Electron Microscopy of Cell Wall Thickening in Local Lesions of Potato Virus-M Infected Red Kidney Bean

J. C. Tu and C. Hiruki

Former Postdoctoral Fellow and Associate Professor, respectively, Department of Plant Science, University of Alberta, Canada. Present address of senior author: Electron Microscope Laboratory, University of Alberta.

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ABSTRACT

The primary leaves of Red Kidney bean (*Phaseolus vulgaris*) were mechanically inoculated with an Alberta isolate (AP-1) of potato virus M. Local lesions were sampled for electron microscopy 8 days after inoculation. Thin sections of local lesions revealed an abnormal thickening in the inner portion of secondary cell wall. Thickening was initiated in those cells at the periphery of the necrotic area in the lesion. In these cells, thickening of 5 times or more the normal cell wall was not uncommon in the late stage of development. These cells also showed individual virus particles. Various developmental stages of the thickening are described, and the significance of cell wall thickening to cell-to-cell movement of virus is discussed. Phytopathology 61:862-868.

Additional key words: ultrastructure, virus-host interaction.

Plant virus infections can be categorized into three groups: systemic, local, and local infection later becoming systemic. The ultrastructure of systemic virus infection has been intensively studied in many virus-host combinations (10). Ultrastructural investigations of local lesions were made mostly in tobacco mosaic virus (TMV)-infected *Nicotiana glutinosa* (4, 5, 17, 18), *N. tabacum* 'Samsun NN' (8), *Chenopodium amaranticolor* (11), and *Datura stramonium* (1). Little information is available on the local lesions of other virus-host combinations.

Studies on the ultrastructure of TMV-induced local lesions focused mainly on the changes of cytoplasmic organelles in an attempt to relate these changes to physiological alterations during local lesion formation (8, 17). Physiological alterations due to host reaction to virus infection (13, 14, 16, 19, 21, 22) have been assumed to provide an unfavorable cellular environment for virus entry or virus multiplication, and to create a barrier to further cell-to-cell movement of the virus (8, 20).

The movement of virus beyond the local lesion is not uncommon. Many viruses which incite local lesions in inoculated leaves are also known to induce systemic symptoms on new growth. Therefore, it may be safe to assume that the rapidity of barrier formation in an infected cell would contribute to the restriction of virus within the local lesion area and influence cell-to-cell virus movement. This process may be physiological as well as physical. Physical alteration, if it exists, would result from physiological changes during pathogenesis. Some physiological changes known to occur in the periphery of the lesion are an increase in respiration (16, 21), accumulation of several metabolites (13, 22), change of some enzyme activities (14), and increase of soluble protein (14).

This investigation was made on potato virus M (PVM)-incited local lesions in Red Kidney bean. Since PVM-infected Red Kidney bean formed only local lesions, special attention was directed to the occurrence of morphological alterations.

MATERIALS AND METHODS.—The primary leaves of Red Kidney bean (*Phaseolus vulgaris* L.) were mechanically inoculated with an Alberta isolate (AP-1) of potato virus M (6, 15). Inoculum was obtained from PVM-infected King Edward potato (*Solanum tuberosum* L.). Inoculated plants were maintained in a 17 ± 2 C greenhouse. Infected plants produced local lesions in 4 days after inoculation. The local lesions were marked and sampled 8 days after inoculation. Healthy leaf tissues were also sampled for comparison. Samples were cut in 1-mm squares with a razor blade and fixed in 3% Formalin-glutaraldehyde mixture in 0.1 M phosphate buffer, pH 7.0. The tissues were subjected to 5 min partial vacuum treatment to remove air from the intercellular spaces, and were left overnight at 4 C. The fixed materials were washed, postfixed in phosphate-buffered 2% osmium tetroxide for 7 hr, dehydrated in graded ethanol-propylene oxide series, and embedded in Araldite.

The tissues were sectioned in a parallel fashion toward the upper surface of the local lesion. Sections were cut 60 to 90 mμ thick with a diamond knife mounted on a Reichart ultramicrotome and picked up with 400 mesh copper grids without Formvar coating. Thin-sections were stained with 2% aqueous uranyl acetate for 2 hr and poststained with 0.2% aqueous lead citrate for 2 min at room temperature. Observations were made in a Philip 200 electron microscope (60 kv).

RESULTS.—The abnormal wall thickenings in the inner portion of secondary cell wall were observed in the peripheral cells, two-four cells in width, around the necrotic area in the PVM local lesion on Red Kidney bean. Cells immediately adjacent to the necrotic area showed more thickenings than those farther away from the necrotic area. Abnormal thickening was not observed four cells away from the necrotic area. Dead cells at the edge of the necrotic area (Fig. 5) also showed some wall thickening.

Virus particles (Fig. 4, 10) could be seen in the living mesophyll cells where thickenings were in progress.

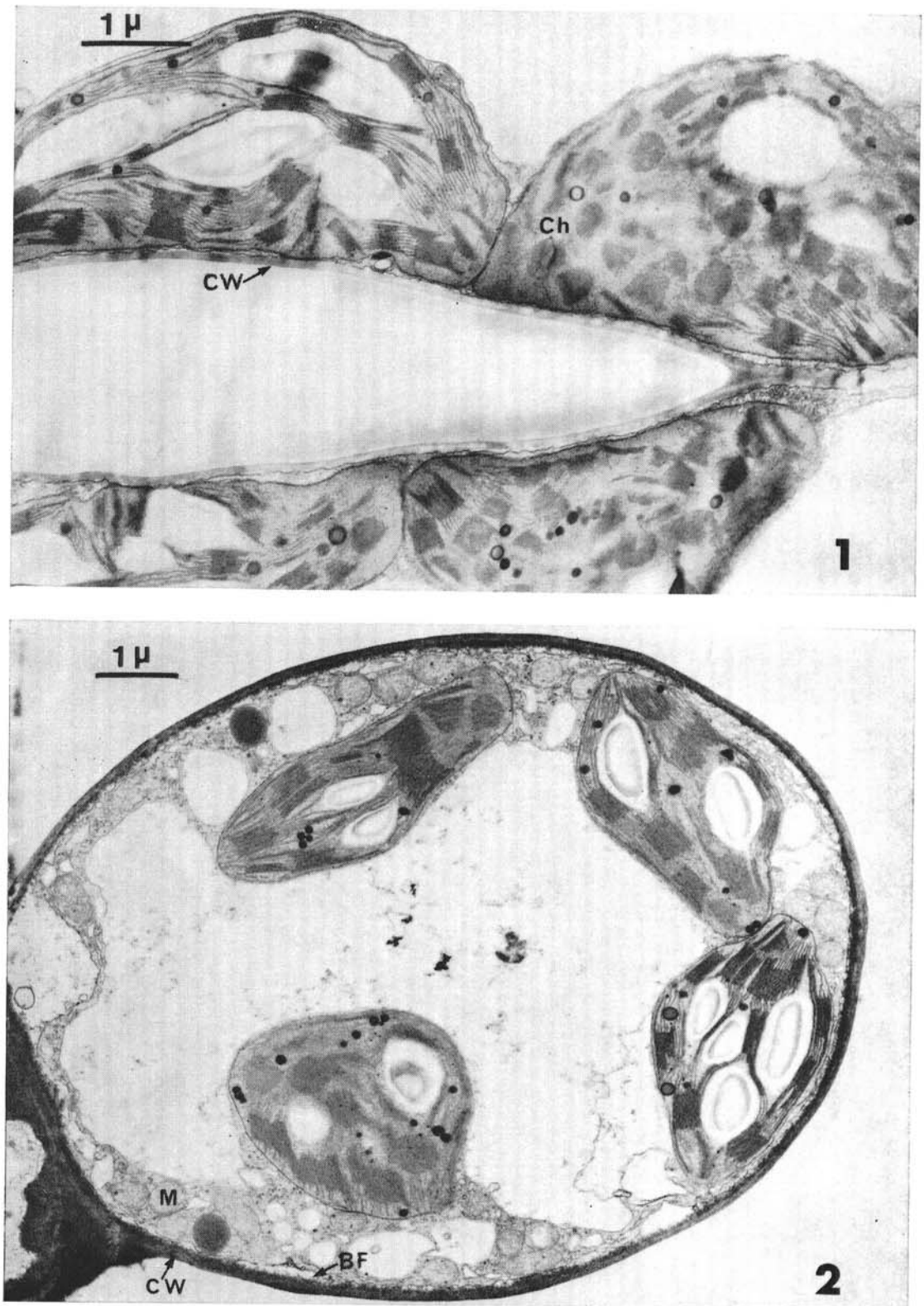

Fig. 1-2. 1) A portion of a healthy cell of a Red Kidney bean leaf in which chloroplast (Ch) and cell wall (CW) appear normal. 2) Evidence of boundary formation (BF) and increased mitochondria (M) numbers in cells infected with potato virus M manifesting the initial stage of abnormal cell wall (CW) thickening.

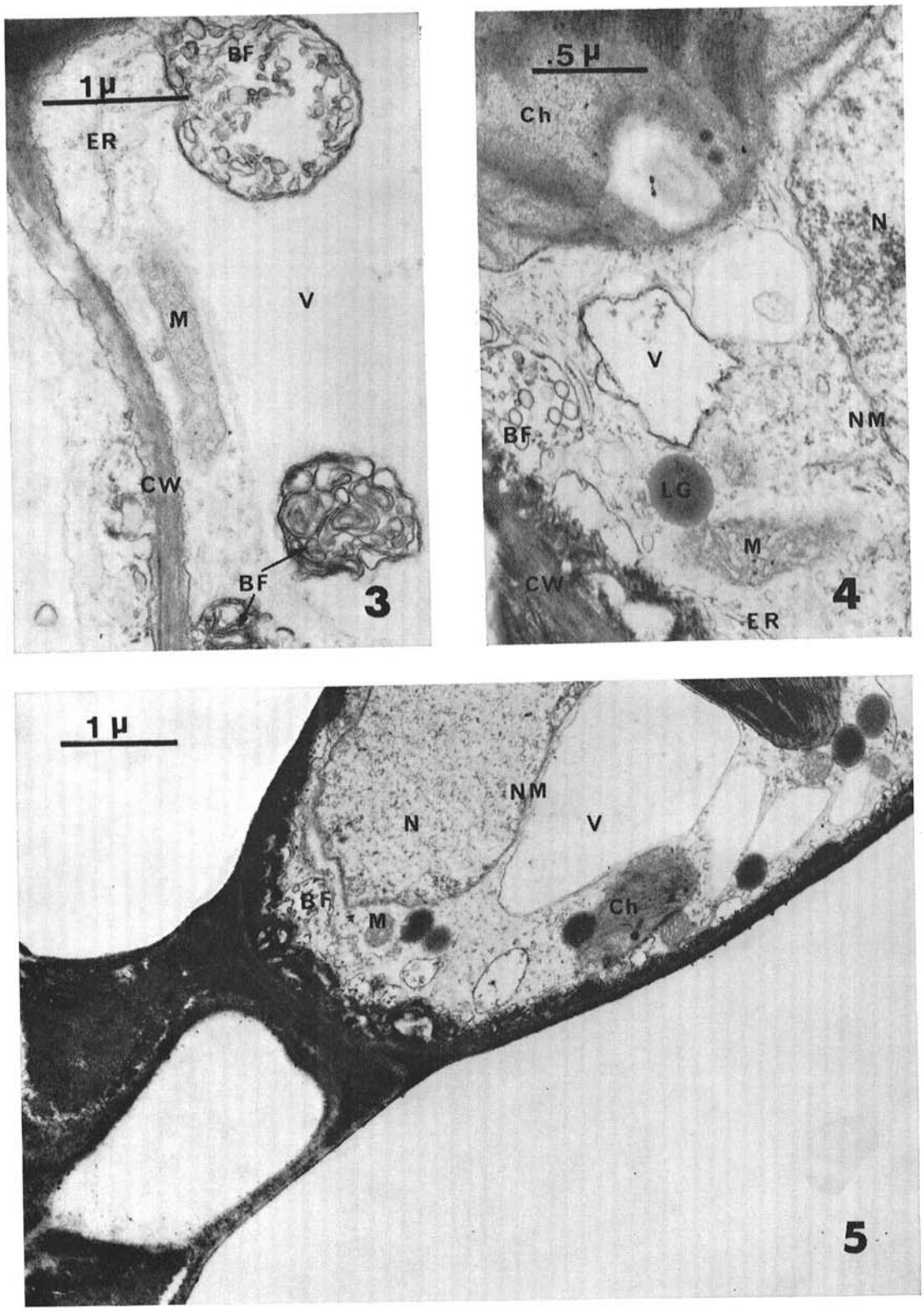

Fig. 3-5. Cells of a Red Kidney bean leaf infected with potato virus M. **3)** Roughening of cell wall (CW) and initiation of boundary formation (BF) (tubules and vesicles) as the primary step of abnormal cell wall thickening. M = mitochondrion; V = vacuole; ER = endoplasmic reticulum. **4)** An intermediate stage of cell wall thickening where secondary wall materials are deposited under the boundary formation (BF). **5)** An intermediate stage of cell wall thickening (at right) and a necrotic cell (at left). Ch = chloroplast; N = nucleus; NM = nuclear membrane; V = vacuole; LG = lipid globule; M = mitochondrion.

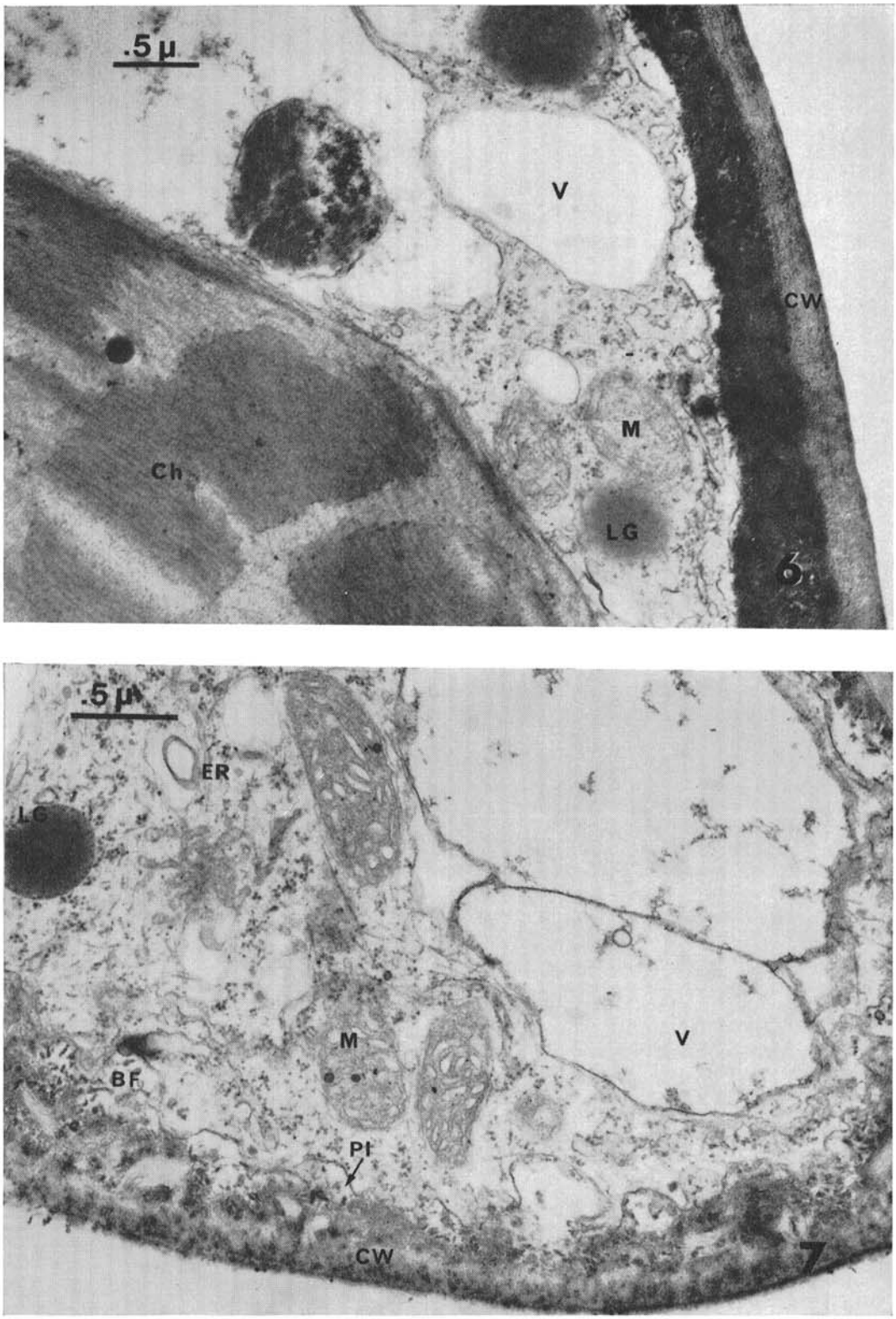

Fig. 6-7. High magnifications of the intermediate stages of Red Kidney bean leaf cells infected with potato virus M showing the presence of virus particles, extension inward of boundary formation (BF), and deposition of wall materials under the boundary formation. **6)** Uniform secondary deposition of wall. **7)** Mosaic type of secondary wall deposition. CW = cell wall; M = mitochondrion; LG = lipid globule; V = vacuole; ER = endoplasmic reticulum; Ch = chloroplast; Pl = plasmalemma.

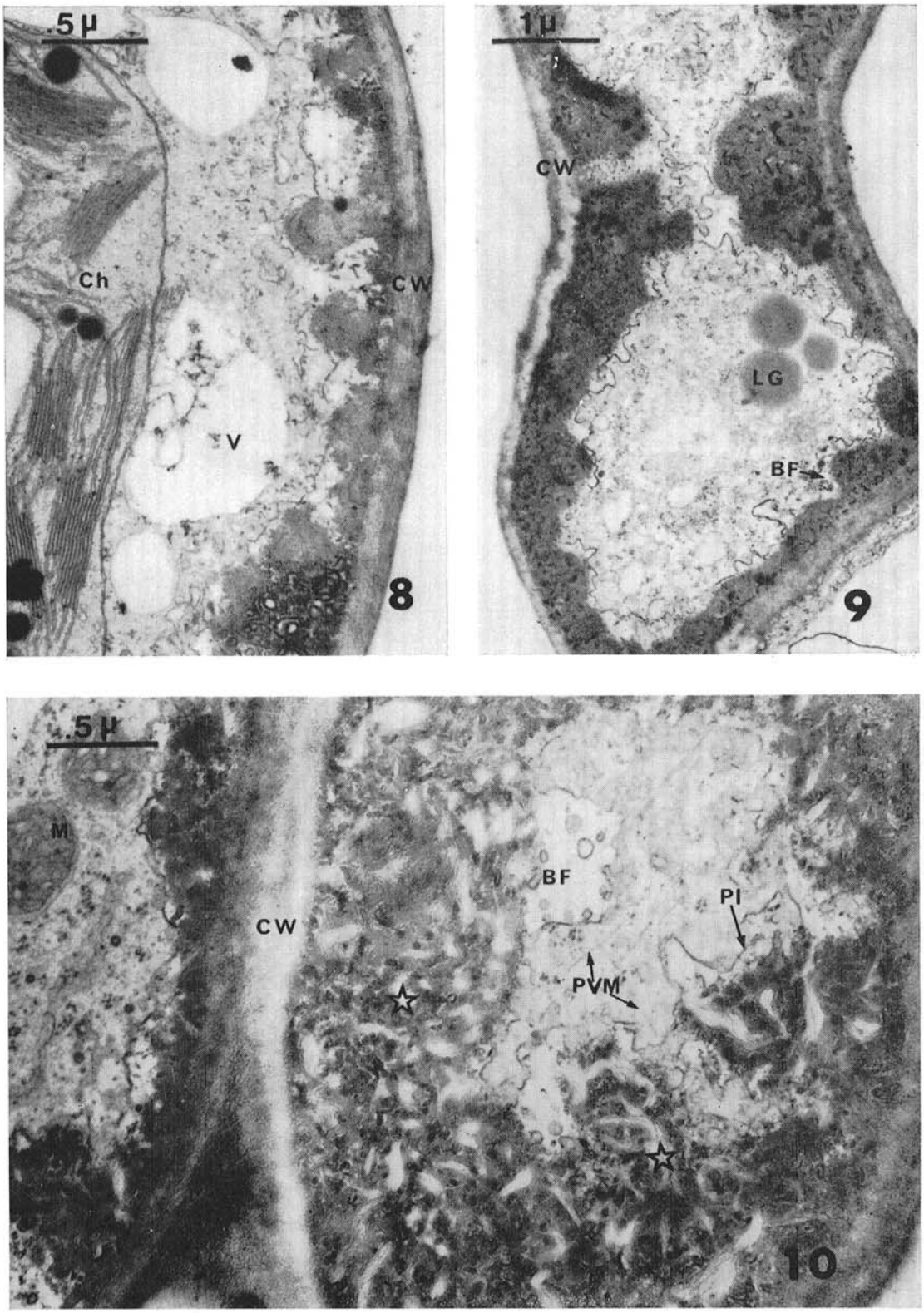

Fig. 8-10. Advanced stages of abnormal cell wall thickening showing details of the thickened cell wall (CW) in Red Kidney bean leaf cells infected with potato virus M. **8)** Mosaic type of secondary wall deposition. **9)** Nonmosaic type deposition. **10)** Mosaic type deposition. PVM particles (→) are clearly seen in cytoplasm. BF = boundary formation; Pl = plasmalemma; Ch = chloroplast; CW = cell wall; LG = lipid globule; M = mitochondrion; V = vacuole; * = the space trapped within the mosaic-patterned area.

The necrotic cells were electron-opaque. There was total blackening of cell contents, and virus was not observed (Fig. 5). Neither thickenings of wall nor virus particles were observed in comparable healthy tissues (Fig. 1).

Different developmental stages of the wall thickening were observed and described in sequence from the initial to the advanced stage. Cell wall thickening was more extensive in cells closer to the necrotic area than those farther away. Thus, material for study of developmental sequence was taken from cells farther from the necrotic area.

Boundary formation (2, 3), accompanied by roughening of the inner cell wall, was the first sign of abnormal secondary wall deposition in virus-infected cells in PVM local lesions (Fig. 2). Vesicles (Fig. 3, 4, 5, 6, 10) were more commonly encountered than tubules (Fig. 9) in the sections. Vesicles and tubules varied considerably in their size. The boundary formation originated from the cell wall and pushed the plasmalemma inward (Fig. 3). Occasionally, the boundary formation appeared to penetrate the thin layer of cytoplasm and protruded into the vacuole (Fig. 3). The structure, however, was still separated from cytoplasm by a continued plasmalemma (Fig. 3).

Two types of boundary formations were observed: (i) Vesicles and tubules were localized in certain portions of the cell rather than being uniformly distributed (Fig. 8). (ii) Vesicles and tubules were somewhat uniformly distributed around the cell wall (Fig. 6). The first abnormal deposition of cellular materials was observed below the boundary formation (Fig. 4) and/or around the vesicles and tubules (Fig. 9, 10).

As the type-(i) boundary formation extended inward, the secondary deposition also extended deeper into the cell, and the bases of the adjacent boundary formations widened (Fig. 5). These widened bases then coalesced with each other and eventually coated the entire inner cell wall (Fig. 8, 9). The type-(ii) boundary formation often resulted in formation of a rather uniform secondary deposit (Fig. 6, 7). Boundary formation not only extended inward but also laterally (Fig. 8, 9), and the secondary deposition developed as a mosaic or anastomosing pattern (Fig. 8, 9). The spaces trapped within the mosaic-patterned area of the secondary deposit initially appeared to lack materials (Fig. 10), but were later filled with fibrous material which was lightly stained when compared to the mosaic-patterned area.

At the advanced stage, the secondary wall deposition sometimes reached more than 5 times the thickness of the normal cell wall (Fig. 9, 10), and a relatively large portion of the cell was occupied by the deposit.

DISCUSSION.—Little is known about the ultrastructure of lesions incited by viruses other than TMV. Observation of PVM in the peripheral cells of PVM local lesion in Red Kidney bean confirmed the presence of virus particles in the peripheral zone of the local lesion. The presence of TMV particles in local lesions, recently confirmed by Milne (11) and Israel & Ross (8), was a controversial issue (4, 17, 18). The difference was later found to be due to the fixatives used (8). The fixative used herein has been proved to be successful in detecting PVM particles in the cell, and has also eliminated the possible failure in detecting boundary formation. Boundary formation was invisible when the plant materials were fixed with glutaraldehyde, $KMnO_4$ series (7).

The most significant finding, however, was the abnormal thickening of secondary cell walls in a few layers of living cells surrounding the necrotic area. This type of thickening at the ultrastructure level has not been previously reported in virus-induced local lesions, and is possibly significant in limiting the virus movement towards the neighboring cells and eventually halting expansion of the local lesion. Wu & Dimitman (20) postulated that the resistance of Pinto bean to TMV depended on the ability of noninfected cells, adjacent to the infected ones, to form a barrier against the entrance of virus, and correlated callose deposition with the resistance of host cells to virus spread. Further investigation is needed to relate this finding to the thickening of the cell wall.

Direct translocation of virus from the infection site to neighboring cells is mainly via plasmodesmata, and the long-distance translocation of virus takes place via the host-conducting system (12). The thickening in the inner portion of secondary wall probably is an indirect host reaction to the PVM infection. If the secondary wall thickening developed fast enough to block the plasmodesmata before the cell-to-cell translocation occurred, the virus would fail to enter the adjacent cells.

The wall thickening of mesophyll cells around the area penetrated by *Puccinia graminis* (stem rust) was found to be a major defense mechanism in immune dent corn (9). The wall thickening was a specific reaction of this corn cultivar to the rust. Thickening of cell walls was also observed in developing giant cells of *Vicia faba* and *Cucumis sativus* induced by root knot nematode (7). The process of the wall-thickening in PVM local lesions was similar to that of giant cell formation induced by root knot nematode. The only difference was that the boundary formation was not uniformly distributed around a secondary wall deposit (7) as it was in our observations. Irregular boundary formation may represent the early stage of the process. The difference in the uniformity of boundary formation is probably not an intrinsic one, but may merely represent a difference due to the location of the cell in relation to where the section was made.

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