed in the special genus *Rhizobium*, do not show any remarkable characters when cultured *in vitro*, and closely resemble other common soil bacteria such as *Radiobacter*, with which they have often been confused. It is their association with the legume host which alone gives them their unique interest.

Strains of *Rhizobium* show a considerable degree of specificity as regards the host plants that they are able to infect and, largely for practical reasons, this character is used in their classification. But specificity is often not absolute and differs in degree in the various infection groups and according to the bacterial strain (Aughtry 1948; Wilson 1939). Moreover, it is not well correlated with other characters. For instance, strains of *Rhizobium* from *Trifolium* and *Pisum* will only cross-inoculate the host plants of each other with great difficulty (Kleczkowska, Nutman & Bond 1944). Yet strains isolated from these two genera may resemble each other antigenetically, while, on the other hand, strains within each group have been found that show no cross-agglutination (Kleczkowski &

Thornton 1944). It seems possible, therefore, that the ability to infect clover and peas has been developed more than once from amongst a soil population of *Rhizobium* that had already become differentiated antigenetically. A similar lack of correlation has been found between host plant specificity and susceptibility to infection by strains of bacteriophage (Conn, Bottcher & Randall 1945; Kleczkowska 1946).

The nodule bacteria are normal constituents of the soil flora and can exist for many years in field soil without their host plant. But its presence induces a marked increase in the numbers of *Rhizobium* in the neighbourhood, and there is evidence connecting this with a stimulatory root secretion (Thornton 1929). Strains differ considerably in the rates at which they multiply in the roots surrounding under the influence of these secretions (Nicol & Thornton 1941).

Microscopic examination of clover or lucerne roots grown in agar and supplied with *Rhizobium*, show masses of the bacteria amongst the root hairs, through which infection normally takes place. Deformation of these root hairs seems to be a necessary prelude to infection and usually takes the form of a curling of the distal end. It can be produced by a sterile filtrate of a culture of *Rhizobium* (Thornton 1936) and is almost certainly due to β-indolyl acetic acid produced by the bacteria (Thimann 1936; Chen 1938). Deformation of the hairs is not strain-specific. It can be produced by strains that have lost the power to invade the plant, by those belonging to other cross-infection groups (McCoy 1932) and on host plants incapable of developing nodules (Nutman 1949). But we have never found infected root hairs in any association of plant and bacteria that for any reason is incapable of resulting in the formation of some nodules. Moreover, even in a compatible association, the percentage of root hairs invaded by the bacteria has been estimated by McCoy (1932) at only 5·5 % in the case of lucerne. Hence the plant puts up considerable resistance to infection at the root-hair surface.

Having surmounted the barrier of root-hair infection the bacteria penetrate root parenchyma and there induce cell division by the secretion of a substance able to diffuse from cell to cell in the host tissues. There is again a limitation imposed by the host plant on the number of nodules that develop. In McCoy's material 1·5 % of the infected root hairs resulted in nodules. The number of nodules produced is a characteristic of the bacterial strain, different strains showing big differences in this character of infectivity, as was shown by Chen (1941). With the same bacterial strain and type of host plant, however, there is a correlation between the dose of bacteria supplied to the root surroundings, the number of infected root hairs and the number of nodules formed (Bhaduri 1951).

Under natural conditions, the root system of the host legume is commonly exposed to invasion by several strains of *Rhizobium*. In fact, different strains can be isolated from nodules on the same individual plant grown in the field (Hughes & Vincent 1942; Purchase & Vincent 1949). When two or more strains are simultaneously present, the proportion of nodules produced by each will be determined by the following factors (Nicol & Thornton 1941):

(1) The relative numbers of each strain in the root surroundings. This may be greatly affected by differential competition between the strains outside the plant.

Such competition can result in one strain being suppressed by another that is dominant in competitive growth.

(2) The relative infectivity of the strains. This factor can be detected where the strains do not compete differentially in the root surroundings.

When one strain forms nodules before the arrival of a second, it may have saturated the nodule-forming capacity of the plant, thus excluding the second strain (Nicol & Thornton 1941). This has led some authors to conclude that nodules formed by one strain exclude the entry of a different strain while allowing continued infection by the same strain (Dunham & Baldwin 1931; Virtanen & Linkola 1947). There seems, however, to be no definitive evidence of such specific immunity.

Individual strains of *Rhizobium* differ not only in characters that influence *infection*, but also in the *effectiveness* in fixing nitrogen of the nodules that they produce on the same type of host plant. The nodules formed by most strains fall into one of two groups, namely:

(1) *Effective nodules* that fix quantities of nitrogen normally adequate for the plant's needs. Such nodules tend to occur mainly on the large roots, to be relatively few in number and large in size.

(2) *Ineffective nodules* that fix little or no nitrogen. These are usually found all over the root system, and are relatively numerous and very small.

Typically effective and ineffective nodules show important differences in the course of their development (Chen & Thornton 1940). In both types of nodule the early stages of growth are usually similar. At first the nodule consists of a small mass of meristem cells usually derived from the root cortex. Most of the central cells become infected with bacteria and cease to divide. External to these a layer of uninfected cells remains meristematic, usually forming a distal cap. The activity of this meristem causes growth of the nodule, the inner layers of newly formed cells being successfully invaded by the bacteria (Thornton 1930*a*). Further differentiation results in the formation of vascular strands connecting the nodule with the central cylinder of the root, and of a secondary endodermis (Bond 1948; Frazer 1942). From about this stage of development the two types of nodule differ.

In effective nodules further growth occurs and results in the formation of a considerable mass of central bacterial tissue. This tissue, when in a healthy and apparently active condition, presents interesting characters. First, the great majority of the bacteria lie within the cytoplasm of the host cells and not in the intercellular spaces. Secondly, these bacteria are often, though not always, swollen, branched or otherwise deformed. In the mature bacterial tissue the bacteria show no evidence of multiplication and, indeed, are said to be non-viable after isolation (Almon 1933). Thirdly, the host cells containing the bacteria themselves become hypertrophied, cease to divide and their nuclei show signs of degeneration. These appearances are shown in figure 1, plate 11. Fourthly, the infected host cells contain haemoglobin. The presence of this haemoglobin is correlated with nitrogen fixation (Smith 1949*a*; Virtanen 1945; Virtanen, Erkama & Linkola 1947), although no satisfactory explanation of its function has yet been given (Smith 1949*a*, *b*), nor is its origin known. It is not produced in the plant without the

bacteria or by the bacteria without the plant. Thus the hypertrophied cells containing 'resting' bacteria and haemoglobin are features of the symbiosis and correlated with the nitrogen fixation.

The duration of the active life of a nodule varies very much with the host plant, bacterial strain and cultural conditions. Clover nodules may remain active for several months but eventually they disintegrate, and this process is of great interest and importance. It usually begins in the older parts of the nodule and is due to a parasitic attack of the bacteria on the host tissue of the nodule (Thornton 1930*b*). During the development of the young nodule, passage of the bacteria from one host cell to the next usually leaves a few bacteria in the middle lamella of the cell wall. These remain in the condition of short rods. In early stages of necrosis these bacteria multiply and spread in the intercellular spaces, causing tissue destruction. The swollen resting bacteria inside the host cells meanwhile break up and disappear (see figure 2, plate 11). In consequence, the old disintegrated nodule contains slime full of short rod-shaped bacteria directly descended from those that infected the root and produced the nodule, while the swollen forms that were responsible for nitrogen fixation disappear.

In typically ineffective nodules, both the meristem and the central bacterial tissue are transient. After a short period, the meristem ceases its activity and growth stops, while the bacterial tissue undergoes rapid disintegration. In ineffective clover nodules internal necrosis is complete when the nodule is about 2 weeks old. This early necrosis may be the reason why ineffective nodules have not been shown to contain haemoglobin (Smith 1949*a*) which may not have had time to accumulate in detectable amounts. Indeed, it is impossible from our observations on nodule histology to tell whether differences in the course of development of the effective and ineffective nodules are the cause or the effect of the failure to fix nitrogen, or whether both result from a common cause. There is, however, some evidence that root systems bearing ineffective nodules contain some substance harmful to the growth of nodule bacteria *in vitro* (Chen, Nicol & Thornton 1940).

Ineffective strains of *Rhizobium* may be abundant in field soil. Umbreit (1944) found that 25 % of the strains isolated from soy beans were ineffective. Leonard (1930) described a case where the failure of a pea crop was related to the abundance of such strains. Ineffective strains of *Rh. trifolii* are abundant in hill pastures in Great Britain, and this raises the question whether the clover growth in such pastures can be improved by inoculation with an effective strain. For such inoculation to succeed it is necessary that the introduced inoculum should be capable of establishing itself in competition with the native population of *Rhizobium* already present in the soil. Hence the strain characters already discussed that determine which of several strains simultaneously present shall infect the plant have considerable practical importance. Indeed, it has been found that strains, when used for clover-seed inoculation in the field, differ in the degree to which they will establish themselves in the crop (Kleczkowska & Thornton 1950).

Like other bacteria, *Rhizobium* is liable to the sudden production of variant forms differing from the parent in one or more characters. Some of these variants

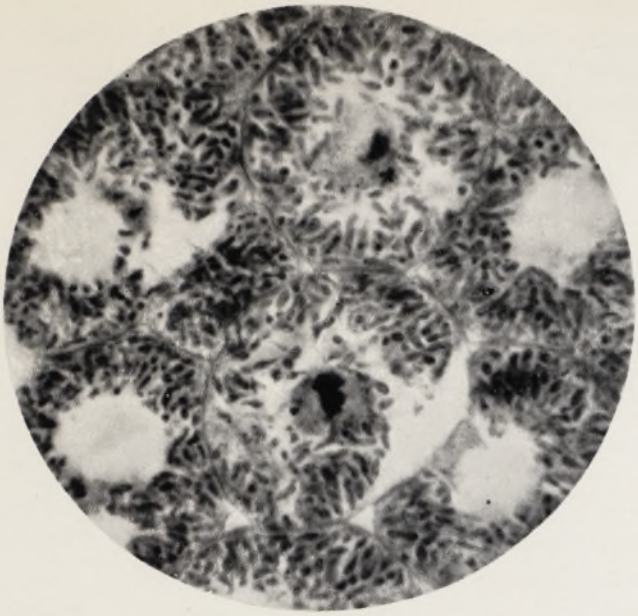

FIGURE 1. Healthy tissue, showing hypertrophied host-plant cells with degenerate nuclei and swollen bacteria in the cytoplasm.

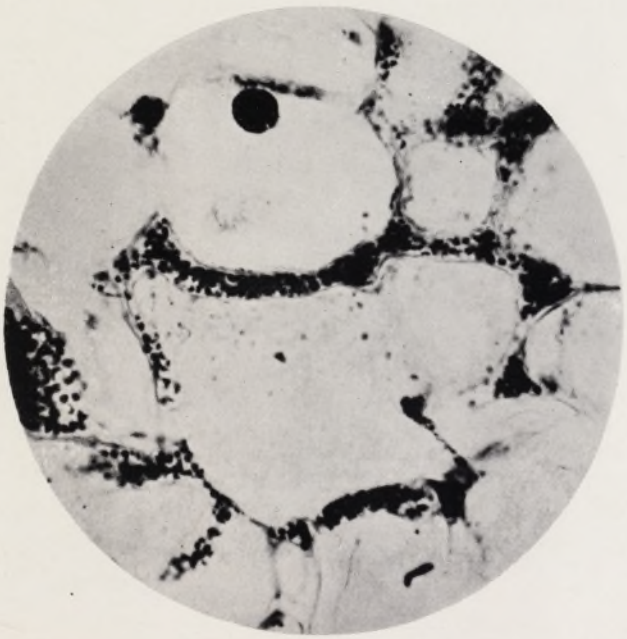

FIGURE 2. Necrotic tissue in an old nodule, to show disappearing cell contents and bacteria invading the cell walls.

Sections of the central bacterial tissue of clover nodules. (Iron haematoxylin, magn. × 1200.)

(*Facing p.* 174)

show complete change in the effectiveness of the nodules that they produce (Nutman 1946). Culture in sterilized soil or at cold temperatures and treatment with bacteriophage have produced completely ineffective variants from highly effective parent strains, while, with greater difficulty, ineffective strains have been induced to produce effective variants by phage treatment (Kleczkowska 1950). These variations in effectiveness also affect the number of nodules produced in the root system. Strains may also lose their ability to infect the root hairs and to produce nodules on their host plant. It is, indeed, possible that soil may contain many such avirulent strains of *Rhizobium* which would never be detected as such, since nodule formation is our only certain diagnostic character.

In any association such as that between *Rhizobium* and its host legume, both partners must have their effects on the mutual relationship. In the present paper I have considered only the influence of bacterial strain differences, assuming the inherent characters and environment of the host plant to be constant. This ideal condition is never encountered in practice. If two sets of plants raised from the same sample of seed and grown under similar conditions are supplied respectively with typically effective and ineffective strains of *Rhizobium* that can infect them, striking and significant differences between the two sets may be expected in the mean number and size of the nodules and in the mean quanity of nitrogen fixed per plant. In the set supplied with the effective strain, individual plants may show wide variation in all these characters, and a few may even show all the features of a completely ineffective association, although bacteria reisolated from such plants have remained unaltered and produce normal, effective nodules on other plants (Nutman 1946). The characters of nodules produced by very ineffective strains tend to show less variability between individual plants in the same set, but strains that are quite ineffective on one plant species may be effective on another. The growth conditions of the plant may also influence the number and character of its nodules.

The effect of the host plant on its association with *Rhizobium* is the subject of Dr Nutman's paper.

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Host factors influencing infection and nodule development in leguminous plants

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[Plate 12]

Dr Thornton has shown that much of the diversity in the symbiosis between the leguminous plant and root-nodule bacteria can be attributed to inherent bacterial strain differences which show themselves, however, only in the symbiosis and are not causally related to any known *in vitro* property of the bacteria. On the plant side also very little of significance has been discovered connecting the physiology or metabolism of the host with symbiotic behaviour.

As a result mainly of studies of environmental effects, it has been established that in general any factor which increases the proportion of available carbohydrate in the host's tissues at the expense of the nitrogen content tends to be associated with an increase in nitrogen fixation (Fred & Wilson 1934; Wilson 1940, and others). On the other hand, factors decreasing the C/N ratio tend to reduce nodule size, and in extreme cases may so change the behaviour of the bacteria within the nodule as to result in tissue disintegration (Brenchley & Thornton 1925; Thornton 1930). This relation is not sufficiently well characterized to throw much light on the nodulation process, nor can it do so until the qualitative differences in the carbon and nitrogen fractions have been established.

The crucial reactions in symbiosis occur within the living infected plant cell where they are protected from the impact of external agents and are directly influenced only by intrinsic bacterial factors and by the internal environment of the host.

The study of these two sets of factors lies primarily in the fields of genetics and cell physiology. The inherent bacterial strain differences have been dealt with in the previous paper, and it is the object of this paper to discuss the effect of