Cytoplasmic Changes During and After Infection of Soybean Root Nodule Cells with Rhizobia

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ABSTRACT

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Root cortical cells adjacent to or invaded by infection threads underwent cytoplasmic changes. These changes included an increase in number of ribosomes, and the extension of endoplasmic reticulum. The changes indicate increased cellular synthetic activities. These cellular changes continued throughout the life of the cells. Upon release of the rhizobia from the infection thread into the cytoplasm, the number of dictyosomes increased. These dictyosomes were arranged in a long chain between the rhizobial bacteroids and the interior nucleus of the cell. Similarly, vesicles of dictyosome origin greatly increased in number, and often fused into a large vesicular body that was deposited around the rhizobia. The content of these vesicles and vesicular bodies had staining properties similar to that of the cell plate or middle lamella, as demonstrated by a differential staining technique. Increased dictyosome activity receded after the rhizobial bacteroids were released into the cells. Therefore, I suspect that the transitory increase of dictyosome activity may be attributed to stimulated production of cell wall materials induced by the invasion of infection threads and the release of rhizobial bacteria.

In legumes, rhizobia enter the root at the root hair (12). After entry, the rhizobia move through the middle lamella of the root hair cells and then through the middle lamellae of the adjacent root cortical cells. It is known that the rhizobia infect tetraploid cortical cells, but not diploid ones. The noninfected diploid cortical cells develop into interstitial tissue cells of the nodules (15).

The development of the root nodule is initiated when the infection thread reaches a tetraploid cell in the cortical tissue. At that point, the rhizobia exert pressure which causes the host cell wall to form an invagination or a tube which extends into the cell. The tubular protrusion later branches (3, 12). In all cases, release of rhizobia takes place at the tips of the tubular protrusions of the infection thread. Since the infection thread is a portion of the host cell wall itself, the released rhizobia first enter the periplasmic space between the host plasma membrane and the wall of the infection thread. The final entry of the rhizobia into a host cytoplasm is by endocytosis (17). This type of entry is unique because bacteria normally gain entrance to the host through wounds or natural openings.

Although the processes of rhizobial infection and nodule formation have been studied in many legumes (3, 5, 6, 12, 18), the cellular responses of host cells to the infection process have been largely neglected.

The present paper describes and comments on changes in the host cell during the infection process.

MATERIALS AND METHODS

Seeds of soybean [*Glycine max* (L.) Merr. 'Amsoy'] were germinated in a petri plate with a moistened filter paper for 24 hours. The seedlings were transplanted to 15-cm diameter pots containing perlite. Watering was done by applying 200 ml of modified Hoagland's solution (4) daily to each pot. Seedlings in their primary leaf stage were inoculated with *Rhizobium japonicum* (Kirchner) Buchanan by applying 100 ml of rhizobial inoculum suspension to each pot. The inoculum was from a 2-day-old liquid culture of rhizobia growing in 2-liter flasks. Each flask contained 0.5 g of K_2HPO_4, 0.2 g of $MgSO_4$, 0.1 g of NaCl, 3 g of yeast extract, and 10 g of mannitol per liter of water (17). The flasks were placed in a water bath shaker operated at 25 C.

After inoculation, plants were kept in a 22 ± 1 C controlled environment programmed for 10 hours of darkness and 14 hours of fluorescent light at 26,000 lux.

Three weeks after inoculation, root nodules about 1 mm in diameter were excised and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0 for 4 hours at room temperature. The materials were washed with three changes of buffer, 15 minutes each, and postfixed for 2 hours at 22 C with 2% OsO_4 in 0.1 M neutral phosphate buffer. Then they were rinsed with the same buffer, dehydrated through a graded series of ethanol, followed with propylene oxide, and embedded in an Epon-Araldite mixture (8).

Thin-sections were cut with a DuPont diamond knife on a Reichert ultramicrotome. A group of sections was picked up on a copper grid coated with 0.3% Formvar, and stained with 2% aqueous solution of uranyl acetate for 2 hours, then with 0.2% aqueous lead citrate for 3 minutes before examination in an electron microscope (16). Another portion of the sections was transferred with a 4-mm diameter platinum loop to a small beaker containing 1% aqueous solution of HIO_4 for 30 minutes of destaining, followed by three transfers of 10 minutes

each through distilled water (13), and then these sections were picked up on 149-μm (100-mesh) Formvar-coated grids. The sections were stained with 1% phosphotungstic acid (PTA) in 10% chromic acid, as described by Roland et al. (13) with two minor modifications. Firstly, Roland et al. (13) handled their sections with loops throughout the staining procedure. I found that this was unnecessary because the sections could be stained equally well while they adhered to the Formvar on a copper grid. Secondly, the duration of staining was increased to 15 minutes. This stained the background cytoplasm darker in a somewhat amorphous manner so that the vesicles, cell plates, and middle lamella showed a distinct contrast.

After staining, the sections were washed by dipping the grids in three changes of distilled water, thirty dips for each change. They were then examined in a Philips EM-300 electron microscope.

RESULTS AND DISCUSSION

General remarks.—The term "infection thread", although in general use, is an ambiguous one. It originated from a light microscopic observation of rhizobial infection processes in which a fine thread containing rhizobia advance from the root hair to the root cortical cells where the rhizobia were released into the cells. Thus the "infection thread" has been considered by some as a long tubular structure enclosing the rhizobia. The tubular structure was thought to grow and advance toward the cortical cells, as the rhizobia multiplied within it. McCoy (7), however, was first to suggest that the "infection threads" were a part of the host cell wall, that the rhizobia in the infection threads were in fact moving in the middle lamella between the two cell walls, and that the tip of the "infection thread" which penetrated the host cell was but a perpendicular fingerlike invagination of the host cell wall. At present, McCoy's hypothesis is generally accepted (6). Nevertheless, for convenience, the term "infection thread" is used in this report.

Cells in association with the infection threads.—The host cells adjacent to the rhizobial infection threads showed many cytoplasmic changes (Fig. 2, 3). These changes included an enormous increase in the number of ribosomes, extension of the endoplasmic reticulum, and an increase in the number of dictyosomes (Fig. 3). These cells appeared to be stimulated by the presence of the infection thread, as evidenced by their increased cellular synthetic activities. The cells which were not adjacent to the infection threads were not altered and were in the typical parenchymatous form (Fig. 1).

The increased vesicular activity of dictyosomes resulted in an increased number of vesicles in the cytoplasm of the host cells. These vesicles contained amorphous electron-dense material similar in staining properties to both the inner layer of infection threads and of cell walls, when stained with uranyl acetate and lead citrate (Fig. 3, compare areas indicated by white and black arrows). This observation was substantiated further when stained with PTA-CrO_3. These vesicles (Fig. 6 inset) together with the infection threads and cell walls remained electron-transparent (unstained) whereas the cytoplasm and all the organelles, except the rhizobia, were amorphously stained (Fig. 6). Further, these vesicles appeared to deposit their contents directly onto the infection threads and cell wall (Fig. 5 arrows), indicating that their contents could be homologous with cell wall materials. It is known that veiscles containing materials which are not homologous with the cell wall usually are deposited in the periplasmic space and form multivesicular bodies which undergo molecular reorientation and digestion by the periplasmic enzymes prior to incorporation into the cell wall (1, 10).

The findings on the similarities in staining properties and the physical continuity between the cell wall and infection threads are consistent with McCoy's hypothesis that the walls of the threads are derived from the host cell wall (7).

The release of the rhizobia into the host cytoplasm.—Upon the release of the rhizobia from the infection threads into the host cells, the number of dictyosomes further increased and then formed a dictyosome chain, which lay between the bacteroids and the interior nucleus of the cell (Fig. 7). This type of dictyosome arrangement is unique and was not observed either in the cells not invaded by the infection threads or prior to the release of the rhizobia. The frequency of occurrence of dictyosome chains in sections of cells with infection threads was approximately 80%.

Vesicles, presumably of dictyosome origin (9, 11), often fused into various sizes of vesicular bodies (Fig. 4, 8). Some of them were as large as or larger than a mitochondrium or a rhizobium [Fig. 6 (inset), 8]. They were present in considerable numbers in the cytoplasm. Some of the vesicular bodies either became attached to (Fig. 3, 5, 6) or surrounded (Fig. 8) the newly released bacteroids. Such a phenomenon is illustrated in Fig. 9, in which bacteroids are seen either partly (Fig. 9-A, B) or fully (Fig. 9-C, D) encircled by numbers of vesicular bodies. The membrane of each vesicular body often remained visible for some time (Fig. 9-C). However, after a large number of rhizobia had entered the cell and multiplied within it, the increased dictyosome activity receded and only a few small vesicles or vesicular bodies were noticeable (Fig. 10).

Dictyosomes are secretory organelles (7, 9, 11) which form vesicles containing cellulose-, hemicellulose-, and pectinlike substances (1, 2, 11). These vesicles are thought to be involved in the formation of the cell plate, and the primary and secondary walls (2). The vesicle-producing activity of dictyosomes seems rather flexible. It depends mainly on the demand for cell wall substances. The demand for increased cell wall formation may arise from either genetically governed cell division or from induced factors; for example, injury, heat treatment, infection, etc. Under both circumstances, dictyosomes are requisite for synthesis of cell wall materials. For cell division, they provide material for the cell plate and the cell wall (2) and, in response to the other stimulations, they provide cell wall materials required for wound healing or for preventing the spread of infection. For example, abnormal thickening of cell walls has been observed in cells surrounding virus-induced local lesions (14, 18).

Since the infection thread is a part of the cell wall itself and its invagination into the cell is caused by the pressure of rhizobia on the middle lamella (6), the increased number and activity of dictyosomes during the infection processes of rhizobia may be induced by the following factors: (i) the infection threads require a portion of the

cell wall to extend and subsequently to branch and proliferate within the cell; (ii) a further stimulation of dictyosomes appears to coincide with the injury or the cracking of the infection threads in the course of releasing rhizobia into the cytoplasm; and (iii) the stimulation of dictyosomes is related to the entry of rhizobia into the cytoplasm. After the infection processes have been completed, bacteroids divide when their host cell divides. Therefore, the progenies of the original bacteroidal cell normally have bacteroids but no infection thread. The increased dictyosome activity in the progeny bacteroidal cells could be a result of this change.

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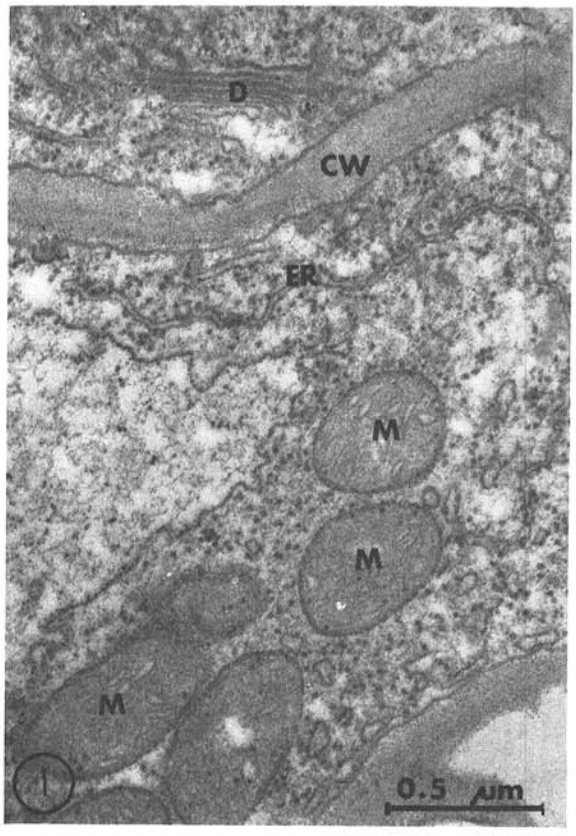
The abbreviations used in Fig. 1-10 are: CW, cell wall; D, dictyosome; ER, endoplasmic reticulum; M, mitochondrion; Pd, plastid; R, rhizobium; Th, infection thread; VB, vesicular body.

Fig. 1-3. Effect of the presence of the infection thread on central tissue cells of root nodules. **1)** A portion of the central tissue cell of the root nodule not invaded with a rhizobial infection thread showing normal cellular organization of a typical parenchymatous cell. **2)** Infection threads from the middle lamella thrusting into the cell. The infected cells have an increased number of ribosomes and extensive endoplasmic reticula. **3)** An infection thread releasing the rhizobia from a crack at its tip. At this stage, the cellulose containing vesicular bodies has become more prominent (white arrows). Note that the inner layer of the infection thread (black arrows) has a staining property similar to that of the vesicular bodies.

Fig. 4-6. Relationship of cell wall materials to the vesicles and vesicular bodies of dictyosomes as seen in a young bacteroidal cell. **4)** A high magnification micrograph taken from a cell at the same stage as in Fig. 3, showing the increased vesiculation of dictyosomes. **5)** A portion of an infected cell in the early bacteroidal stage showing direct deposition of vesicular bodies onto the cell wall (solid arrow) and onto the bacteroids (broken arrows). **6)** Infected cells in the bacteroidal stage, stained with a mixture of phosphotungstic acid (PTA) and chromic acid showing that the host cell wall, the infection thread, and the vesicular bodies (arrows) are all generally unstained. The amorphous background is a result of PTA-CrO_3 staining of the ground cytoplasm. Inset is a portion at higher magnification, showing the deposition of vesicular bodies (arrows) on or around the bacteroids.

Fig. 7-8. Host cytoplasmic changes after rhizobia are released into the cytoplasm from the infection thread. **7)** Portions of newly infected cells showing dictyosomes arranged in chainlike fashion and their association with the bacteroids. **8)** Presence of numerous vesicular bodies of various sizes in the newly infected cell.

Fig. 9-10. Bacteroids with varying degrees of deposition of vesicular bodies and also those free of deposition. **9)** A composite micrograph showing various amounts of deposition on the vesicular bodies (arrows) which partly (A and B) and fully (C and D) surround the bacteroids. **10)** Portions of infected cells in the late bacteroidal stage, showing very few vesicular bodies.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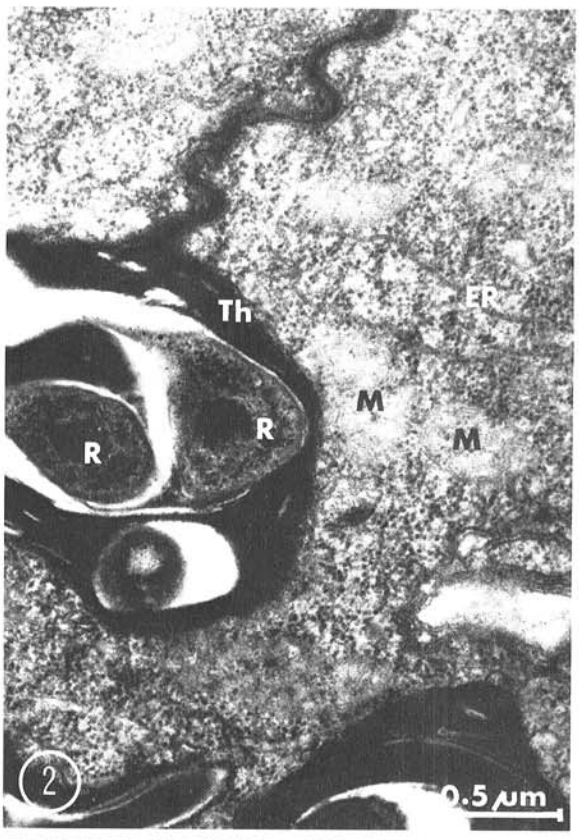
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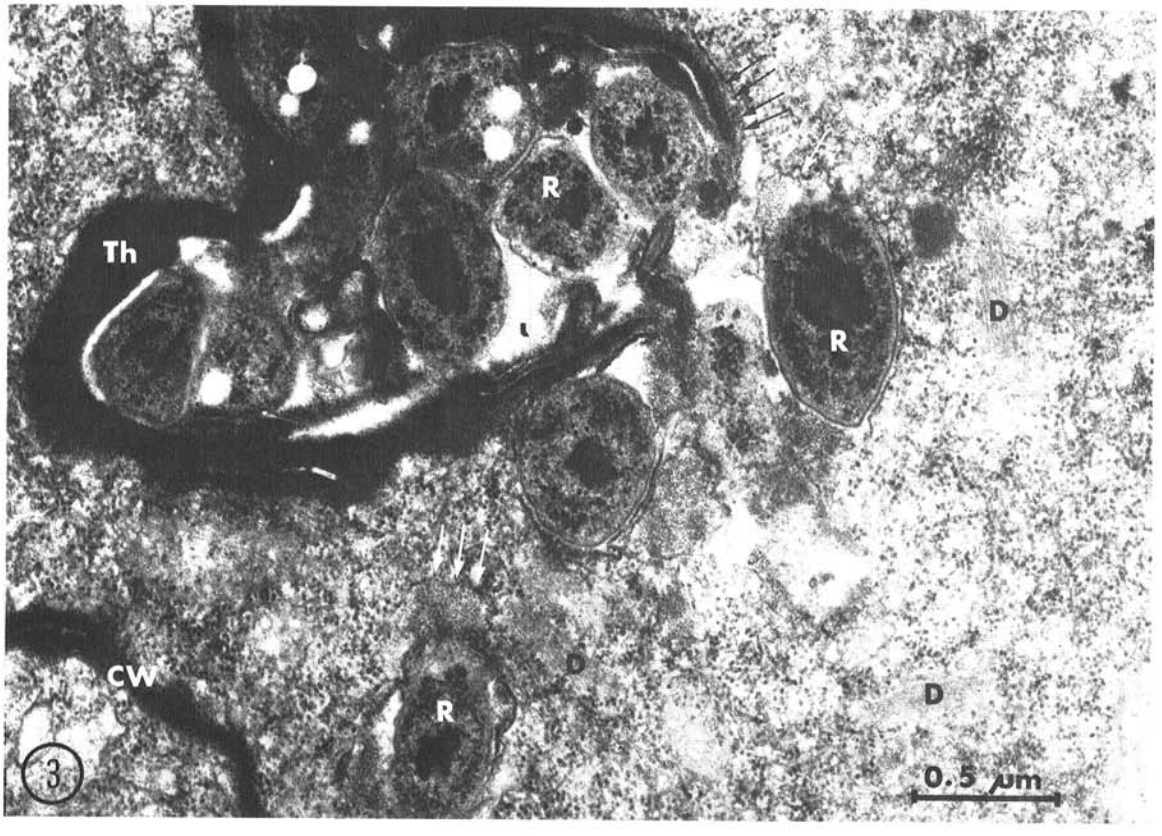
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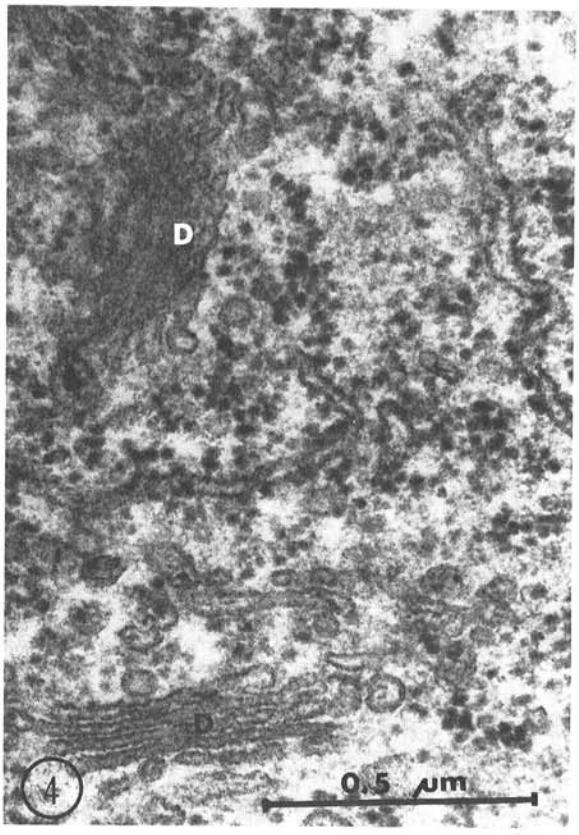
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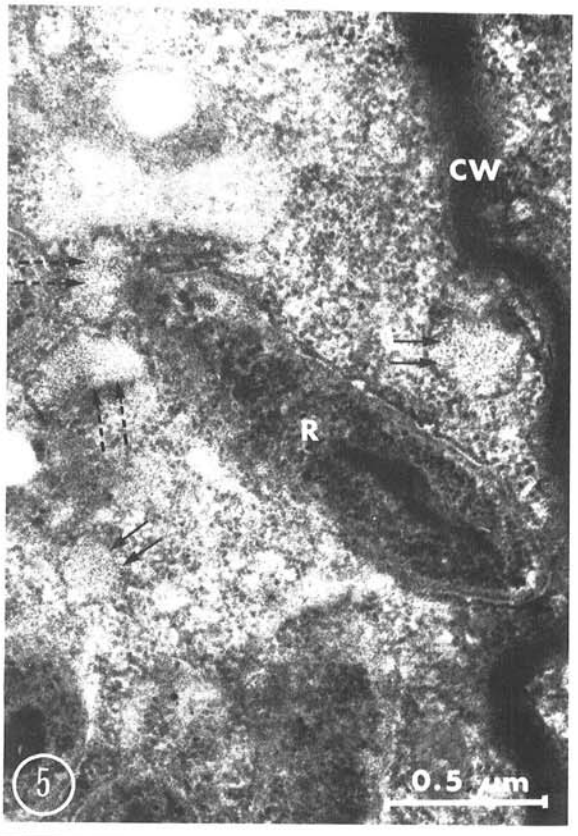
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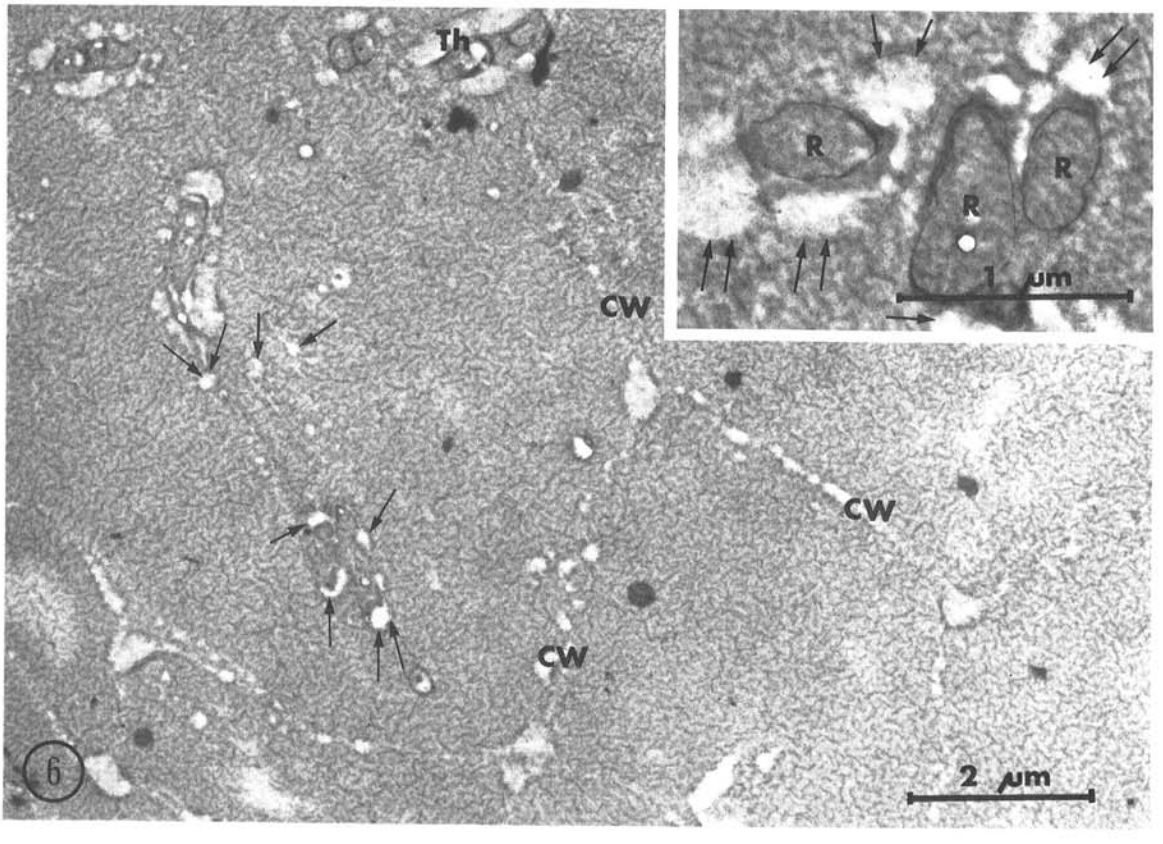
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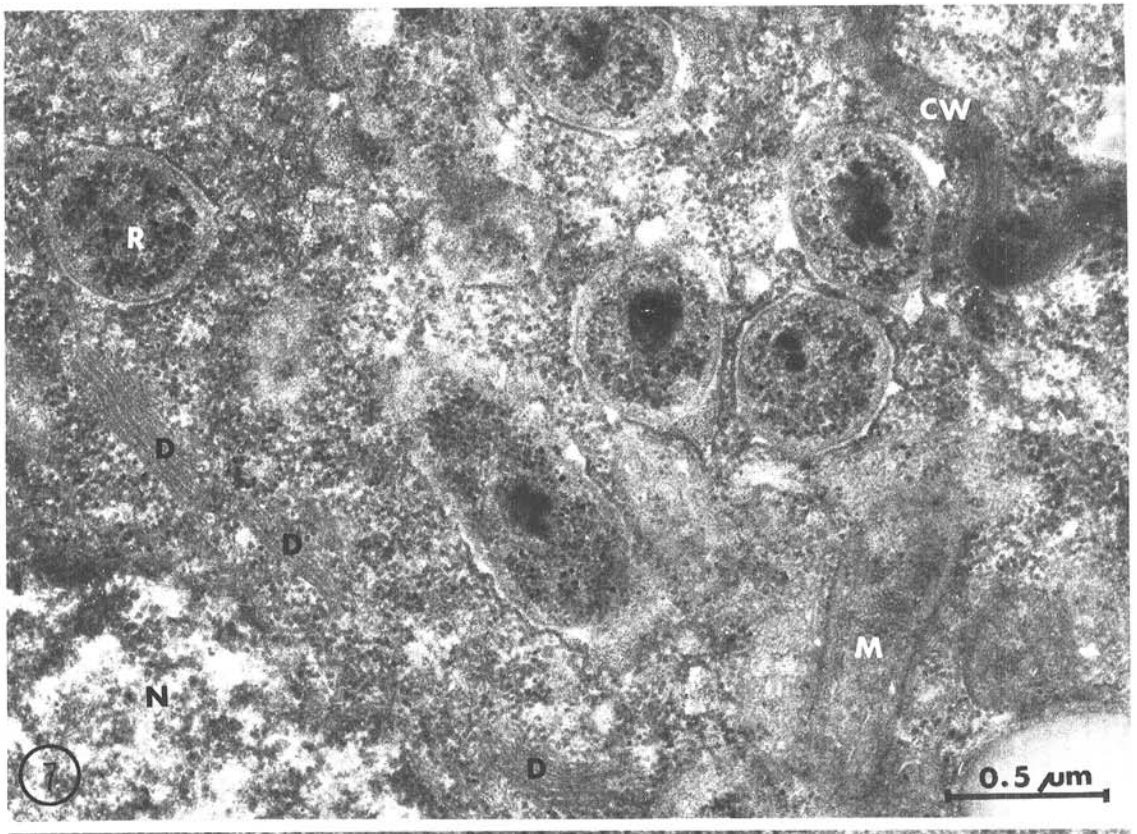
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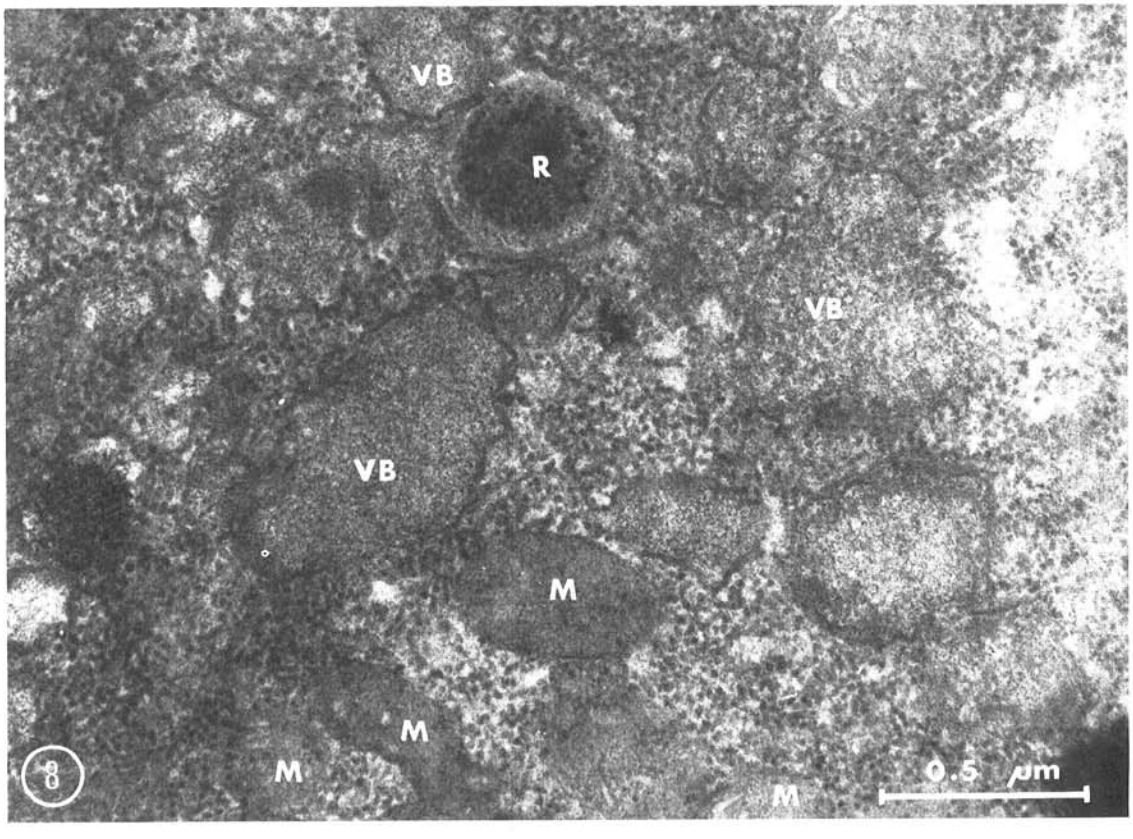
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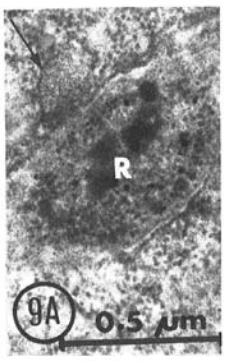
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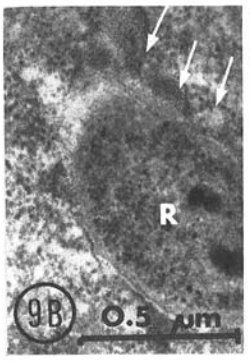
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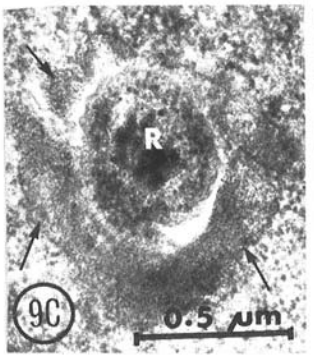
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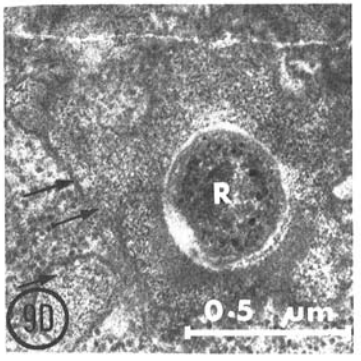
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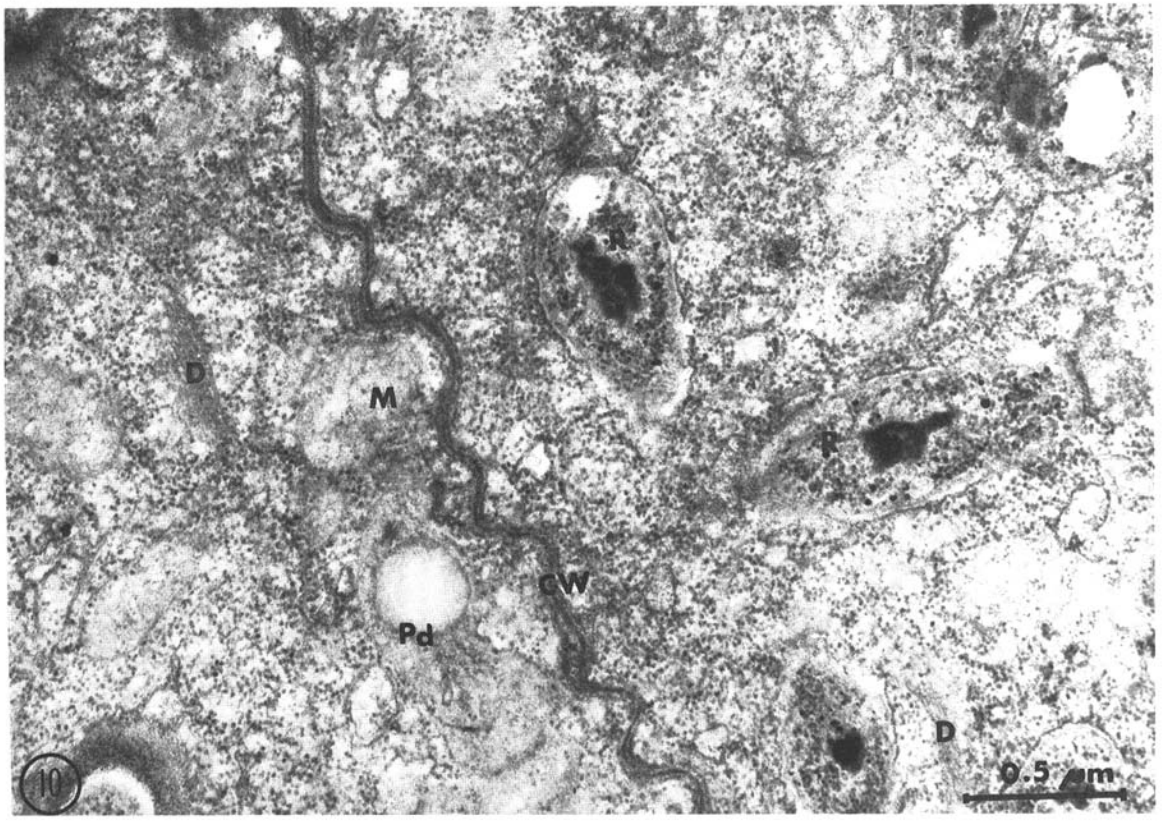
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