Electron Microscopy of Soybean Root Nodules Infected with Soybean Mosaic Virus

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ABSTRACT

Thin sections of developing central tissue cells of soybean mosaic virus (SMV)-infected soybean root nodules were studied. The development of rhizobial infection threads and the processes of releasing rhizobia into nodule tissue cells appeared to be little affected by SMV infection.

The infection threads of rhizobia were usually present in the young central tissue cells near the meristem of the nodule, and in these cells small SMV aggregates, pinwheels, and bundle inclusions were also found. Cells, free of rhizobial infection threads, were generally infected with SMV.

SMV infectivity in root nodules was consistently higher than that in the root tissues. The presence of virus aggregates in the root nodule cells further evidenced that SMV could multiply in the root nodule cells despite the presence of *Rhizobium*.

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Symbiotic association between legumes and rhizobial bacteria in root nodules appears to be affected by plant virus infection. Reduction in size and number of root nodules has been shown in soybean mosaic virus (SMV)-infected soybeans (7, 8). Reduced effectiveness in N-fixation and reduced efficiency in N-utilization have also been reported, respectively, in the root nodules of clover phyllody diseased clover (4) and SMV-infected soybean (7). Recently, Tu et al. (8) found that crude saps from root nodules of SMV-infected plants were infective upon being introduced into healthy soybeans. This finding suggested that SMV was present in nodule tissue of virus-infected soybean.

The interaction of virus and rhizobia in root nodules of a virus-diseased system is not yet well understood. The present investigation, therefore, provides information on SMV-*Rhizobium japonicum*-soybean system to elucidate the interrelationship among virus, rhizobium, and root nodule cells.

MATERIALS AND METHODS.—Soybean (*Glycine max* [L.] Merr. 'Amsoy'), soybean rhizobium (*Rhizobium japonicum* [Kirchner] Buchanan), and soybean mosaic virus (SMV) were used in this study. SMV used was the strain 'O' obtained from R. E. Ford, Ames, Iowa. This strain was originally isolated from soybean collected at Ottumwa, Iowa (6).

Seeds of soybean were dipped in sterilized 2% skimmed milk and then coated with rhizobial inoculum powder. These seeds were immediately sown in a steamed soil mixture consisting of sand, peat, and loam (1:1:2). Four seeds were planted in each 6-inch clay pot.

Soybean seedlings at their primary leaf stage, approximately 2 weeks after sowing, were mechanically inoculated with SMV. The inoculum consisted of a crude sap of virus-infected leaves, diluted 1:10 with 0.01 M phosphate buffer, pH 7.0. The seedlings were dusted with 600-mesh Carborundum, and the inoculum was rubbed onto the primary leaves. Control plants were rubbed with neutral phosphate buffer. After inoculation, the leaves were immediately rinsed with tap water. Both control and inoculated plants were kept in a greenhouse with temperatures ranging 24 ± 3 C.

Assay for virus infectivity.—Crude saps from SMV-infected root nodules and root tissues were clarified by centrifugation at 2,000 *g* for 10 min, then were assayed for dilution end points (DEP). Samples consisting of the terminal one-third of the root system (excluding the nodules) were taken for virus assay at 2-week intervals starting 2 weeks after virus inoculation. The nodules were collected separately for assaying. These saps were then diluted with an appropriate amount of 0.01 M phosphate buffer, pH 7, to obtain 1/10, 1/100, 1/500, 1/1,000, 1/2,000, 1/4,000, 1/8,000, and 1/10,000 dilutions. Each dilution was assayed on 20 soybean seedlings at primary leaf stage. These assays were repeated.

Electron microscopy.—Ten nodules each of healthy and SMV-infected plants were picked randomly 10 weeks after sowing. Each nodule was cut into four equal pieces through the center; thus, 40 pieces of healthy and SMV-infected nodules were available. They were fixed for 6 hr

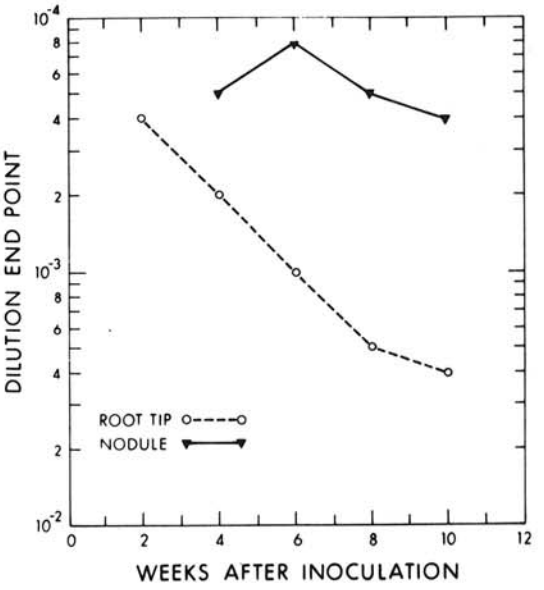


Fig. 1. Changes in infectivity in clarified sap of root nodules and roots following inoculation of soybean mosaic virus to the primary leaves of soybean.

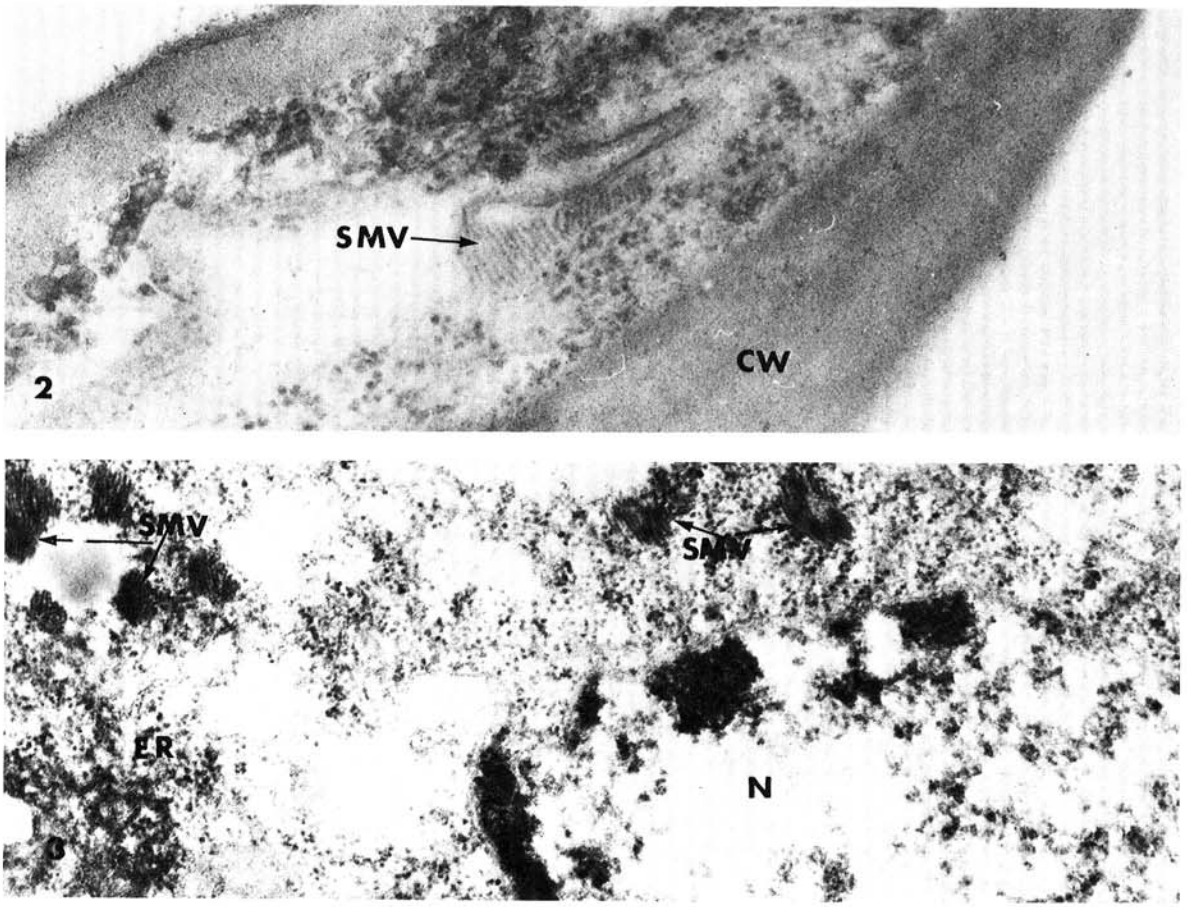


Fig. 2-3. 2) Portion of root nodule cell infected only with soybean mosaic virus (SMV) showing SMV particles aggregated along the cytoplasmic membrane (× 58,000) CW = cell wall. **3)** Small aggregates of soybean mosaic virus (SMV) in the root nodule cell infected with both SMV and *Rhizobium* (× 39,000). CW = cell wall, ER = endoplasmic reticulum, N = nucleus.

in a 1:1 mixture of 3% glutaraldehyde and 3% formaldehyde in 0.1 M phosphate buffer, pH 7.0. Fixed materials were washed for 30 min with several changes of the same buffer, postfixed with buffered 2% osmium tetroxide for 4 hr, dehydrated in a graded ethanol-propylene oxide series, and subsequently embedded in Araldite (9). At least 10 blocks each of healthy and SMV-infected nodules were sectioned for electron microscopy. Sections were cut on a Reichert ultramicrotome, stained with 2% aqueous uranyl acetate, followed by 0.2% aqueous lead citrate, and examined in Phillips EM 200 and EM 300 electron microscopes.

RESULTS.—*Infectivity assay.*—Virus infectivity was determined by periodical dilution end point assays of clarified saps from roots and root nodules. SMV infectivity in root nodules was consistently higher than in the terminal region of roots. Virus infectivity in root nodules was highest (DEP = 1/8,000) 6 weeks after virus inoculation and showed little change throughout the experiment (DEP's between 1/4,000 and 1/8,000). SMV titer in roots was highest (DEP = 1/4,000) 2 weeks after virus inoculation and dropped gradually with increasing age of soybean plants (Fig. 1). These results suggest that virus multiplication took place in situ in the nodules.

Electron microscopy.—In a SMV-infected soybean plant, the nodules were systemically infected with the virus but not all the cells were infected with rhizobia. Young meristemic cells of root nodules were usually free from rhizobial infection.

The presence of SMV in infected nodules, in addition to the infectivity assays, was proven by the sectioning of nodule tissue. These sections showed the presence of SMV particles (Fig. 2), and aggregates (Fig. 3), and the presence of pinwheel and bundle inclusions (Fig. 4, 5, 8) which were characteristic of infection by SMV (5) and other members of the potato virus Y group (1).

1) *SMV-infected cells with or without rhizobial infection.*—Nodule cells free of rhizobia had fewer ribosomes. On the other hand, the cells with rhizobial infection threads had enormous numbers of ribosomes (Fig. 4, 5, 8) and an extensive rough endoplasmic reticulum (ER) (Fig. 6, 7). Although pinwheels and bundles were visible, their detailed structures were difficult to resolve because of the high density of ribosomes and ER (Fig. 4-7). Mitochondria appeared to be more numerous in cells infected with rhizobia and were in orthodox conformation [see Hackenbrock (3)] which was suggestive of high metabolic activity.

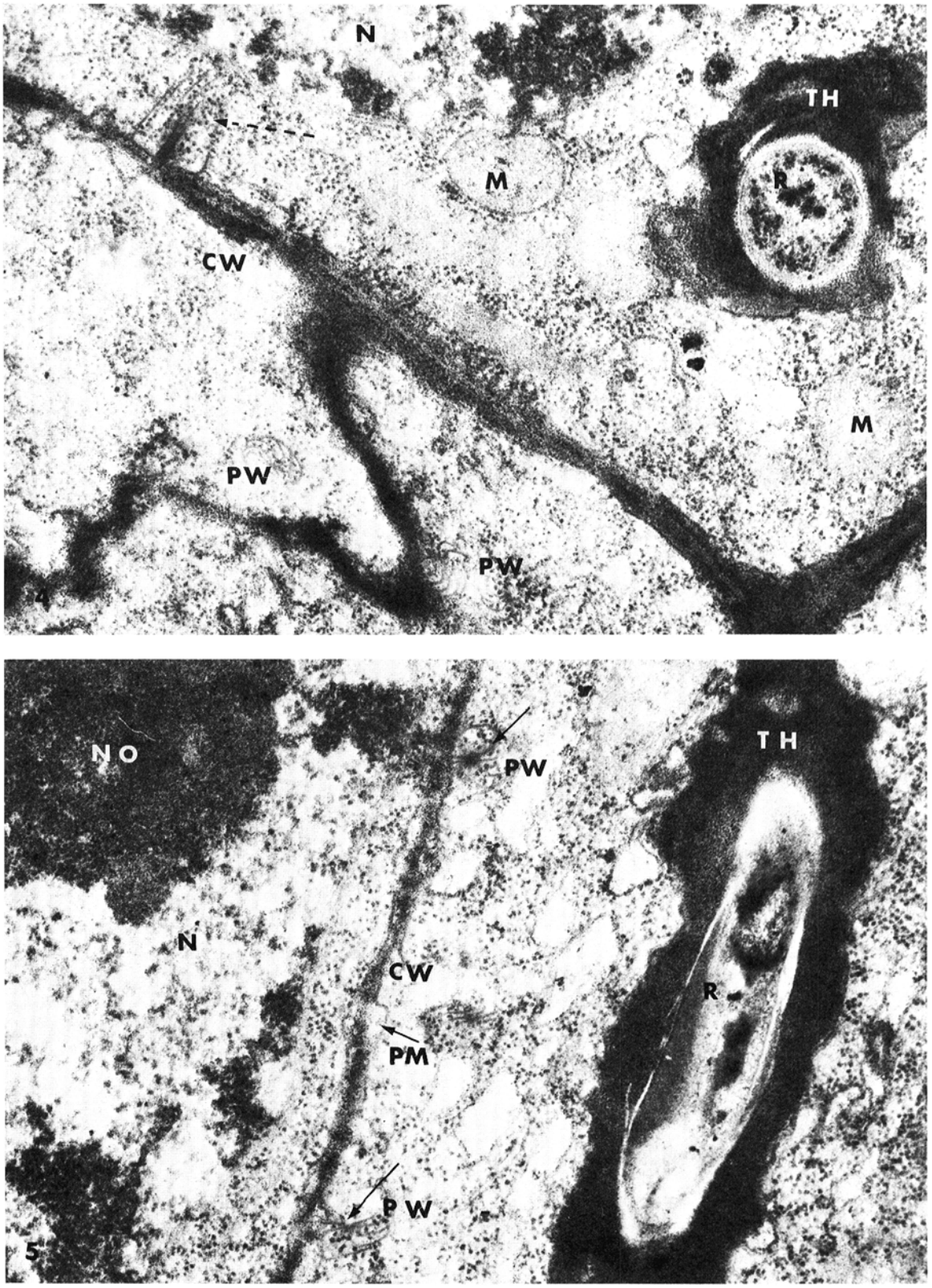
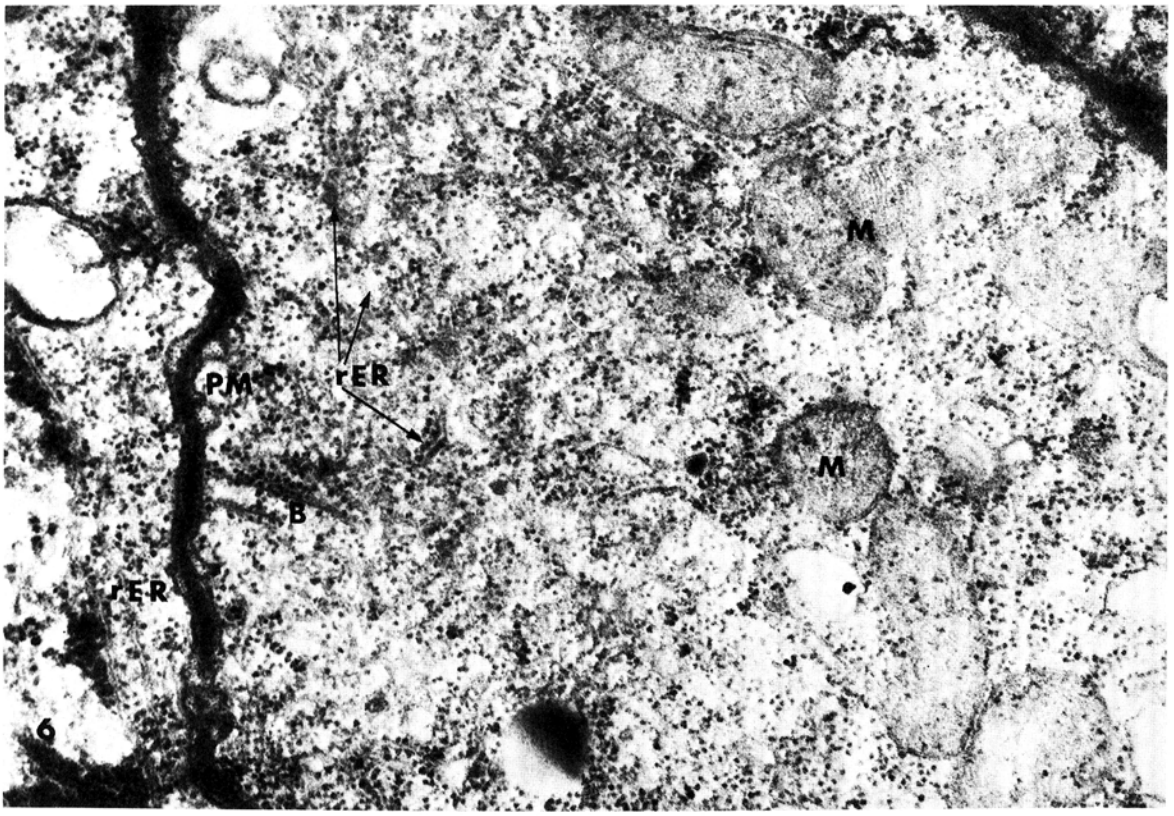


Fig. 4-5. Portions of root nodule cells infected both with soybean mosaic virus and *Rhizobium* showing a high density of ribosomes, membrane-bound rhizobial infection threads (TH) and pinwheel inclusions (PW). Some pinwheels are seen to be in close association with the plasma membrane (PM) and cell wall (CW) (arrow). Also note the bundle inclusion (broken arrow) (× 39,000). M = mitochondrion, No = nucleolus, R = rhizobium.


M
PM
rER
M
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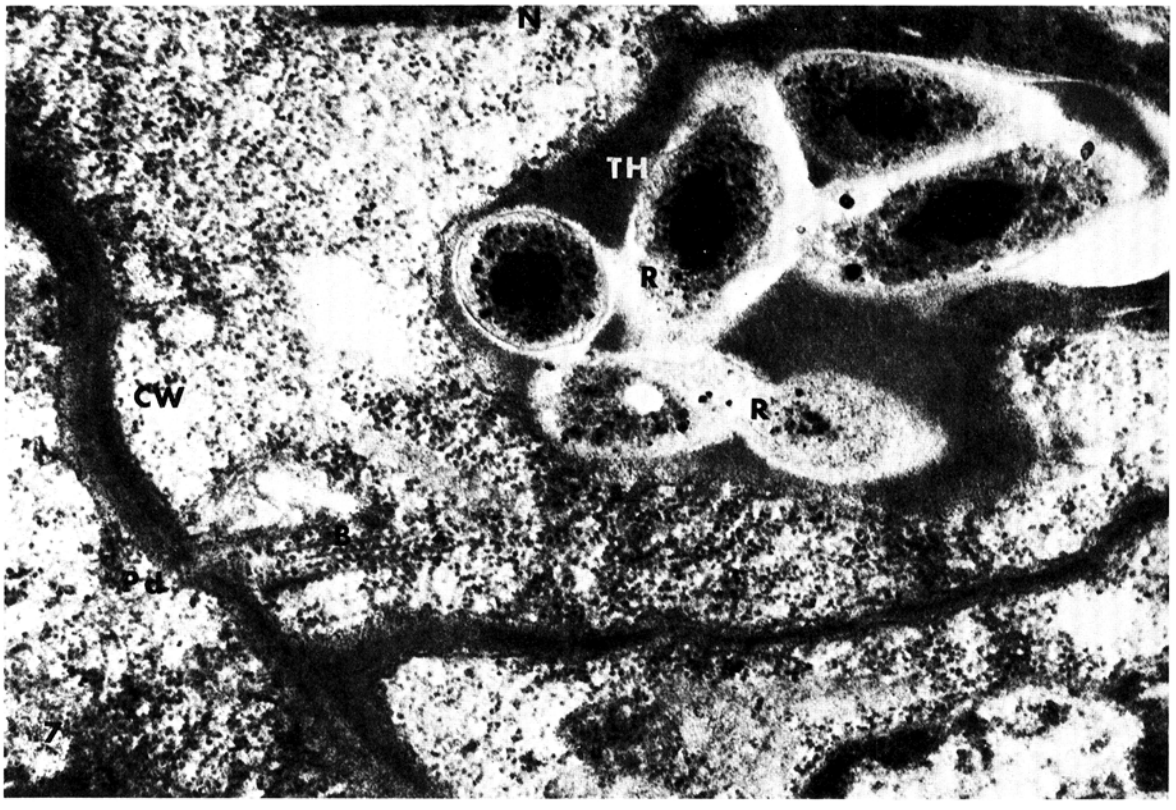
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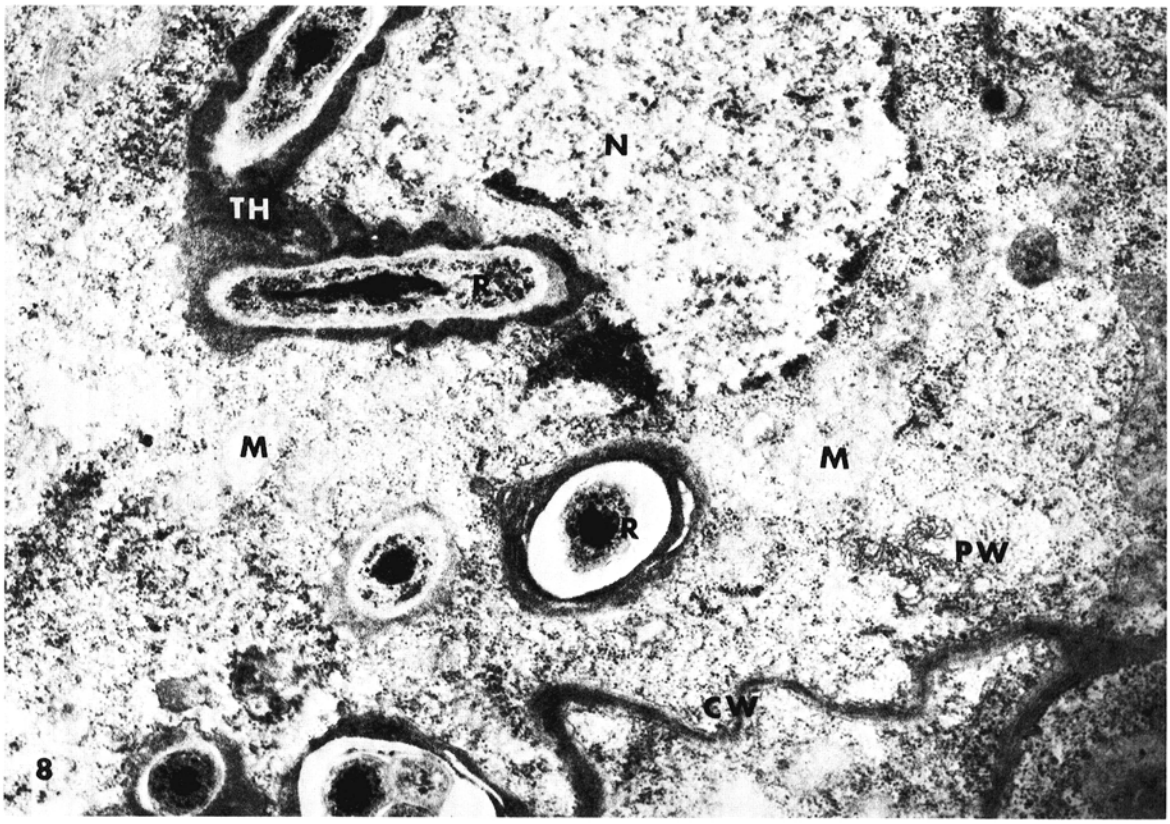


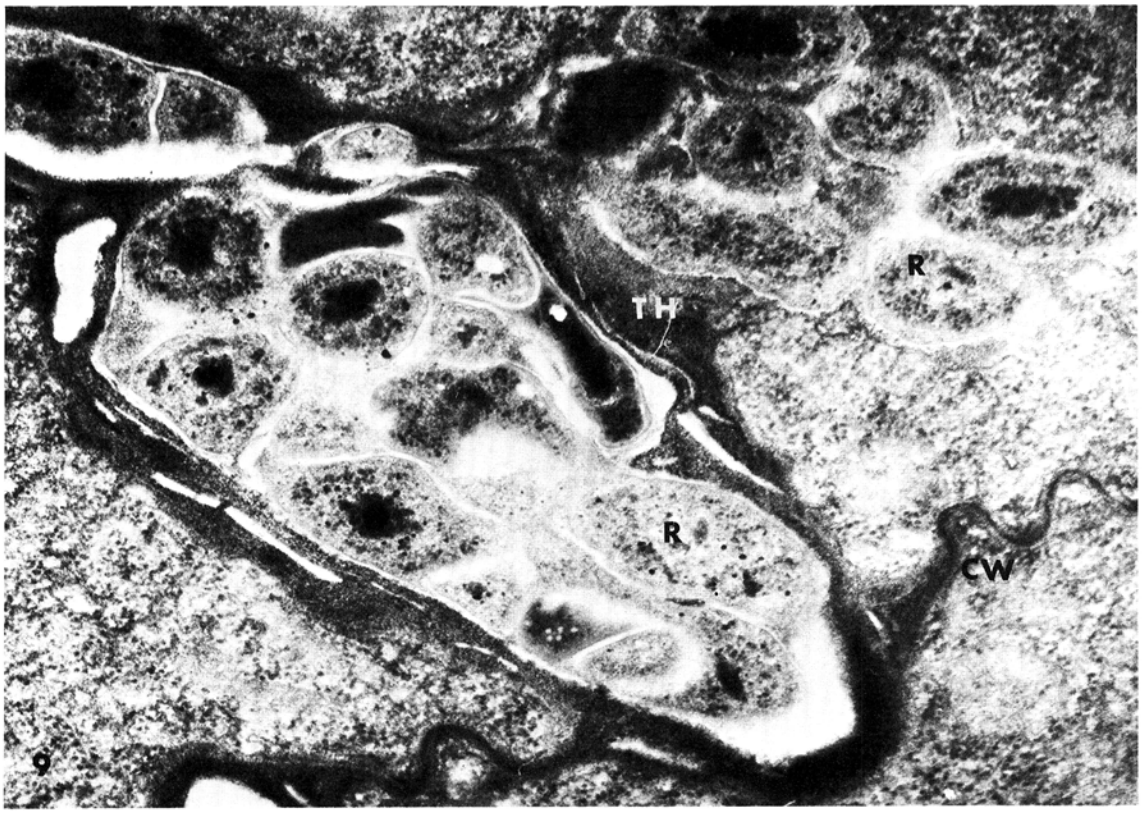
Fig. 8. Portion of a root nodule cell infected with soybean mosaic virus and rhizobial infection threads showing the presence of a membrane of a rhizobial infection thread and pinwheels (PW) in a cell (× 30,000) CW = cell wall.

2) *Rhizobia in SMV-infected cells.*—SMV infection appeared to have little effect on the development of infection threads and release of bacteria from threads into the host cells. Infection threads of rhizobia were seen in SMV-infected central tissue cells. The infection threads passed through the intercellular spaces and penetrated the cell wall. The bacteria were enclosed in the thread wall. The thread walls were membrane-bound and were often thicker than the cell wall of central tissue cells (Fig. 8, 9). Even though the bacteria were still enclosed in the infection threads and were not being released into the cytoplasm, metabolic changes of host cells apparently had been triggered. Unlike the uninfected cells, rhizobium-infected cells were typified with abundant ribosomes and an extensive endoplasmic reticulum complex (Fig. 8).

The mode of release of bacteria from the infection thread into the cytoplasm was described as follows. Firstly, a lateral wall-enclosed bulge developed on the thread and became filled with bacteria (Fig. 9); secondly, the bulge then extended but the wall material was no longer deposited (Fig. 9); and finally, bacteria came in touch with the membrane surrounding the bulge and the membrane eventually folded around each bacterium as it moved into the cytoplasm (Fig. 10). This observation in respect to the development of rhizobial infection thread and the mode of releasing bacteria from the thread into the cytoplasm agreed closely with the processes described by Goodchild & Bergersen (2) in a virus-free system.

DISCUSSION.—This investigation shows that SMV multiplies in situ in the central tissue cells. However, SMV infection did not seem to affect the growth of rhizobial infection threads and the processes of releasing into the nodule cells. These rhizobia later became bacterioids. Since SMV multiplication took place in situ in root nodules, physiology of these nodule cells could be affected as some of the soluble N-compounds synthesized through soybean-rhizobium symbiosis could be used as building blocks of viral protein and turned into insoluble viral protein. The higher total N contents in nodules found in SMV-diseased soybeans than that of healthy ones reported by Tu et al. (7, 8) could, therefore, be

Fig. 6-7. Portions of root nodule cells infected both with soybean mosaic virus and rhizobial infection threads. **6)** Showing the presence of extensive rough endoplasmic reticulum complex (rER), large numbers of mitochondria (M), and bundle (B) inclusion attached perpendicularly to the plasma membrane (PM) (× 39,000). **7)** Showing rhizobia (R) in the rhizobial infection thread (TH) and the bundle inclusion close to a plasmodesmatum (Pd) (× 39,000).


TH
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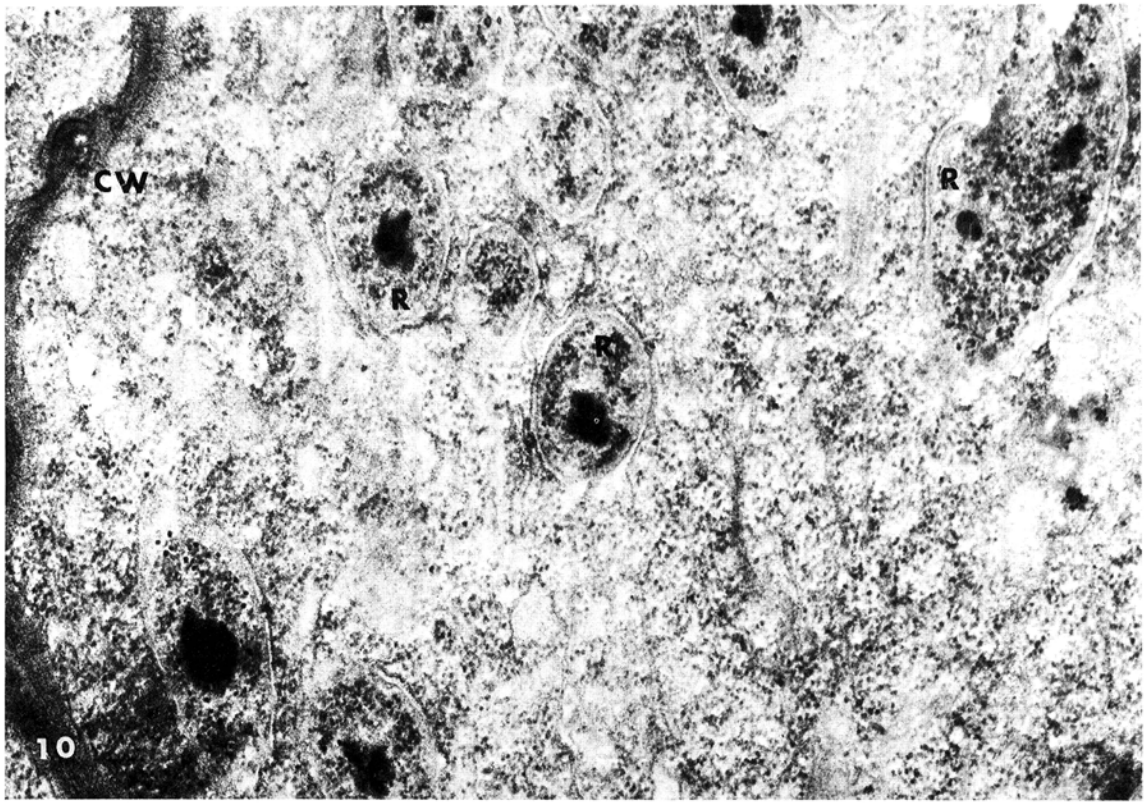
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Fig. 9-10. Portions of root nodule cell infected with soybean mosaic virus and rhizobia. **9)** Showing the release of rhizobia (R) from the infection thread (TH) into the cytoplasm of nodule cell (× 39,000) **10)** Showing rhizobia (R) in the bacterioid state (× 39,000) CW = cell wall.

explained in part by the presence of large portions of insoluble viral protein. The synthesis of viral protein could decrease the translocation of soluble N-compounds to other parts of the plant and result in yield reduction of soybean.

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