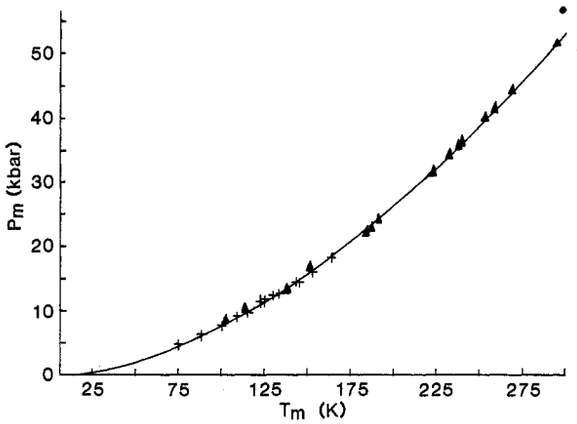

Fig. 2. A plot of P_m versus T_m for normal hydrogen: ▲, present work; x, from (*8*); ●, from (*9*); and the solid curve–modified Simon equation from (*8*).

in the sample chamber after repeated temperature and pressure cyclings above 300 K.

The melting curve of normal hydrogen was determined up to 19 kbar by Liebenberg *et al.* (*8*), using a piston-cylinder apparatus such that the absolute pressure could be measured. A modified Simon melting equation (where P_m has the dimension kilobars)

$$P_m = -0.2442 + 2.858 \times 10^{-3} T_m^{1.724}$$

was proposed as a least-squares fit to their data. The same equation describes our results well and is shown as a solid curve in Fig. 2; results obtained by Liebenberg *et al.* are shown for comparison. The surprisingly good agreement between the modified Simon equation proposed by Liebenberg *et al.* and our results suggests that the ruby pressure scale is an absolute scale, at least below 175 K. Mao and Bell (*9*) reported a P_m of 57 kbar at 298 K, in contrast to the 53 kbar from this investigation. It should be noted that the intense laser beam used to excite the ruby fluorescence can drastically enhance the local temperature of the sample and thus can introduce a significant error to T_m, particularly when method 1 is used. In our experiment, the laser power was kept as low as possible (*10*).

Ross (*11*) has calculated P_m based on an effective pair potential obtained from analyzing the shock compression data at high pressure and temperature. The value of P_m so calculated was 78 kbar at 250 K, in contrast to 39 kbar from our study. The large disagreement may be attributed to the basic difference of hydrogen (*1*) at low (a quantum solid) and high (a classical solid) temperatures or to the difference in pressure scales at high and low pressure. Grigorev *et al.* (*12*), using an equation of state in the Mie-Grüneisen form with experimentally determined parameters, showed that a value of 41 kbar could be predicted for P_m at the same temperature. The disagreement with our value is small but exceeds the experimental uncertainty. Since the equation of state was an empirical one, the disagreement may be ascribed to the difference in pressure scales.

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Uninfected Cells of Soybean Root Nodules: Ultrastructure Suggests Key Role in Ureide Production

Abstract. *In soybean root nodules, which export recently fixed nitrogen mainly as the ureides allantoin and allantoic acid, cells uninfected by rhizobia undergo a pronounced ultrastructural differentiation not shown by the infected cells, including enlargement of the microbodies and proliferation of smooth endoplasmic reticulum. Since some of the enzymes contributing to ureide synthesis occur in these subcellular components in root nodule preparations, the uninfected cells may participate in ureide synthesis and thus play an essential role in the symbiosis between host and bacterium.*

In legume root nodules, the central tissue enclosed by the cortex consists not only of cells that become greatly enlarged and heavily infected with rhizobia but also of many smaller uninfected cells interspersed among the infected ones (*1*). We have found that in soybean (*Glycine max* L.) inoculated with effective strains of *Rhizobium japonicum* (*2*), an ultrastructural differentiation takes place in the uninfected cells which is distinctly different from changes in the infected cells. The principal changes are a marked enlargement of the microbodies and a proliferation of smooth endoplasmic reticulum (ER). We suggest that the ultrastructural responses of the uninfected cells are related to the participation of these cells in metabolic transformations of compounds arising from recently fixed N_2 in the nodule.

Our observations are relevant to current attempts to clarify the major pathway of nitrogen metabolism in soybean root nodules. Legumes are divisible into various groups based on the major nitrogenous compounds transported from the nodules to the shoots. For example, pea (*Pisum*), vetch (*Vicia*), and lupine (*Lupinus*) belong to a group that transports fixed nitrogen mainly as asparagine (*3*). Soybean (*Glycine*), bean (*Phaseolus*), and cowpea (*Vigna*), on the other hand, belong to a group in which the ureides allantoin and allantoic acid are major products of nitrogen assimilation in the nodules (*3–5*) and the principal form of transport of the nitrogen to the shoots (*5, 6*). The rationale for our assumption that the uninfected cells of the soybean nodule are involved in ureide formation depends in part on correla-

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tions between our ultrastructural observations and recent evidence on the subcellular localization of the enzymes involved in the final steps of ureide production.

Early in soybean root nodule development, when only infection threads are present in the infected cells, microbodies in the central tissue of the nodule have the small size and ultrastructural characteristics of those in normal root tissues (*7*). As the infected cells become greatly enlarged and filled with bacteroids, the microbodies disappear almost completely from the cytoplasm, while the mitochondria and plastids increase greatly in number and become crowded into the cell periphery (Figs. 1 and 2). Microbodies are only rarely encountered among these organelles. Their almost complete absence from infected cells may explain why they appear to have received no attention in studies of legume root nodule ultrastructure.

In the uninfected cells, microbodies undergo rapid enlargement and within 2 to 3 weeks are prominent in the cell profiles (Figs. 1 and 3) (*8*). They also appear to increase in number during this period. The largest microbodies have diameters ranging up to 1.5 μm, and thus are comparable in size to the leaf peroxisomes of C_3 plants (in which the first product of photosynthesis is a three-carbon acid), which heretofore were the largest plant microbodies reported (*9*). Enlarged microbodies are still present in the uninfected cells in nodules 4 to 8 weeks old. We have used the reactivity of microbodies toward diaminobenzidine (*10*) to establish that they contain catalase, a marker enzyme for the organelle (Fig. 4). We have also documented the development of the enlarged microbodies through intermediate stages from small microbodies characteristically associated with ER.

Smooth ER also undergoes remarkable development in the uninfected cells of young nodules, so that after 2 to 3 weeks much of the cytoplasm is filled with cisternal and tubular components of this membrane system (Fig. 3). In the infected cells, little or no smooth ER can be identified (Fig. 2). In this respect the infected cells resemble most plant cells, in which rough ER predominates and smooth ER is a very minor component, usually difficult to identify. Smooth ER is especially well developed in secretory cells, including the tapetal cells of anthers and those of various plant glands.

We suggest that the ultrastructural specialization of the uninfected cells reflects their participation in ureide synthesis. Recently the pathway of ureide synthesis in soybean and cowpea root nodules was examined in several laboratories. These studies are in agreement that the ureides are produced in the nodules from purines (*11–14*), which in turn arise from amino acids that incorporate ammonia produced from N_2 by the bacteroids (*15*). In the oxidation of purines to ureides, there is first a conversion of xanthine to uric acid catalyzed by xanthine dehydrogenase (*11, 14, 16, 17*). Uric acid is then converted to allantoin by uricase, and allantoin to allantoic acid by allantoinase (*11, 12, 16, 17*). The action of uricase generates toxic H_2O_2, whose destruction requires the action of catalase.

These enzymes occur in substantial amounts in soybean and cowpea root nodule cells (*5, 11, 12, 16, 17*). There is reason to expect that two of these enzymes, catalase and uricase, will be found in microbodies (peroxisomes) of the nodule host cells. Catalase is found in all peroxisomes, both plant and animal (*18, 19*), while uricase is present in many animal and plant microbodies (*18, 20*). Recently, Hanks *et al.* (*14*) obtained from soybean root nodule homogenates a peroxisome (microbody) fraction with catalase and uricase activity. They also found that allantoinase activity is confined largely to the microsome fraction (including ER).

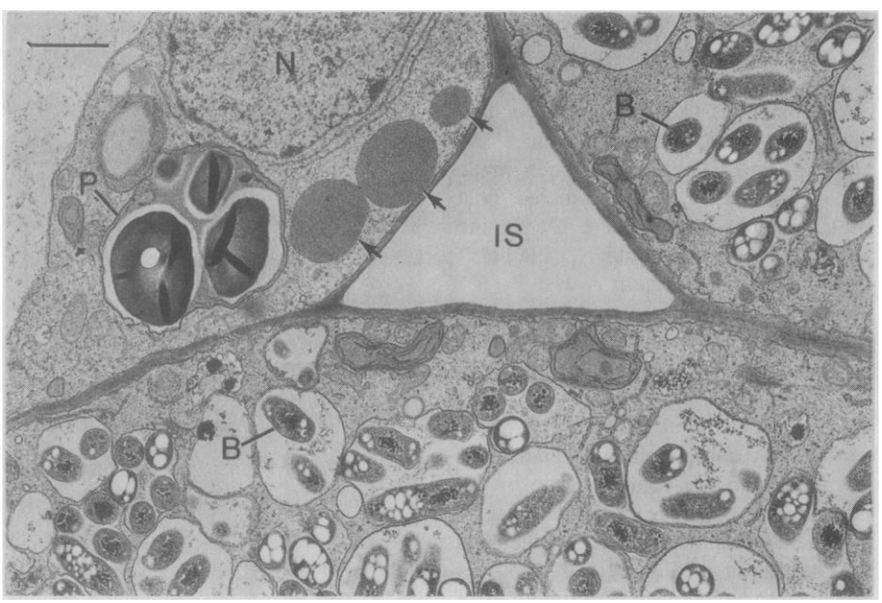

Fig. 1. Portions of infected and uninfected cells. Arrows indicate microbodies in a portion of an uninfected cell. At bottom and upper right are infected cells with numerous bacteroids (*B*) in vacuoles. Abbreviations: *IS*, intercellular space; *N*, nucleus; and *P*, plastid with several starch grains (scale bar, 1.0 μm).

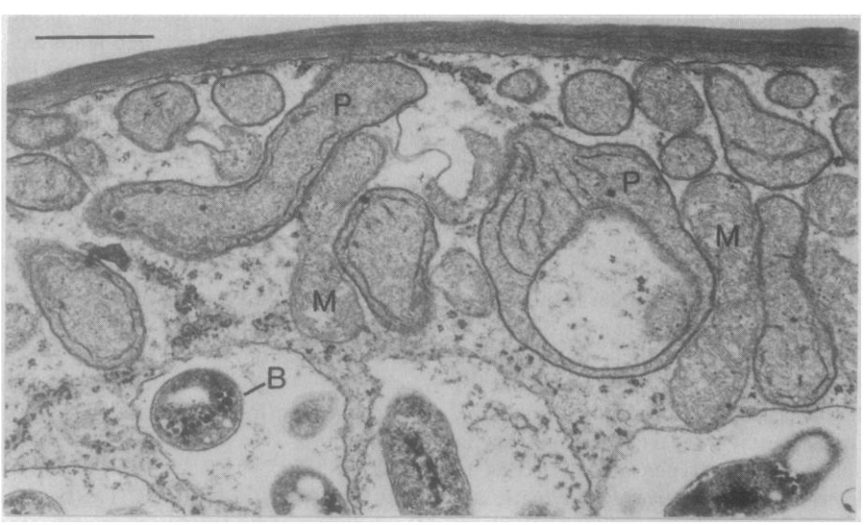

Fig. 2. Small portion of an enlarged cell infected with rhizobia. The numerous plastids and mitochondria (*M*) are present at the cell periphery while bacteroids occupy the central region. No microbodies are present. Cell is from a nodule 3 weeks after infection (scale bar, 1.0 μm).

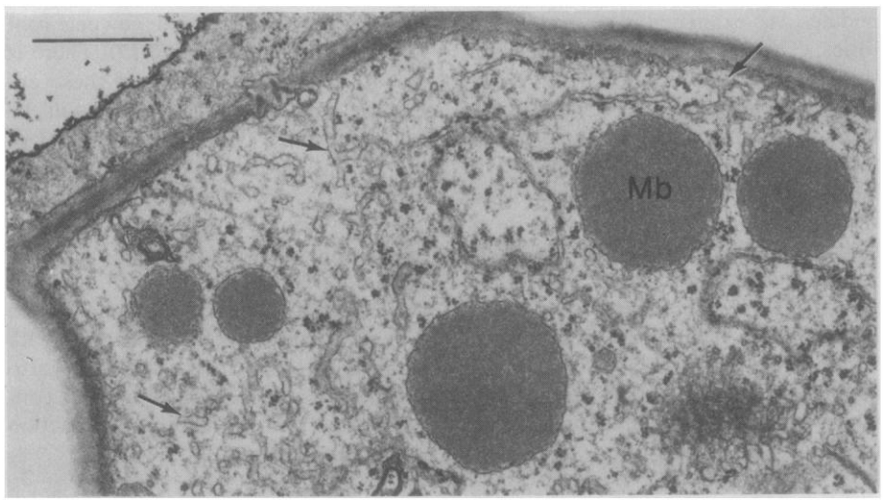

Fig. 3. Portion of a small uninfected cell near the infected cell shown in Fig. 2. Five microbodies (*Mb*) can be seen. Arrows point to elements of smooth ER (scale bar, 1.0 μm).

In the studies to date on the localization of enzymatic activity in legume root nodules, no distinction has been made between infected and uninfected host cells. When reference is made to the "host cells" or the "bacteroid-filled host cells," all cells of the central tissue of the nodule are included, infected and uninfected alike. However, we find that enlarged microbodies and an abundance of smooth ER are characteristic only of the uninfected cells. Our observations imply a previously unrecognized specialization of function in the central tissue of the nodule. The evidence strongly suggests that the development of microbodies and smooth ER in the uninfected cells is a response to an increase in compounds of recently fixed nitrogen reaching them from neighboring infected cells. We infer from these ultrastructural changes that at least the final two steps in ureide formation take place in the uninfected cells, with uric acid being converted to allantoin by uricase (plus catalase) in the microbodies, and allantoin to allantoic acid by allantoinase in the smooth ER.

We hypothesize that the infection process induces in the uninfected cells a well-defined pattern of differentiation, equipping them to play an essential role in the nitrogen metabolism of the soybean nodule. The uninfected cells have been estimated to be similar in number to the infected cells (*1*) or in a ratio of about 1 to 3 (*21*). Thus their numbers are probably sufficiently high to account for the observed rates of ureide production. To test the hypothesis, it is necessary to isolate the two types of cells so as to determine the pattern of enzymatic activity in each. This should reveal where the individual steps in ureide formation occur on a cellular level, and consequently whether at certain stages in the metabolic sequence the compounds derived from nitrogen fixation are moving from infected to uninfected cells. By limited treatment of root nodule slices with cell wall–degrading enzymes (*22*), we have been able to prepare a suspension of infected and uninfected cells separated from the cortex and detached from one another (*23*). These preparations are being used in attempts to separate the two cell types by density gradient centrifugation.

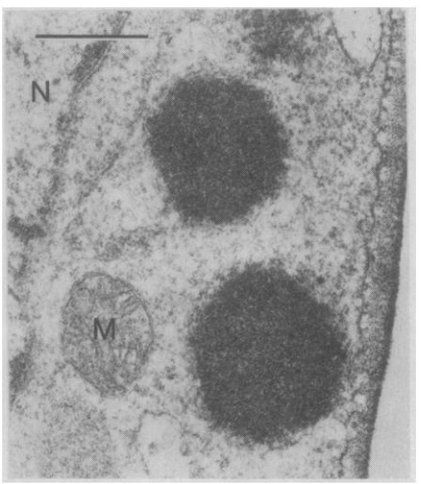

Fig. 4. Two microbodies showing a positive reaction in the diaminobenzidine test for catalase activity. Only remnants of the bounding membranes are visible. The cell wall also shows a positive reaction (scale bar, 0.5 μm).

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