Effects of Soybean Mosaic Virus Infection on Ultrastructure of Bacteroidal Cells in Soybean Root Nodules

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ABSTRACT

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Differences between the rhizobial bacteroids *(Rhizobium japonicum)* in situ in the bacteroidal cells of root nodules in healthy and in soybean mosaic virus (SMV)- infected soybean plants *(Glycine max)* were investigated using thin-sectioning and freeze-fracturing techniques. The bacteroids in SMV-infected cells differed significantly from those in healthy cells. These differences were evident in both the initial stage and, to a lesser degree, in the intermediate stage of bacteroid development. In the advanced stage of bacteroid development, differences between bacteroids in healthy and in SMV-infected cells were no longer apparent. Differences in bacteroidal cells in SMV-infected versus healthy plants include (i) a decrease in endocytotic and exocytotic vesiculation on the membrane envelope of the bacteroids and on the plasma membrane of bacteroidal cells, (ii) a decrease in number of vesicles in the space between the bacteroid and the membrane envelope, and (iii) a decrease in the space between the bacteroid and the membrane envelope. The possible significance of these changes relative to the decreased efficiency of N_2-fixation is discussed.

Rhizobial root nodules of soybeans appear to be affected by soybean mosaic virus (SMV), for it is known that SMV-diseased soybean plants produce smaller, fewer, and less effective root nodules than healthy plants (7, 15, 16). It also is known that the virus is actually present in root nodule cells of SMV-infected plants (11). However, little is known about the effect of SMV on the rhizobia in situ.

Infection by rhizobia can be divided into two phases; nonbacteroidal and bacteroidal. During the first phase, an infection thread develops and the rhizobia are encased in the infection thread and are later released into the host cytoplasm. After the rhizobia enter the host, each cell acquires a membrane envelope (ME). The rhizobia with ME are termed bacteroids (12, 13). They develop and multiply in the cytoplasm of the host cell. In a previous paper, Tu (11) showed that, once rhizobial infection is initiated, the development of the infection thread and the processes of releasing the rhizobia into the cytoplasm, that is, the first phase, are not affected by SMV infection. However, as stated, the effects of SMV on the bacteroidal cells remain to be studied. The presence of SMV may affect the fine structure of the bacteroidal cells and thus may cause a change in their activity, particularly their efficacy of N_2-fixation.

This paper reports fine structural differences between bacteroids in root nodules of SMV-diseased plants and healthy plants. Of particular interest is the structure of the ME. Rhizobia in vitro do not possess this membrane (12). Since the ME is known to be involved in N_2-fixation, and since SMV pathogenesis affects N_2-fixation, it is conceivable that the structure of the ME is altered by SMV.

MATERIALS AND METHODS

Seeds of soybean, *Glycine max* (L.) Merr. 'Amsoy', were germinated on wet filter paper in petri plates for 3 days and transplanted to 14-cm diameter plastic pots containing perlite. The plants were watered daily with balanced Hoagland's solution (6). When plants reached the primary leaf stage, one-half of the plants was inoculated with SMV. This was done by dusting the leaves with 0.22-μm (600-mesh) Carborundum and then rubbing them with a crude sap preparation obtained from infected leaves. Both healthy and SMV-inoculated plants were maintained in a 22 ± 1 C controlled environment. Five weeks later, root nodules from healthy and SMV-diseased plants were sampled (4 wk after rhizobial inoculation). At least 10 root nodules were detached from the roots and dropped into a fixative consisting of 2% formaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0). The surfaces of root nodules are covered by layers of dead cortical cells (14); therefore, the root nodules were cut into quarters to expose their interiors to the fixative. The tissues remained in the fixative for 2 hr and then were given 3, 15-min washes in phosphate buffer. Each sample, healthy and infected, was divided into two groups, one to be freeze-fractured, the other to be thin-sectioned.

Freeze-fracturing.—The samples to be freeze-fractured

were placed in 25% glycerol for 2 hr at room temperature. The samples were frozen in liquid Freon-22, transferred to liquid nitrogen, and processed in a Balzers BA 360 M high vacuum freeze-etch unit. They were then freeze-fractured at −100 C and Pt-C replicas were prepared immediately after fracturing as previously described (13). In each experiment, four replicas were prepared from root nodules of healthy and SMV-diseased plants. The experiment was repeated three times. The replicas were cleaned, picked up on 74-μm (200-mesh) copper grids and examined in a Philips EM-300 electron microscope at 80 kV.

Thin sectioning.—The samples were postfixed in 2% OsO_4 in 0.1 M phosphate buffer for 4 hr, rinsed in the same buffer, dehydrated in a graded ethanol and propylene oxide series, and embedded in Araldite (8, 11). Thin sections were cut with a DuPont diamond knife mounted on a Reichert ultramicrotome. For each experiment, at least five blocks were cut and examined from healthy and SMV-diseased root nodules. The experiment was repeated once. The sections were picked up on Formvar-coated grids, stained with 2% aqueous uranyl acetate followed by 0.2% aqueous lead citrate (9), and examined in a Philips EM-300 electron microscope.

RESULTS AND DISCUSSION

General remarks.—The interpretation of freeze-fractured replicas has been detailed by Branton (3) and Branton et al. (4).

The micrographs that are presented and the results discussed are limited to bacteroidal cells at the early and intermediate stages of the second phase as defined in the introductory section. During the early stage, each bacterium in the host cytoplasm is enclosed in an ME; during the intermediate stage, several bacteria are enclosed together in an ME; during the advanced stage, bacteria have no ME and the host membranes have

Fig. 1. A general view of thin-sectioned bacteroidal cells at the early stage of rhizobial infection in a SMV-infected root nodule. Black and white dash-lined areas are shown at higher magnifications in Fig. 2 and 3, respectively.

Fig. 2. An enlarged portion of Fig. 1 showing several SMV aggregates (arrows).

Fig. 3. An enlarged portion of Fig. 1 showing details of the bacteroids. The space between the bacteroid and the ME is very narrow.

Fig. 4. A portion of a bacteroidal cell seen in a freeze-fractured replica of a SMV-infected root nodule. Most of the bacteroids have a small space between the bacteria and the ME.

Fig. 5. A portion of a bacteroidal cell at the early stage of rhizobial infection in a healthy root nodule, with a clear view of both vesicles (arrowheads) and protrusions (arrows) on the ME of the bacteroid. Note: there is a large space between the ME and the bacterium.

Fig. 6. A portion of a bacteroidal cell seen in a freeze-fractured replica of a healthy root nodule showing the presence of vesicles (arrowheads) in the space between the bacteria and ME. Inset is a clear view of such vesicles.

Fig. 7. A portion of a bacteroidal cell seen in a freeze-fractured replica of a portion of a bacteroidal cell of a healthy root nodule showing a convex fractured view of an ME with the broken neck of a protrusion (arrowheads) and several other protrusions (arrows).

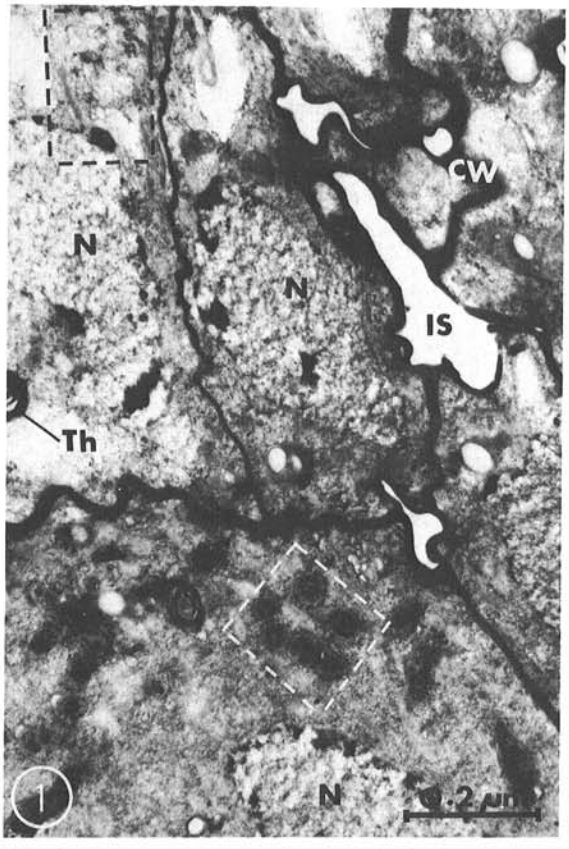
Fig. 8. Portion of freeze-fractured plasma membrane of a bacteroidal cell from a healthy root nodule, showing many depressions (arrows) protruding out to the cell wall side and protrusions (arrowheads) dipping into the cytoplasm side on a concave fractured face (face EF) of a plasma membrane.

Fig. 9. Portions of freeze-fractured plasma membranes of bacteroidal cells from a SMV-infected cell. Note: only a few depressions (arrows) and protrusions (arrowheads) can be seen on the concave-fractured faces.

Fig. 10. Portions of two bacteroidal cells (intermediate stage) and an interstitial cell from a healthy root nodule showing the presence of vesicles (arrows) in the space between the bacteroids and the ME.

Fig. 11. Portions of a bacteroidal cell (intermediate stage) and two interstitial cells from a SMV-infected root nodule. Few vesicles occur in the space between the bacteroids and the ME.

Abbreviations and symbols used: B, bacteroid; BCW, cell wall of bacteroid; BPl, bacteroid plasma membrane; Ω, convex-fractured face; ∪ , concave-fractured face; CW, cell wall; EF, exoplasmic half of a fractured membrane; IS, intercellular space; M, mitochondrion; ME, membrane envelope; N, nucleus; Pd, plastid; PF, plasmic half of a fractured membrane; Pl, plasma membrane of bacteroidal cell; SMV, soybean mosaic virus; St, starch grain; Th, infection thread; and V, vacuole.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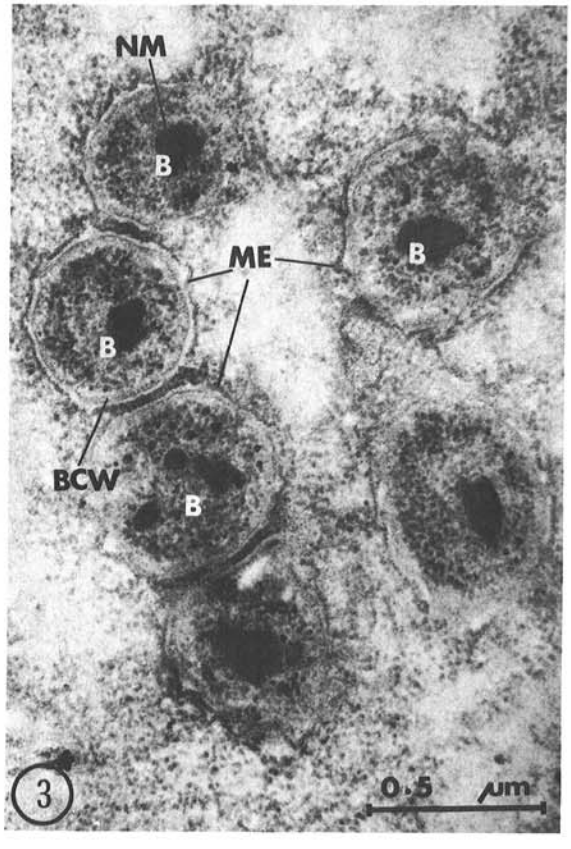
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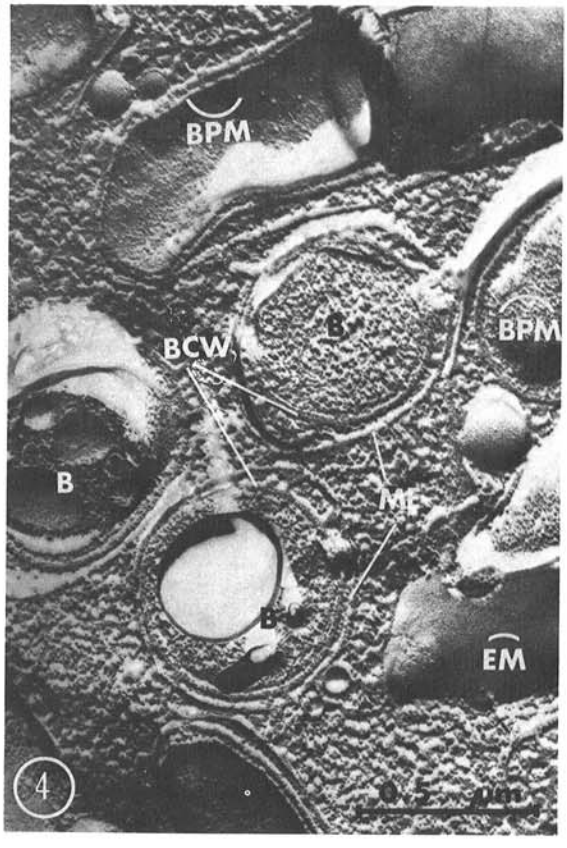
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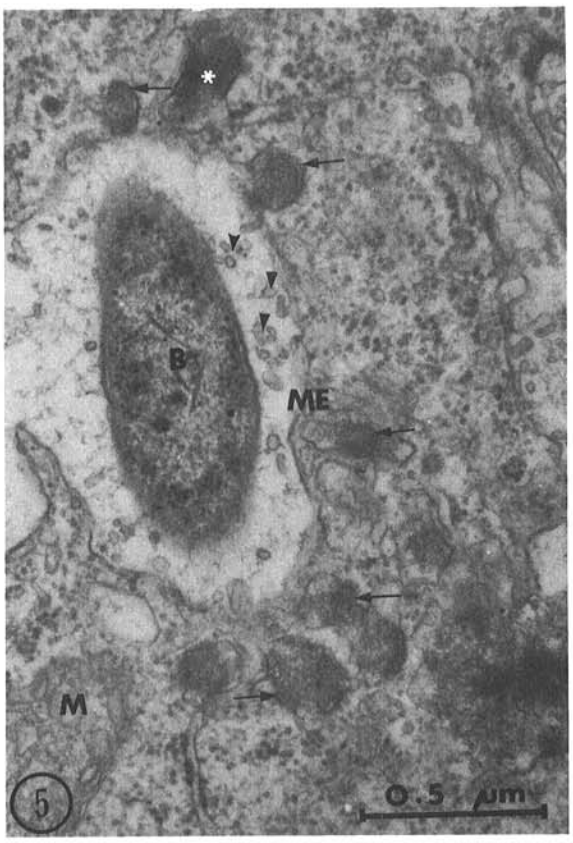
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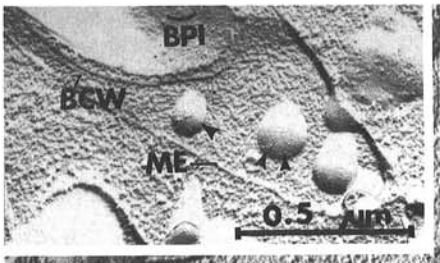
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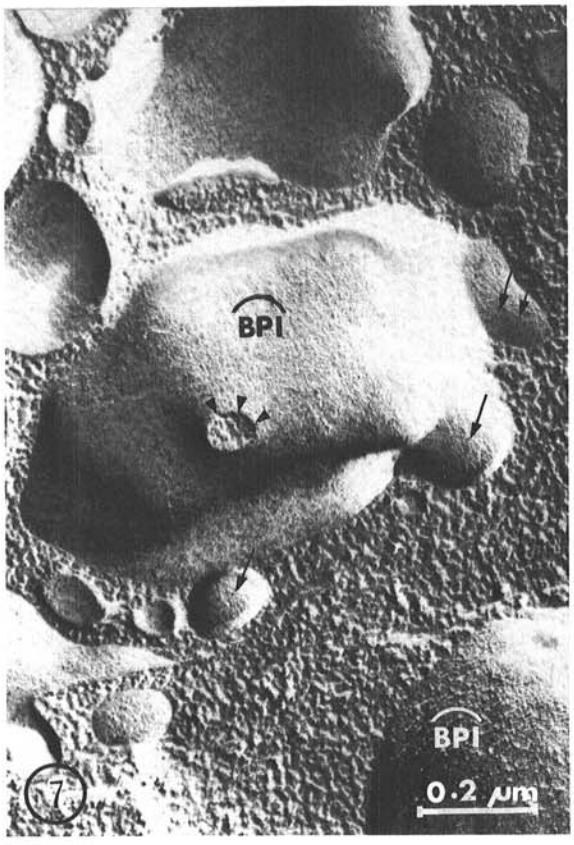
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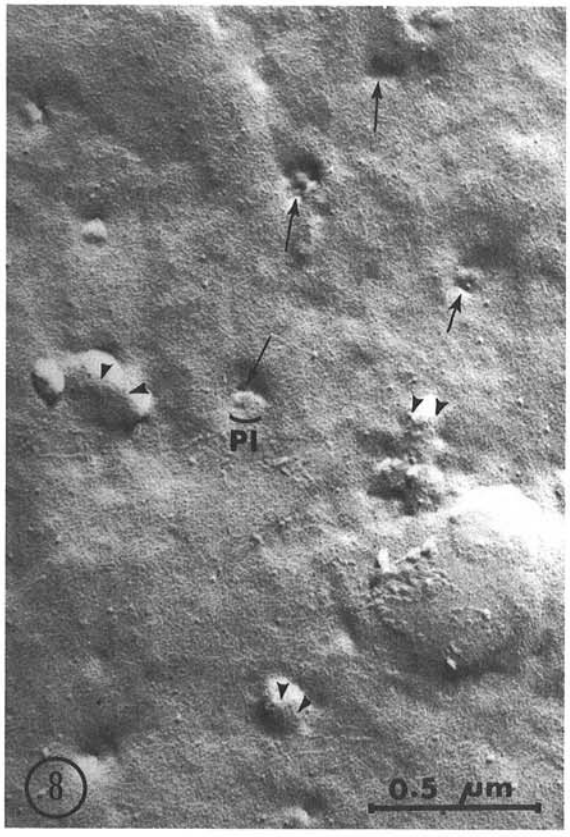
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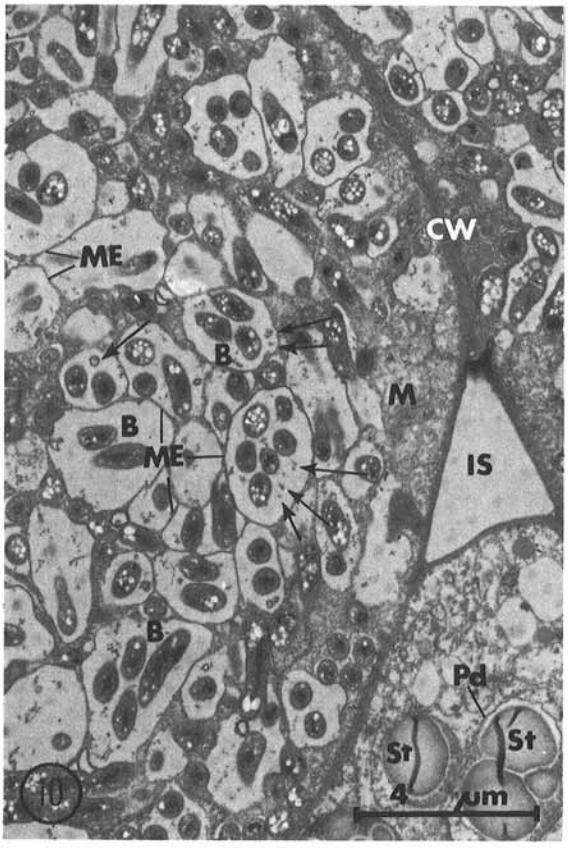
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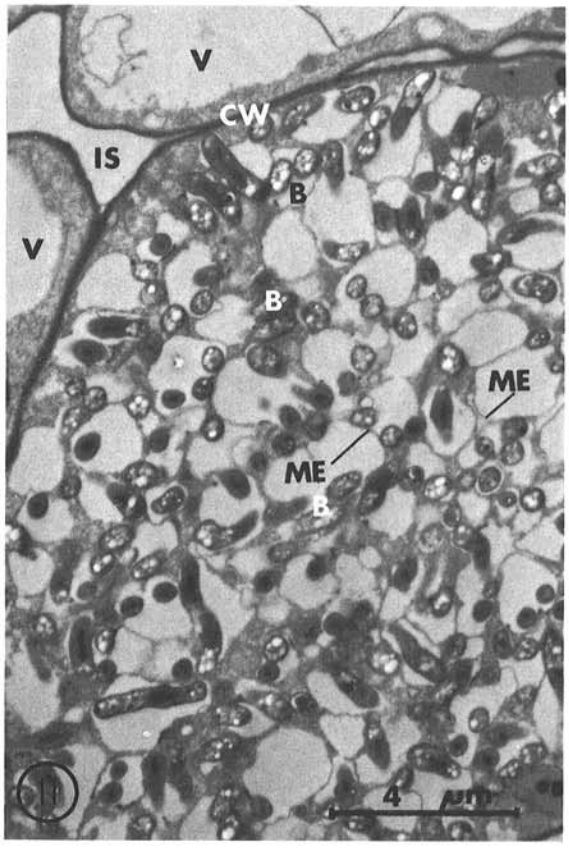
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deteriorated (14). During the advanced stage, no symbiotic interaction is apparent and the bacteria appear to live at the expense of the host (14). Therefore, an investigation into the interactions between the bacteroids and the living host cell's cytoplasm can be carried out in bacteroidal cells at the early and intermediate stages only.

Bacteroidal cells at the early stage.—It is at the early stage that differences between SMV-infected and healthy bacteroidal cells are most noticeable. Figure 1 is a low-magnification micrograph of a SMV-infected bacteroidal cell. The presence of SMV particles in the SMV-infected bacteroidal cell is readily discernible (Fig. 2). One obvious and important difference is the greater space between the bacteria and the ME in healthy cells than in SMV-infected cells (compare Fig. 3, 4 with 5, 6). This may be significant in view of the results (1, 2, 5, 10) which indicate leghaemoglobin (a myoglobin-like haemoprotein involved in N_2-fixation and responsible for the pinkish color of the central tissue of legume root nodules) is located in the space between the symbiotic bacteria and the ME in the bacteroidal cells. In these spaces, a network of lightly stained fibrillar material is clearly observed in the bacteroid cells in healthy root nodules (Fig. 5).

A second difference is that in bacteroidal cells of healthy plants, the vesicles in the space between the bacteria, and the ME in bacteroidal cells are more numerous than those in cells of plants infected with SMV (compare Fig. 3 and 5). This is also demonstrated in freeze-fractured preparations (Fig. 6). Counts made on cross-sectional views of bacteroids on randomly-chosen micrographs show that there are approximately three times more vesicles extending from the ME of the bacteroids in healthy plants than there are in SMV-infected plants (Table 1).

TABLE 1. Comparison of the numbers of vesicles[a] present in the space between the bacteria and the ME, and protrusions attached to the ME at the initial stage of bacteroidal cell development in healthy and in SMV-infected root nodules

Type of vesicle	Vesicles or protrusions per bacterium (no.)	
	Healthy	SMV-infected
Vesicle	22	2
Protrusions	5	0.5

[a]The vesicle counts were made on thin-sectioned preparations. At least 100 bacteria were counted in both healthy and SMV-infected cells

TABLE 2. Comparison of the numbers of protrusions and depressions[a] seen in the concave fractured face (EF) of the plasma membranes of the bacteroidal cells in healthy and SMV-infected root nodule

Type of membrane distortion	Distortions per μm^2 No.)	
	Healthy	SMV-infected
Protrusions	4	2
Depressions	8	3

[a]The counts were made on 20 randomly selected areas of 1 μm^2 in both healthy and SMV-infected cells.

Assuming that the vesicles are related to endocytosis and exocytosis, then a higher vesicle density in the healthy soybean bacteroidal cell would indicate a higher movement of material between the bacteria and the host cytoplasm.

A third difference between healthy and SMV-infected bacteroidal cells is that healthy cells have a much larger amount of unevenly thick amorphous layer of electron-opaque material lining the inside of the ME (Fig. 5, asterisk). It is in these electron-dense portions of the ME that protrusions are formed (Fig. 5). These protrusions are also evidenced in the micrograph of freeze-fractured material (Fig. 7).

A fourth difference is in the concentration of vesicles associated with the plasma membrane of the bacteroidal cell. Such vesicles presumably can be detected in freeze-fractured replicas as depressions and protrusions (Fig. 8 and 9). Depressions and protrusions on freeze-fractured replicas were about twice as numerous in plasma membrane replicas from healthy plants as in those from SMV-infected plants (Table 2). These results suggest a higher rate of material movement in and out of the bacteroidal cell.

Bacteroidal cells at the intermediate stage.—At the intermediate stage, the differences between the bacteroidal cells of healthy and SMV-infected plants are fewer and less obvious than those noticed at the early stage. However, the number of vesicles enclosed by the ME and the bacteroidal cells of the healthy plants (Fig. 10) was greater than the number of such vesicles in the corresponding area in the cells of SMV-infected plants (Fig. 11). In the freeze-fractured replicas, fewer depressions or elevations were seen on the plasma membrane or ME than were seen at the early stage.

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