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Anatomy and ultrastructure of root nodules of *Lupinus luteus*

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Abstract

The paper presents anatomic structure of root nodules of lupine (*Lupinus luteus* L. cv. Express) and ultrastructure of cells infected by *Rhizobium*. The inside of cells from the infected nodule region was filled with numerous bacteria; only centrally located cell nucleus was free of bacteria. *Rhizobium* was present mostly in the form of "transforming bacteria" (according to the terminology by Ching et al. 1977), characterized by visible nucleoid areas, numerous ribosomes, and polyphosphate granules, although typical bacterioids with poly-β-hydroxybutyrate were also found.

INTRODUCTION

Root nodules, the effect of symbiosis between root cells of certain plants and bacteria belonging to the genus *Rhizobium*, constitute a place of atmospheric nitrogen fixation. This phenomenon is of high importance. Consequently, it constituted a subject of numerous studies (among others, Steward 1956; Gołębiowska and Sypniewska 1962; Jordan and Grinyer 1965; Dart and Mercer 1966; Kidby and Goodchild 1966; Pate et al. 1966; Burns and Hardy 1975; Beevers 1976; Newcomb 1976; Newcomb et al. 1977; Broughton et al. 1978; Werner and Mörschel 1978).

Current studies are mainly concentrated on the structure of the bacteria enclosing membrane, location and size of mitochondria in infected and uninfected cells, accumulation (or its lack) of poly-β-hydroxybutyrate in ageing bacterioids, and the division rate and shape of bacterioid cells.

The present work was aimed at obtaining more knowledge on the anatomy of root nodules in lupine, and clarification of selected problems as above.

MATERIALS AND METHOD

Root nodules were obtained from 1 month-old lupine seedlings (*Lupinus luteus* L. cv. Express), and fixed in FAA. Sections for light microscopic studies were made with paraffin method and stained with safranin and fast green. Material for electron microscopy was fived in 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.2 for 2 hrs. After washing with the same buffer, materials were postfixed with 2% OsO_4 in the buffer for 2 hrs. Then the material was dehydrated with a series of acetones and propylene oxide and embedded in Epon 812. Ultra-thin sections cut on microtome LKB "Ultratom III" were contrasted in lead citrate (Reynolds 1963) and uranyl acetate. Electronograms were made on electron microscope JEM 7A.

RESULTS

In young lupine seedling nodules were found at the main root. In nodule sections the following zones could be distinguished: epidermal tissue, nodule cortex, layer of infected cells, parenchyma with vascular bundles of the nodule, parenchyma with root cortex, and root central core (Fig. 1).

Epidermal tissue of the nodules is composed of one layer of cork-like, flaking off cells. Parenchyma cells of the nodule cortex are strongly vacuolized, with thin layer of cytoplasm (Fig. 2). Among these cells sometimes smaller, only slightly vacuolized cells were noted. The latter possessed dense cytoplasm with centrally located nucleus (Fig. 4).

Infected region of the nodule is compact, sharply separated from the remaining layers. Fragment of the border zone between nodule cortex and infected region is presented in Fig. 3. Bacteria occurred in all cells of the infected region, contrary to similar areas of other plants (for instance, bean nodules), in which cells with bacteria occur along with cells without bacteria (unpubl. data). Spaces between cells of the infected region were rather small (Fig. 7A). Protoplasts of neighbouring cells were connected by plasmodesmata.

Numerous starch grains were present in the peripheric areas (Fig. 7A). Frequently, the same zones contained starch-like areas with small-grained fibrillous material (Fig. 9). It is possible that they represented places in which starch was digested, although no visible traces of plastids were found. Central part of cells was occupied by a nucleus with a nucleolus, and typical for lupine chromocenters (Fig. 6). The remaining part of the cytoplasm was filled with *Rhizobium*. The cytoplasm of host cells was rich in ribosomes and endoplasmic reticulum. Large

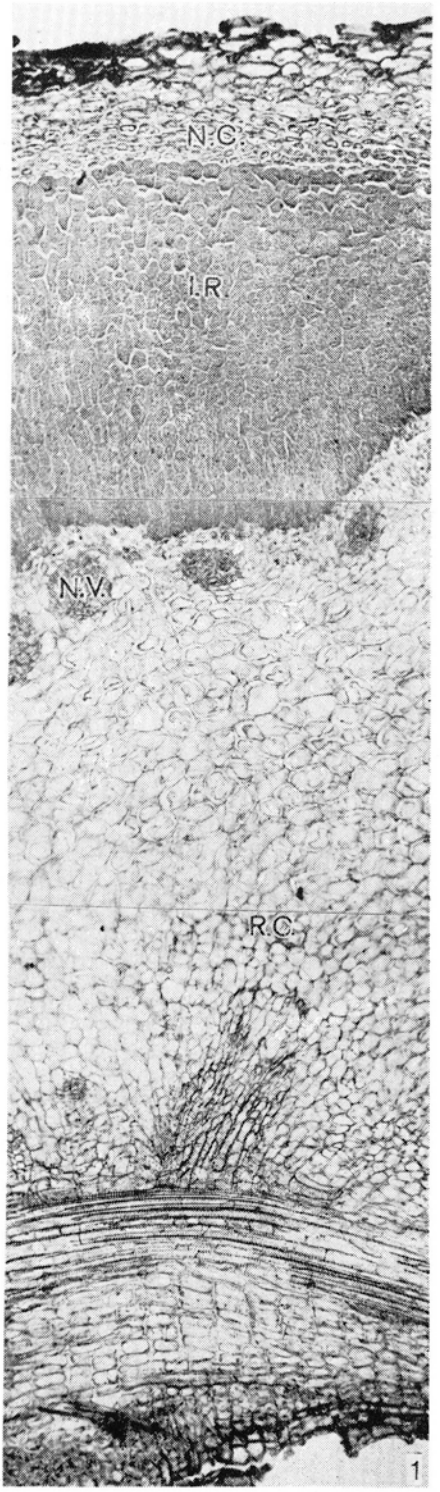
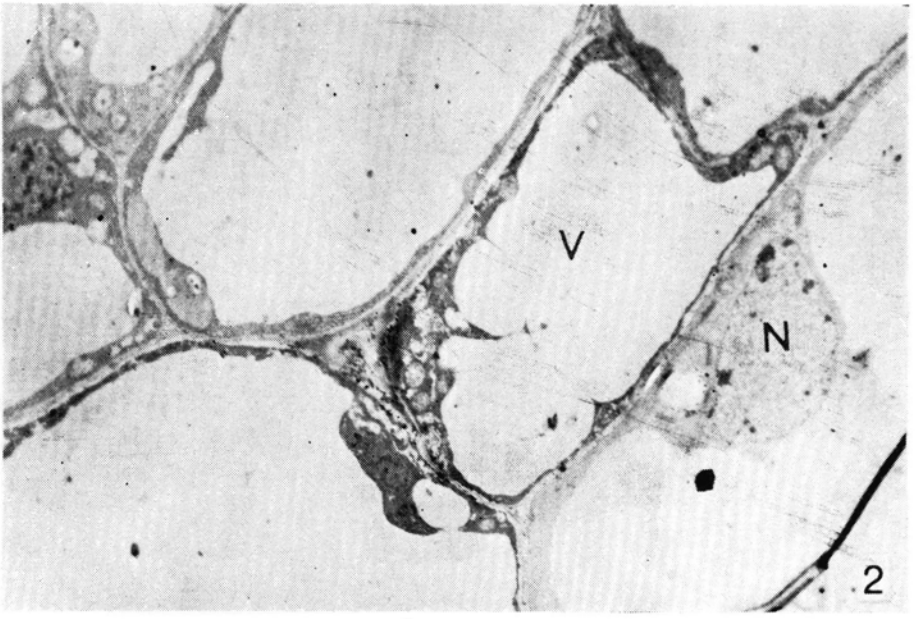


Fig 1. Section of the nodule (longitudinal plane of the root). N. C. — nodule cortex, I. R. — infected region, N. V. — nodule vascular bundle, R. C. — root cortex. Paraffin section stained with safranin and fast green. Magn. ×300.



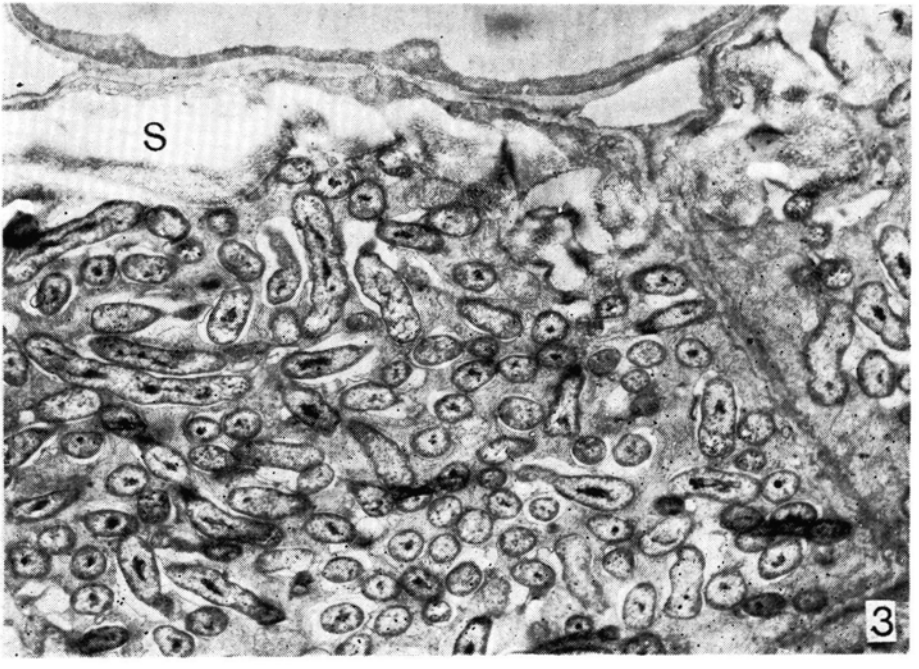
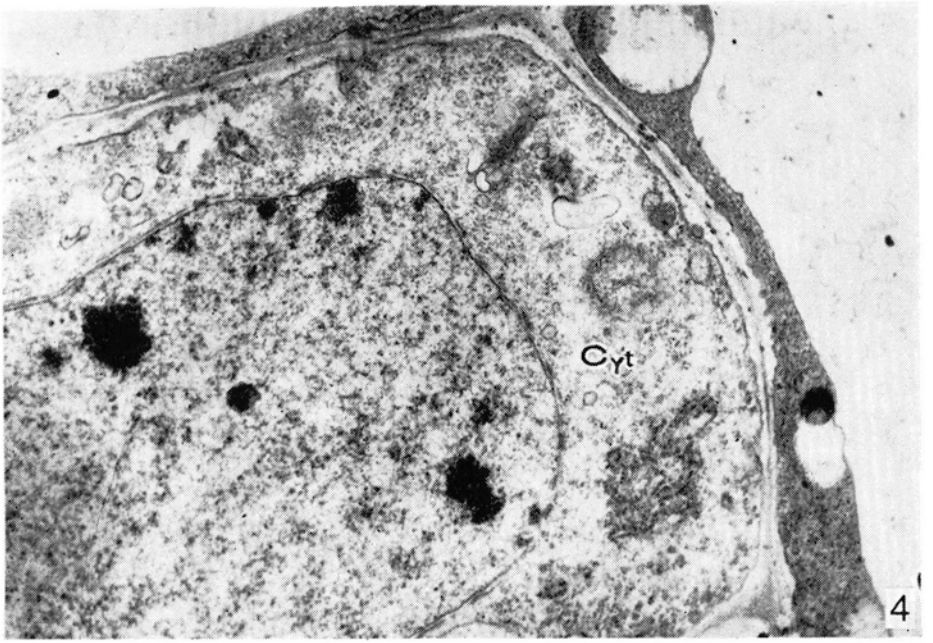


Fig. 2. Parenchyma cells of nodule cortex with large vacuoles (V), narrow layer of cytoplasm close to the cell wall, and prominent nuclei (N). Magn. ×5000.
Fig. 3. Fragment of a nodule cortex cell with neighbouring infected cells. Starch (S) visible at the periphery of the cytoplasm. Magn. ×7000



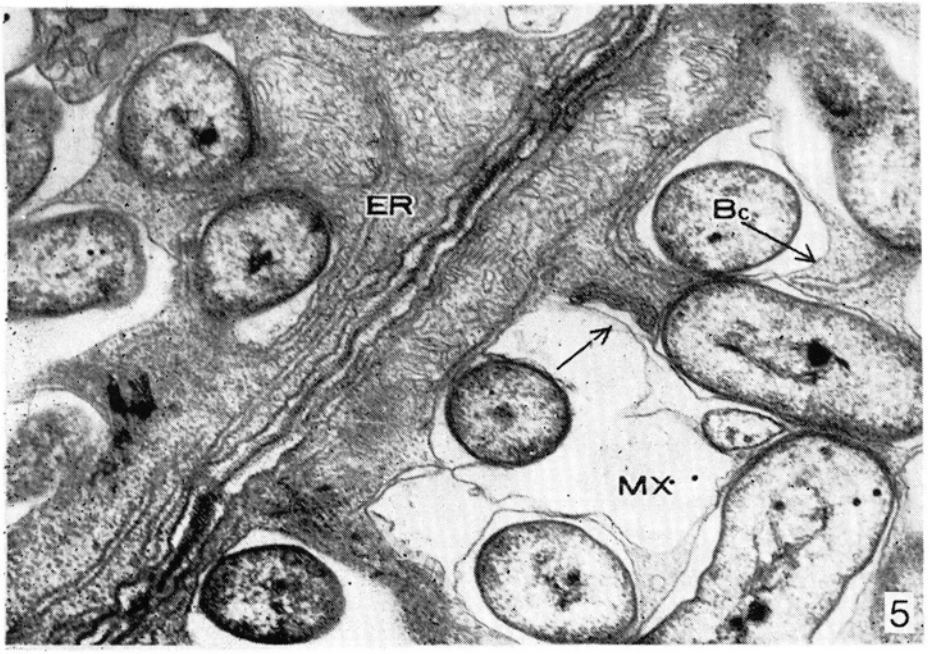


Fig. 4. Cells of nodule cortex with dense cytoplasm (Cyt) and a nucleus (N). Magn. ×17000.

Fig. 5. Fragments of infected cells with visible bacteria (Bc) in electron-light matrix (Mx), surrounded by a membrane (arrows). Cytoplasm with mitochondria (M) and endoplasmic reticulum (ER). Plasmodesmata (PL) visible in the cell wall. Magn. ×30000.

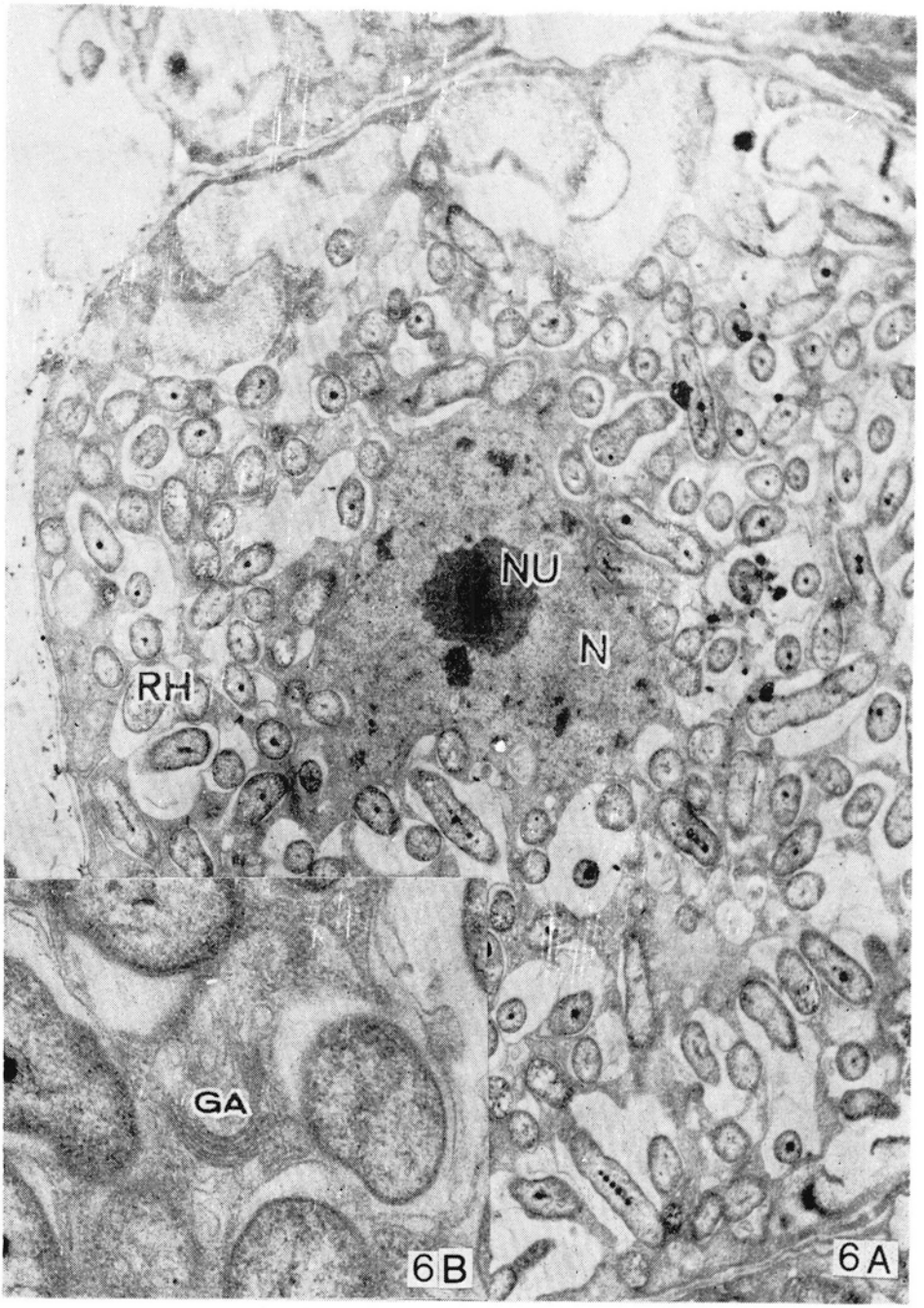


Fig. 6 A. Peripherial cell of infected region. N — nucleus, NU — nucleolus, RH — Rhizobium. Magn. ×9000. Fig. 6 B. Cell fragment with Golgi apparatus (GA). Magn. 40000.

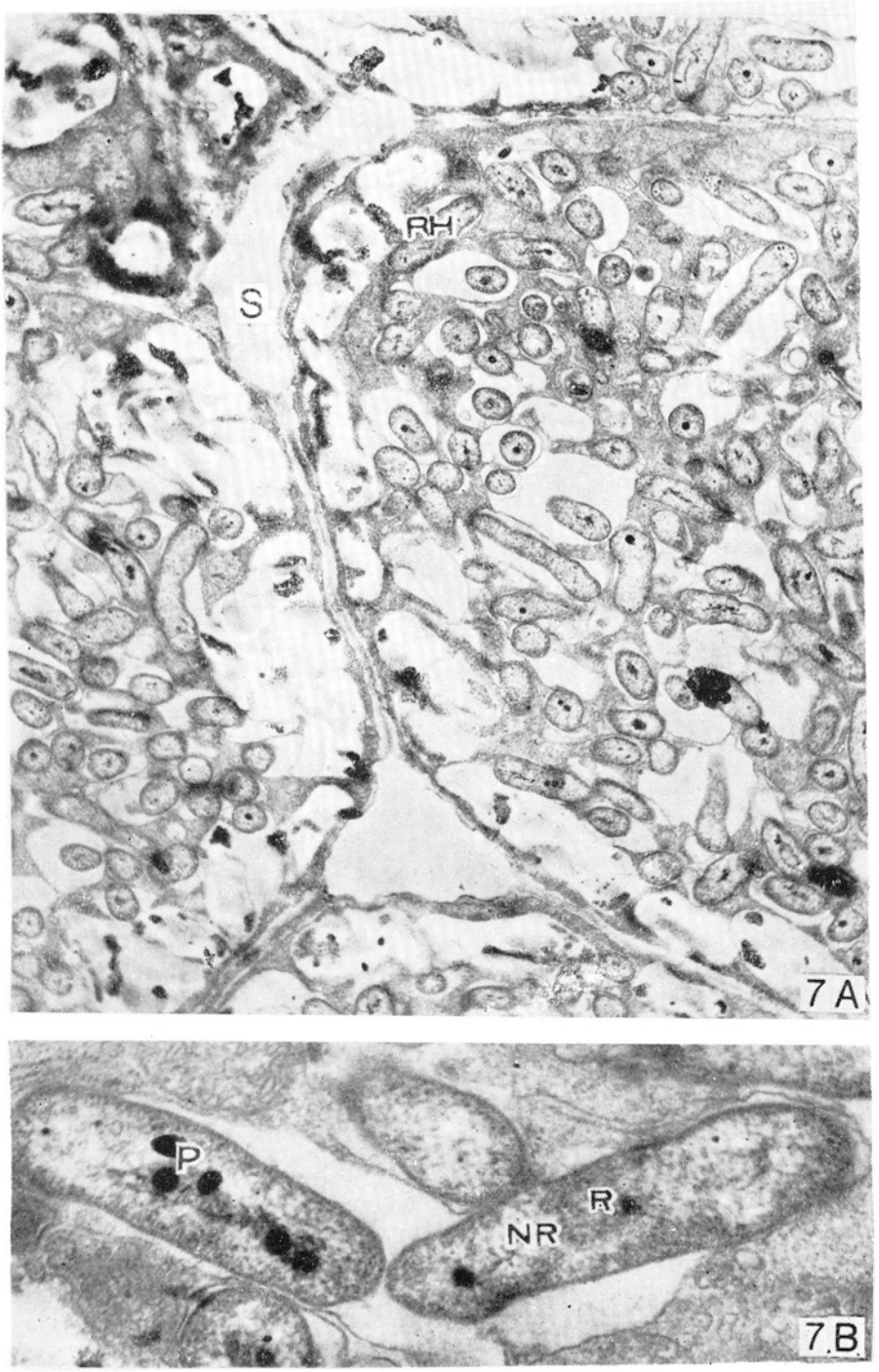
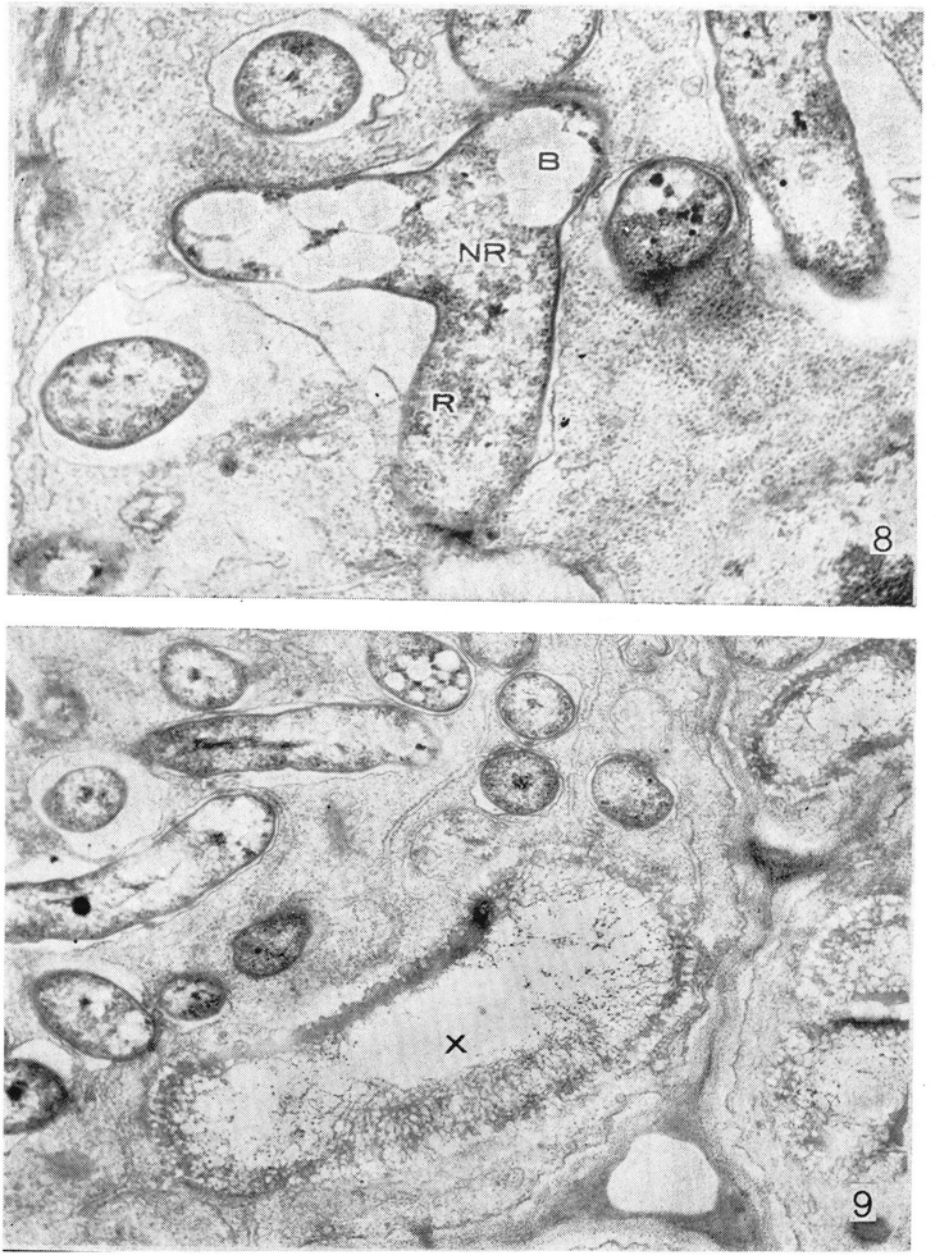


Fig. 7A. Centrally located cells of infected region with small intracellular spaces. Numerous *Rhizobium* (RH) are in a transforming form between bacteria and bacterioids (transforming bacteria). S — starch. Magn. ×12000.

Fig. 7B. Magnified forms of transforming bacteria with nucleoid region (NR), polyphosphate granules (P), and ribosome (R). Magn. ×32000.



Figs 8 and 9. Cells with bacterioids containing granules of poly-β-hydroxybutyrate (B), nucleoid-like regions (NR), and ribosomes (R). Magn. ×32000. In Fig. 9 an area probably after starch digestion (X) can be seen. Magn. ×21000.

mitochondria, rich in tubules, were grouped in cytoplasm lying close to the cell wall (Fig. 5). Active Golgi structures were also found close to bacteria (Fig. 7B).

Various forms of *Rhizobium* were found in lupine nodule cells, similary as in case of soybean (Ching et al. 1977): bacteria, bacterioids, and transforming bacteria. Their ultrastructure was visibly different. In most root nodules under study transforming forms (according to the classification by Ching et al. 1977) predominated. These forms possessed clear nucleoid areas, ribosomes, and polyphosphate granules located in cell center. Cell walls of these forms were very difficult for identification (Fig. 7B). Forms known as bacterioids (Newcomb 1976 and his references) were rare and characterized by the presence of numerous poly-β-hydroxybutyrate granules, usually at one pole of bacterial cell (Fig. 3, 8, and 9). In these cells nucleoid areas were rather numerous, unlike soybean bacterioids (Ching et al. 1977). They were frequently of the "Y" form; cell walls were not noted. All *Rhizobium* forms noted in lupine root nodules occured singularly, more rare in pairs, in an electron-light matrix surrounded by a membrane (Fig. 7B).

Ultrastructure of lupine root nodules, presented in this work, constitutes a starting point for further physiological and biochemical studies, which are now being undertaken.

DISCUSSION

Structure of lupine root nodules presented in this work generally does not differ from the scheme given by Steward (1966). The only significant difference consisted of the fact that vascular system of the nodules was located close to the infected layer, but only at the side of the root. Compactness of the infected region of lupine root nodules has been already discussed in the literature. Usually it is said to be due to the facility of bacteria penetration through walls of very young cells (Milovodov 1926; Schaede 1967). Lupine *Rhizobium* occurred within a homogenous, light matrix surrounded by a membrane. According to Jordan and Grinyer (1965) and Kidby and Goodchild (1966) memraneous envelope in lupine surrounds only one bacteria. During present observations it was noted, however, that sometimes also 2 bacteria were surrounded by one continuous membrane. The same was noted by Dart and Mercer (1966) for *Lupinus angustifolius*. Nevertheless, it seems that only an analysis of a series of sections would allow for clarifying whether we deal here with two separate cells, or with an "Y" from of the bacterioid. Also the origin of the bacteria enclosing membrane remains unexplained. Dixon (1964) states in his works on root nodules of pea and clover that membrane surrounding the

bacteria originates from plasmalemma. The same conclusion was drawn by Goodchild and Bergersen (1966) from their studies on nodules of *Glycine* max. In our opinion the presence of active Golgi apparatus in the vicinity of bacteria in lupine nodules also suggests that it takes part in the formation of matrix and membrane surrounding the bacteria.

Another unsolved problem is the presence, or absence, of poly-β-hydroxybutyrate in the bacteria-form cells of lupine root nodules. This compound has been defined as a reserve material of bacteria, in this also of *Rhizobium* (McRae and Wilkinson, 1958; Patel and Gerson, 1974).

In their studies on lupine nodules ultrastructure Kidby and Goodchild (1966) did not find any bacteria containing granules of poly-β-hydroxybutyrate. On the other hand, Ching et al. (1977) noted a strict relationship between poly-β-hydroxybutyrate and some electron-light areas in symbiotic bacteria. The authors identify these areas with poly-β-hydroxybutyrate. The latter paper gave us the basis for the assumption that also in our observations (Fig. 3, 8, and 9) areas light for electrons represent areas containing granules of poly-β-hydroxybutyrate. Presence of this compound in root nodules of lupine was also confirmed by Romanov et al. (1974) and Gerson et al. (1978). Hence, it can be taken that there is enough evidence for the presence of poly-β-hydroxybutyrate in lupine *Rhizobium*.

Localization of mitochondria in infected cells close to the cell wall was observed by several authors (Dixon 1964; Goodchild and Bergersen 1966; Newcomb 1976; Werner and Mörschel 1978). The mitochondria are characterized by a tubuli-rich internal structure, resembling mitochondria found in organs with high rate of metabolism, such as heart muscle, and not those typically observed in plant cells. This phenomenon most probably results from high energetic requirements of nodule cells, caused — among others — by the multiplication of bacterioids, as well as by high activity of numerous enzymes (Werner and Mörschel 1978).

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Anatomia i ultrastruktura brodawek korzeniowych Lupinus luteus

Streszczenie

Przedstawiono budowę anatomiczną brodawki korzeniowej łubinu i ultrastrukturę komórek zainfekowanych bakteriami. Wnętrze komórek strefy zainfekowanej było wypełnione licznymi bakteriami, wolne od nich było jedynie jądro komórkowe położone centralnie. *Rhizobium* występowało głównie w formie bakterii pośrednich (transforming bacteria — wg terminologii Chinga i wsp. 1977), charakteryzujących się wyraźnymi obszarami nukleoidowymi, licznymi rybosomami i granulami polifosforanowymi, choć spotykano także typowe bakterioidy z granulami poli-β-hydroksymaślanu.