BIOLOGIA PLANTARUM (PRAHA)

17 (3) : 214—218, 1975

Invaginations in Plant Cell Protoplasts Containing Mycoplasma-like Bodies (MLO)

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Abstract. MLO containing invaginations were found in protoplasts of phloem parenchyma cells in symptomless young leaves of *Ribes houghtonianum* JANCZ. infected with a yellows disease. The invaginations originate between the cell wall and plasmalemma, usually at plasmodesmata, and change apparently into superficial vesicles in the protoplast; they are entirely or partially limited by host plasmalemma. The formations mentioned occur in parenchyma cells which contain normal organelles. Sometimes they are divided by a smooth membrane system enclosing MLO. Besides MLO the invaginations contain in some cases slimy fibrils resembling the P-protein in sieve tubes. The MLO bodies seen in invaginations have usually a diameter of 50—250 nm and their plasmalemma (unit membrane) is identical with the plasmalemma of MLO bodies occurring in sieve tubes. However, only few MLO bodies in invaginations are electron dense, so that they resemble naturally degenerated forms of MLO. Similar MLO containing invaginations were formerly described from some leafhoppers transmitting MLO.

Additional index words: parenchyma cells; plasmalemma; *P*-protein; *Ribes houghtonianum* JANCZ.; yellows disease.

Mycoplasma-like organisms (MLO) considered to be causal agents of plant yellows diseases frequently occur in sieve tubes of diseased plants; several authors found them also in companion cells and in phloem parenchyma cells. The first paper dealing with phytopathogenous MLO by DOI *et al.* (1967) presented MLO in cytoplasm of phloem parenchyma cells. However, many other papers describing MLO in companion cells (GOURRET 1970, WORLEY 1970 and others) did not prove that the cells in question were undergoing cytoplasmic degeneration. A very good evidence of MLO present in plant host cytoplasm, along with apparently normal organelles was published by BOWYER and ATHERTON (1970) who concluded that at least at some stage of infection, the occurrence of MLO bodies in intact cytoplasm suggests a more highly specialized host-parasite relationship.

Some further results dealing with an association of host plasmalemma with MLO are important for our contribution. HIRUKI and DIJKSTRA (1973) published an electron micrograph of a sac-like formation at the cell wall limited by plasmalemma and containing MLO, degenerated material, and

Received September 2, 1974

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a lipid body. PROTSENKO and SURGUCHEVA (1974) observed similar vesicles even in intact cells of *Ribes nigrum* L. and suggested their function in MLO infection process. We want to compare our results especially with the paper by MAILLET (1970) discussing the cycle of MLO in a leafhopper vector. In the periphery of salivary glands of *Euscelis lineolatus* BRULLÉ MAILLET observed invaginations in secretory cells containing MLO. This phenomenon could be interpreted as a cellular infection by endocytosis, *i.e.* as a transition of the extracellular stage of MLO cycle in the leafhopper into the intracellular one. It indicated a mode of transition of MLO from haemolymph into cells of salivary glands. Then, individual MLO bodies could be observed envacuolized inside the endoplasmic reticulum.

Investigating a yellows disease of *Ribes houghtonianum* JANCZ. we have observed a lot of MLO in sieve tubes of fruit stems (RAKÚS *et al.* 1974). Because this disease caused symptoms in flowers and fruits only, we also investigated some other plant organs showing no symptoms. In this paper some peculiarities found in phloem tissue of young growing leaves are described.

Material and Methods

Twigs of diseased currant plants *Ribes houghtonianum* JANCZ. were supplied by Ing. D. Rakús (Plant breeding and research station Bojnice, Slovakia). Veins were cut out from small young symptomless leaves growing near bunches carrying yellows malformed berries and treated according to procedure described generally by SABATINI *et al.* (1963) and MILNE (1967) as follows:

Specimens were fixed for 2 h in 5% glutaraldehyde (in phosphate buffer) at 0—5 °C. The fixation was accelerated using vacuum 133 mbar. After washing the specimens were postfixed for 1 h in 2% osmiumtetroxide and transferred continuously into 70% acetone saturated with uranylacetate and kept in it at night. Then the specimens were transferred into water-free acetone and for 1 h into propylenoxide, and finally for 12 h into the nonactivated Durcupan ACM diluted with 5 vol. of propylenoxide. After evaporation of propylenoxide the medium was replaced by the activated mixture of Durcupan ACM and the specimens were embedded in blocks in silicone forms SF. Ultrathin sections made by an ultramicrotome Reichert OM-U2 were stained with 2% uranylacetate for 2 min and with lead citrate for 10 min (RAYNOLDS 1963). Electron micrographs were taken by means of an electron microscope JEM 100 B.

Results

Phloem parenchyma cells in which normal organelles were present and in which there was no evidence of a cytoplasmic degeneration (usually it was difficult to determine the type of cells involved) showed invaginations of protoplasts starting at the cell wall and protruded into protoplasts either funnel-like (Figs. 1, 2, 3, 4) or, more often, bulb-like (Figs. 5, 6). Many invaginations looked like encysted formations (Figs. 7, 8, 9). Some electron micrographs indicate the probable origin of invaginations by pressing plasmalemma into protoplast (Figs. 3, 10). The presumptive development of these formations begins with a funnel-like invagination (Fig. 2) which alters in an open bulb-like form (Fig. 6) and finally in a vesicle fully enclosed by the protoplast (Figs. 7, 8, 9). One cell may contain several invaginations which also occur in cells partially vacuolized. Inside, the invagination may be

Abbreviations used: Cp — chloroplast, CW — cell wall, ER — endoplasmic reticulum, G — Golgi body, INV — invagination, M — mitochondrion, N — nucleus, Pd — plasmodesma, PP — P-protein, SP — sieve plate, ST — sieve tube, UM — unit membrane, V — vacuole.

divided by large smooth membrane systems separating a portion of the content of an invagination (Figs. 7, 9).

The invagination may contain sparse fibrous mass (Figs. 5, 6, 7, 9) which is very like the proteinaceous component — usually called slime fibrils and occurring in sieve tubes (Fig. 14) — which CRONSHAW and ESAU (1968) proposed to name P-protein.

All invaginations contain spherical or oval bodies limited by a three-layer unit membrane (Figs. 8, 11) identical with the unit membrane of MLO occurring in sieve tubes (Fig. 15). The shape and size of these bodies correspond to small MLO bodies in sieve tubes (cf. Figs. 14, 15). Some electron micrographs (Figs. 4, 6, 10, 11, 12) also show the similarity of the internal contents.

The MLO accumulation in invaginations need not be limited by the host cell plasmalemma entirely (Figs. 11, 12). The MLO bodies are usually small (50—250 nm in diam.). In the host plant cells containing invaginations normal organelles are present, *e.g.* nucleus, mitochondria, endoplasmic reticulum, ribosomes, Golgi body, chloroplasts (Figs. 1, 2, 4, 5, 6, 7, 10). The invagination usually lies at the cell wall from which it is not separated by a membrane (Figs. 1 to 6, 10 to 13). In such cases the invaginations were observed near plasmodesmata (Figs. 6, 8, 13) and sometimes opposite in two adjacent cells (Fig. 13).

Discussion

Invaginations in protoplasts of plant host cells contained MLO bodies of only 50—250 nm in diam. and limited by a unit membrane. The internal structure of these MLO bodies sometimes resembled the content of MLO occurring in sieve tubes, but usually they have merely sparse cytoplasmic contents. Such bodies were similar to vesicles going out of a large MLO body, as described by HIRUMI and MARAMOROSCH (1972), who suggested that these "vesicles" lacking internal contents were possibly nonviable debris. MLO bodies having approximately the same size and internal structure also occurred in sieve tubes together with elementary bodies and large bodies possessing normal internal structure.

Several authors (*e.g.* GOURRET and MAILLET 1969) proved the passage of MLO through sieve plate pores, but a reliable information on the mode of infection and on infection process of parenchyma cells is lacking. LOMBARDO *et al.* (1970) observed in young parenchyma and companion cells MLO whose diameter ranged between 50 and 400 nm, *i.e.* smaller bodies than in mature sieve elements; the observed cytoplasmic vacuolization of the invaded host cells also corresponds with our results.

LOMBARDO *et al.* (1970) found in one of the youngest leaves a limited portion of a vein in which MLO were visible, in a young parenchyma cell of the phloem, between plasmalemma and cell wall and in peripheral cytoplasmic vacuoles. The plasmodesmata connecting infected cells very often contained masses of electron dense material. They suggested that MLO could be extruded by the cell both by lysis of the plasmalemma, as is the case in degenerating cells, where MLO and cytoplasmic material are often seen together outside the plasmalemma (a similar hypothesis contains the paper by PROTSENKO and SURGUCHEVA 1974), and, perhaps, by reverse pinocytosis, as

suggested by the presence of MLO in peripheral cytoplasmic vacuoles. The passage of MLO through the plasmodesmata could be inferred from the electron opaque content of the latter, suggesting the presence of foreign material inside them. This hypothesis of MLO transmigration through plasmodesmata suits very well the interpretation of our findings.

Invaginations in plant cell protoplasts, changing apparently in vesicles containing MLO, strongly suggest a very similar effect of MLO in leafhopper tissues described by MAILLET (1970) and interpreted as endocytosis. A similar effect of MLO on the leafhopper *Macrosteles fascifrons* (STÅL) carrying MLO (clover phyllody agent) was observed by SINHA and PALIWAL (1970). They sometimes found the "vesicles" in the basement membrane of the mid intestinal wall; these vesicles which were filled with MLO cells may represent a pathological effect produced by MLO in this tissue.

Since we did not find in invaginations any MLO whose shape would suggest MLO reproduction (*e.g.* filamentous bodies, budding, binnary fission, chains of dense coccoid bodies *etc.*), we can present a comparison of our results with the published facts merely as a hypothesis.

References

BOWYER, J. W., ATHERTON, J. G.: Observations on the relationship between *Mycoplasma*-like bodies and host cells of legume little leaf-diseased plants. — Aust. J. biol. Sci. **23** : 115—125, 1970.

CRONSHAW, J., ESAU, K.: *P* protein in the phloem of *Cucurbita*. I. The development of *P*-protein bodies. — J. Cell Biol. **38** : 25—39, 1968.

DOI, Y., TERANAKA, M., YORA, K., ASUYAMA, H.: Mycoplasma- or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. — Ann. phytopathol. Soc. Japan **33** : 259 to 266, 1967.

GOURRET, J. P.: Ultrastructure et micro-écologie des mycoplasmes de phloème dans trois maladies des pétales verts. — J. Microsc. **9** : 807—822, 1970.

GOURRET, J. P., MAILLET, P. L.: Ultrastructure des mycoplasmes dans le phloème du cotonnier atteint de virescence. — Coton Fibres trop. **24** : 325—326, 1969.

HIRUKI, C., DIJKSTRA, J.: An anomalous form of mycoplasma-like bodies in periwinkle infected with sandal spike agent. — Neth. J. Plant Pathol. **79** : 112—121, 1973.

HIRUMI, H., MARAMOROSCH, K.: Natural degeneration of mycoplasmalike bodies in an aster yellows infected host plant. — Phytopathol. Z. **75** : 9—26, 1972.

LOMBARDO, G., BASSI, M., GEROLA, F. M.: Mycoplasma development and cell alterations in white clover affected by clover dwarf. An electron microscopy study. — Protoplasma **70** : 61—71, 1970.

MAILLET, P. L.: Contribution à l'étude du cycle des mycoplasmes, à propos des "poches à mycoplasmes" dans les glandes salivaires d'*Euscelis lineolatus* BRULLÉ, vecteur de la phyllodie du trèfle. — Comp. rend. Acad. Sci. (Paris) Sér. D **270** : 731—733, 1970.

MILNE, R.: Electron microscopy of leaves infected with sowbane mosaic virus and other small polyhedral viruses. — Virology **32** : 589—600, 1967.

PROTSENKO, A. E., SURGUCHEVA, N. A.: [Mycoplasma origin of black currant reversion disease.] In Russ. — Sel'skokhoz. Biol. **9** : 80—83, 1974.

RAKÚS, D., KRÁLÍK, O., BRČÁK, J.: Mykoplazma v *Ribes houghtonianum* JANCZ. nakažené „plnokvětosti". [Mycoplasma-like organism in *Ribes houghtonianum* JANCZ. infected with a yellows disease.] — Ochrana Rostlin (Praha) **10** : 307—309 1974.

RAYNOLDS, E. S.: Use of lead citrate at high pH as an electron-opaque stain in electron-microscopy. — J. Cell Biol. **17** : 209, 1963.

SABATINI, D. D., BENSCH, K., BARRNETT, R. J.: Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. — J. Cell Biol. **17** : 19—58, 1963.

SINHA, R. C., PALIWAL, Y. C.: Localization of a mycoplasma-like organism in tissues of a lea hopper vector carrying clover phyllody agent. — Virology **40** : 665—672, 1970.
WORLEY, J. F.: Possible replicative forms of mycoplasmalike organism and their location in aster yellows diseased *Nicotiana* and aster. — Phytopathology **60** : 284—292, 1970.

Figures at the end of the issue.

O. KRÁLÍK, J. BRČÁK (Praha): **Invaginace protoplastu rostlinných buněk obsahující tělíska připomínající mykoplazmu (MLO).** — Biol. Plant. **17** : 214—218, 1975.

V lýkové části cévního svazku bezpříznakých mladých listů rybízu *Ribes houghtonianum* JANCZ., nakaženého žloutenkovou chorobou „plnokvětostí", byly v parenchymových buňkách zjištěny vchlípeniny protoplastu obsahující MLO. Tyto invaginace vznikají mezi buněčnou stěnou a plasmalemou obvykle u plasmodesmů. Z invaginací zřejmě vznikají vesikuly v povrchu protoplastu obsahující MLO; jsou úplně nebo jen částečně uzavřeny plasmalemou hostitele. Tyto invaginace a vesikuly se vyskytují v parenchymových buňkách obsahujících normální organely a někdy jsou rozčleněny systémem hladkých membrán, v němž bývá též uzavřen jejich obsah MLO. Uvedené útvary někdy obsahují kromě MLO též fibrilární hmotu, která připomíná P-protein v sítkovicích. Tělíska MLO v invaginacích mají nejčastěji průměr 50—250 nm, jejich plasmalema je stejná jako u tělísek MLO v sítkovicích; pouze některá tělíska mají hustší obsah, takže připomínají přirozeně degenerované formy MLO. Podobné invaginace s MLO jsou známy z křísů přenášejících MLO.

BOOK REVIEW

SHUL'GIN, I. A.: **Rastenie i Solntse** [Die Pflanze und die Sonne]. — Gidrometeoizdat, Leningrad 1973. 251 S. Rbl. 1,92.

Die wichtigste Energiequelle in unserer Welt — die Sonnenstrahlung — und deren Einfluss auf die Pflanzen, auf die Photosynthese, das Wachstum und die Entwicklung sind das Thema dieses Buches, welches eine erweiterte und umgearbeitete Ausgabe eines 1967 erschienenen Buches desselben Verfassers ist. Das erste Kapitel behandelt die physikalischen Grundlagen der Sonnenstrahlung wie Spektralverteilung, Energiegehalt, Absorption, Reflexion, Transmission, Verteilung der Sonnenstrahlung in verschiedenen Pflanzengesellschaften, optische Eigenschaften des Blattes usw. Im 2. Kapitel verfolgt der Verf. den Einfluss der Sonnenstrahlung event. Licht verschiedener spektralen Zusammensetzung auf die Pflanze: auf das Längenwachstum der Pflanzenorgane, die anatomische Struktur des Blattes, den Pigmentgehalt und in Kapitel 3 den Einfluss auf Photosynthese und Produktivität der Pflanzen. Ein besonderer Abschnitt behandelt das Wachstum und die Entwicklung der Pflanzen bei künstlicher Beleuchtung, ein weiterer die photoperiodischen Reaktionen der Pflanzen. Aspekten der evolutionären Adaptation der Pflanzen zur Sonnenstrahlung ist das letzte 4. Kapitel gewidmet. Das Buch schliesst mit einer russischen und englischen Zusammenfassung und einem ausführlichen Literaturverzeichnis (515 russische und 286 anderssprachige Zitationen, in den fremdsprachigen Zitationen leider Druckfehler). Zu bedauern ist, dass weder Autoren- noch Sachregister ausgearbeitet wurden. Verf. trug in diesem Buch eine grosse Menge faktischen Materials (801 Zitationen, 71 Abbildungen und 85 Tabellen) zusammen und verarbeitete es zu einem Text, der den Spezialisten auf diesem Gebiet bezeugt. Das Buch ist Interessenten auf dem Gebiet der landwirtschaftlichen Meteorologie, Biogeographie, Botanik (Physiologie und Ökologie), Landwirtschaft, Geobotanik sowie Studenten bestimmt.

INGRID TICHÁ (*Praha*)

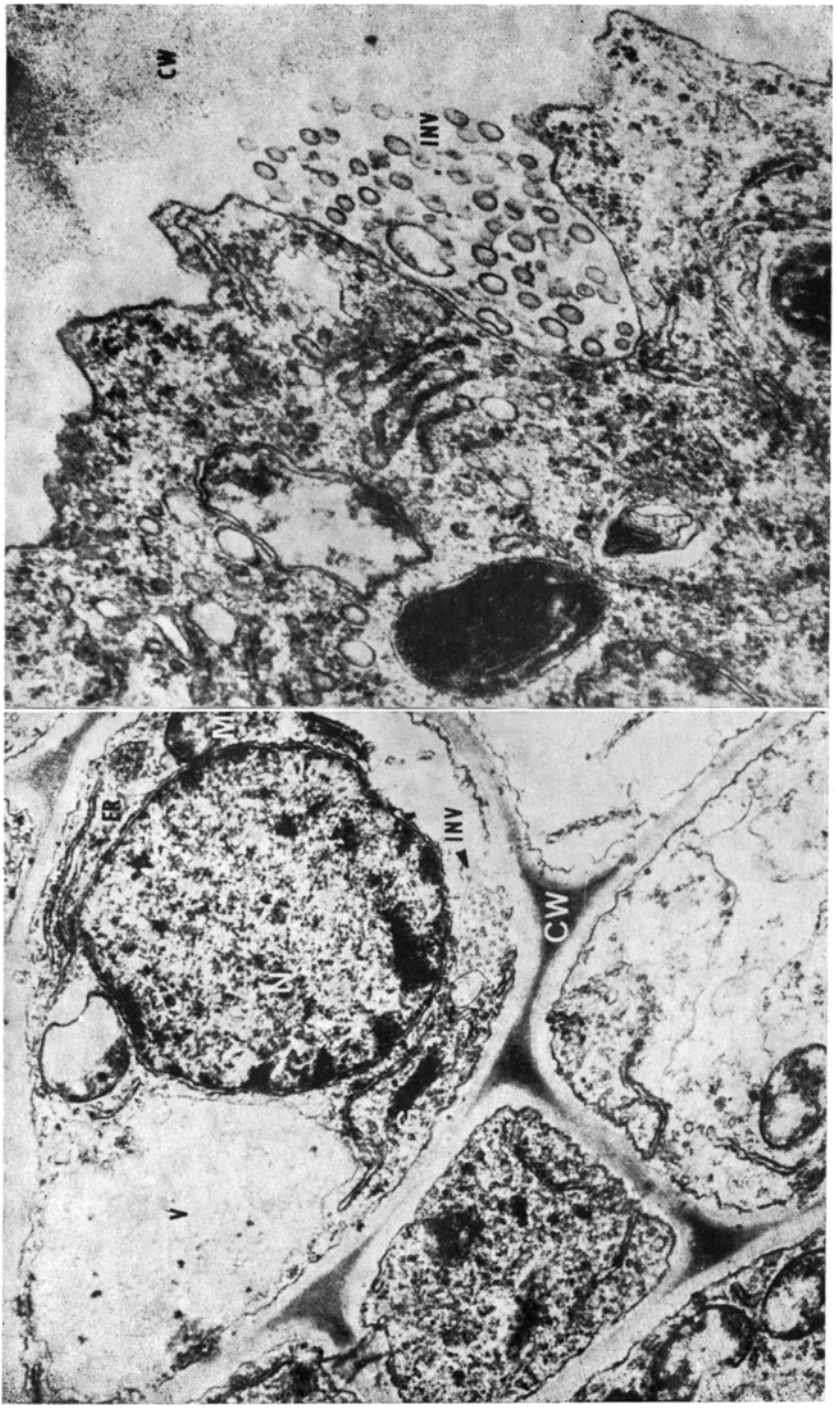

Fig. 1. A funnel-like invagination protruding into the protoplast near nucleus and Golgi body. (14 500×). **Fig. 2.** An invagination at the cell wall containing small MLO and limited by host cell plasmalemma (30 000×).

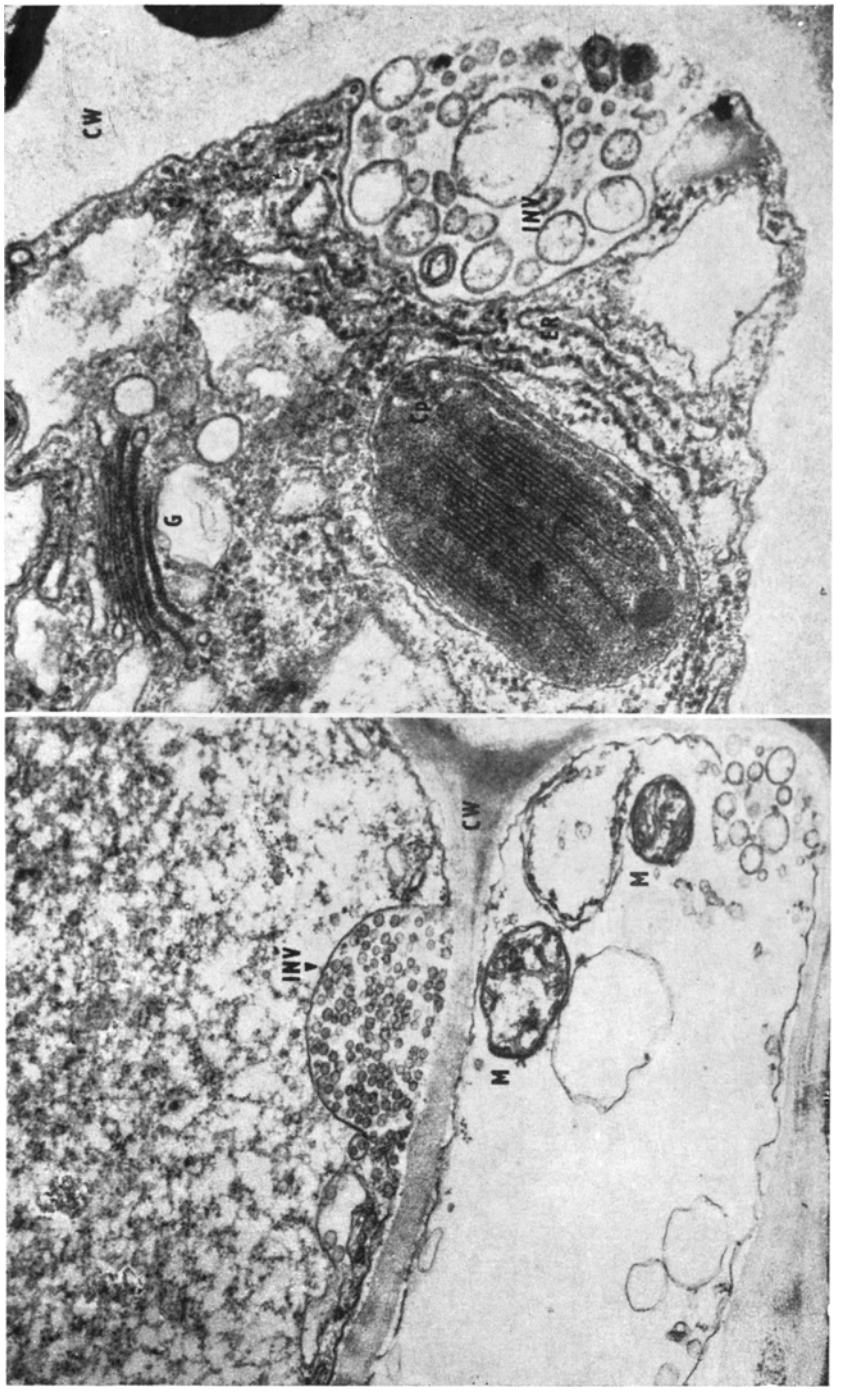

Fig. 3. MLO containing invagination limited by host plasmalemma in a cell adjoining an almost degenerated cell. (18 000×).
Fig. 4. A small invagination formed at the cell wall in a parenchyma cell (35 000×).

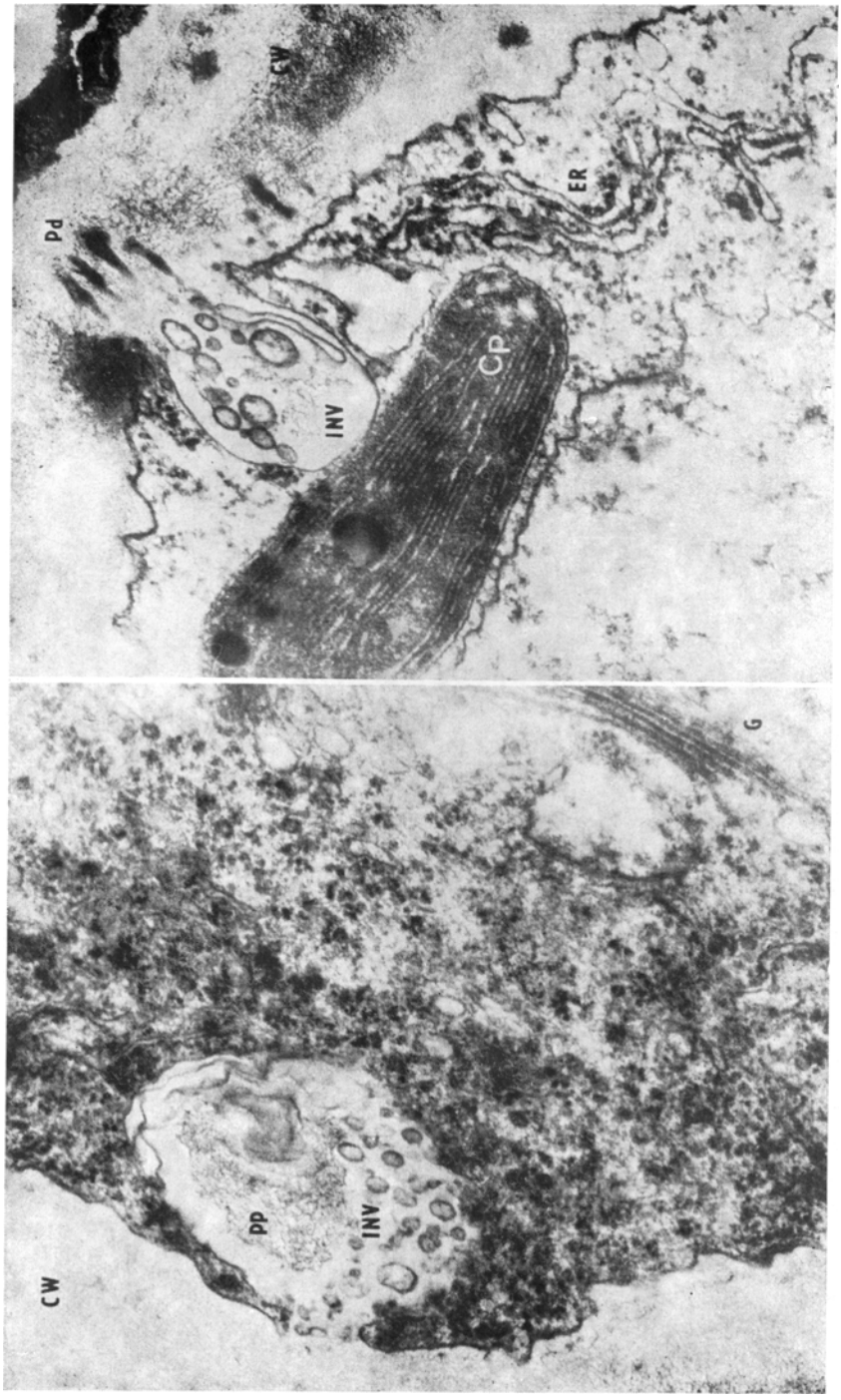

Fig. 5. An almost enclosed bulb-like invagination containing small MLO, membrane systems, and P-protein-like mass (35 000×).
Fig. 6. A bulb-like invagination situated near plasmodesmata and containing MLO and P-protein-like substance (35 000×).

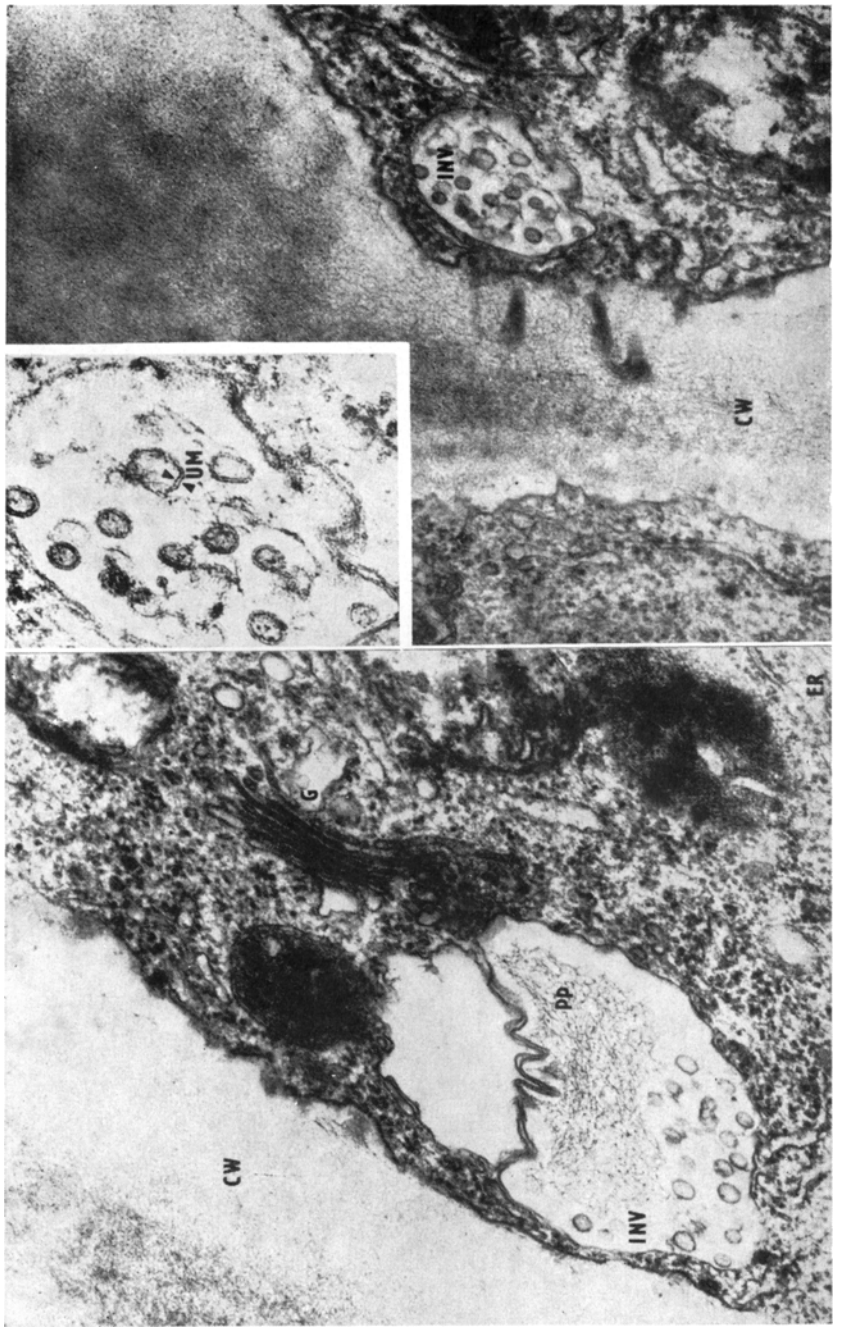

Fig. 7. A vesicle-like invagination divided by membranes; one part of it contains slimy fibrils and small MLO (35 000×). Fig. 8. Unit membrane of small MLO in a vesicle-like invagination (35 000× and 77 000×).

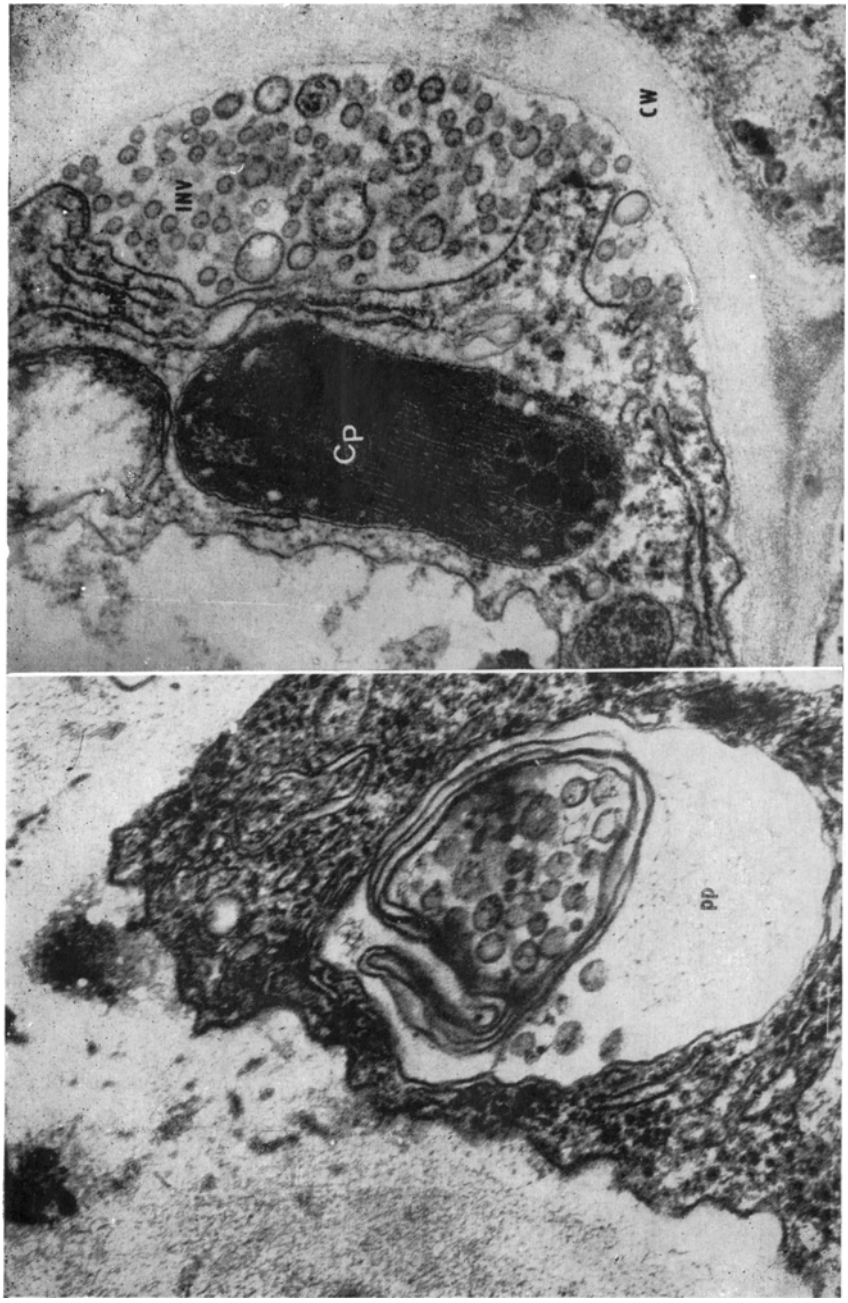

Fig. 9. A smooth membrane system enclosing most MLO inside an invagination; slimy fibrils in the outer part. (35 000×).
Fig. 10. An invagination lying between the cell wall and host plasmalemma and containing MLO of various size and inner structure (30 000×).

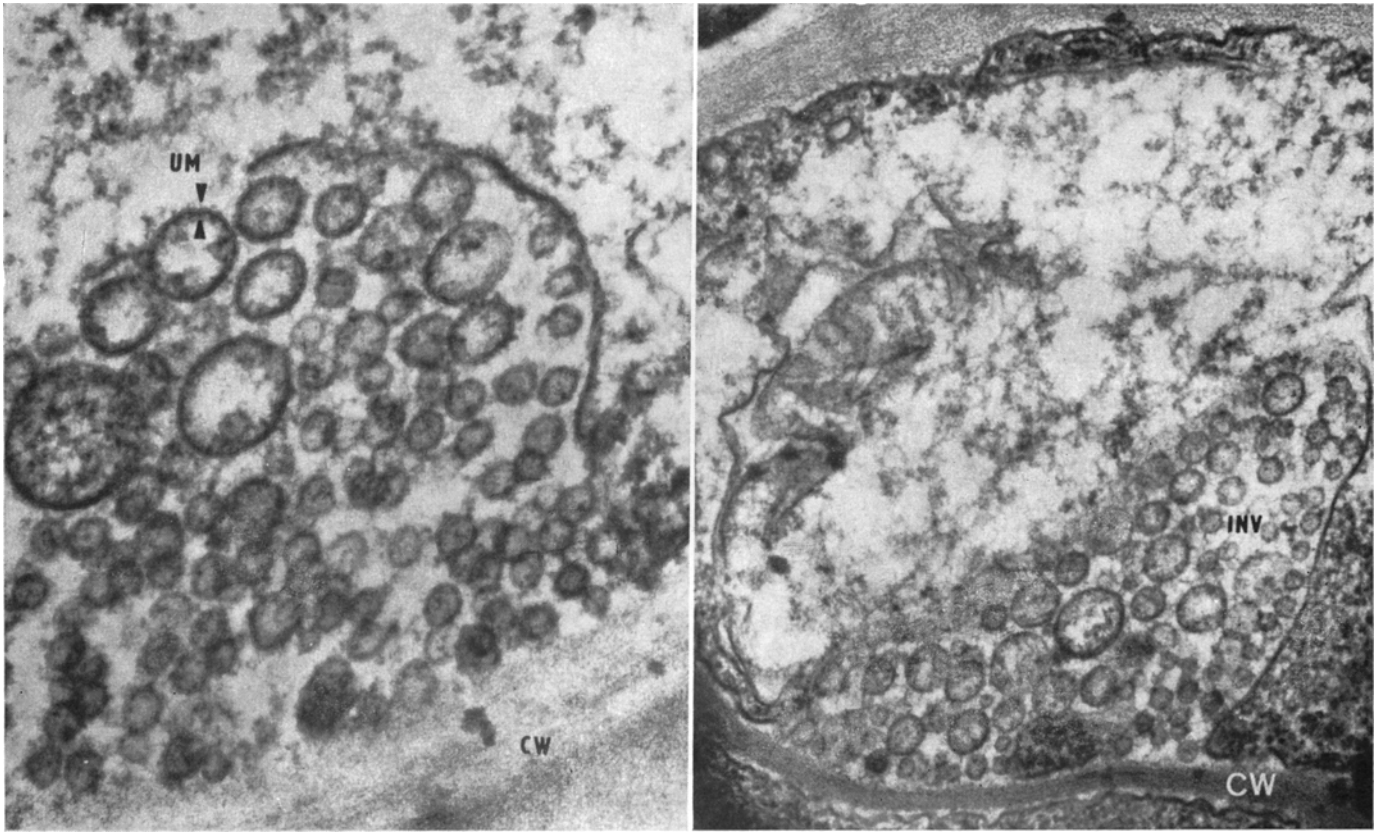

Fig. 11. MLO in an invagination which is only partially limited by host cell plasmalemma (73 500×). Fig. 12. A partially "open" invagination containing MLO of various size (35 000×).

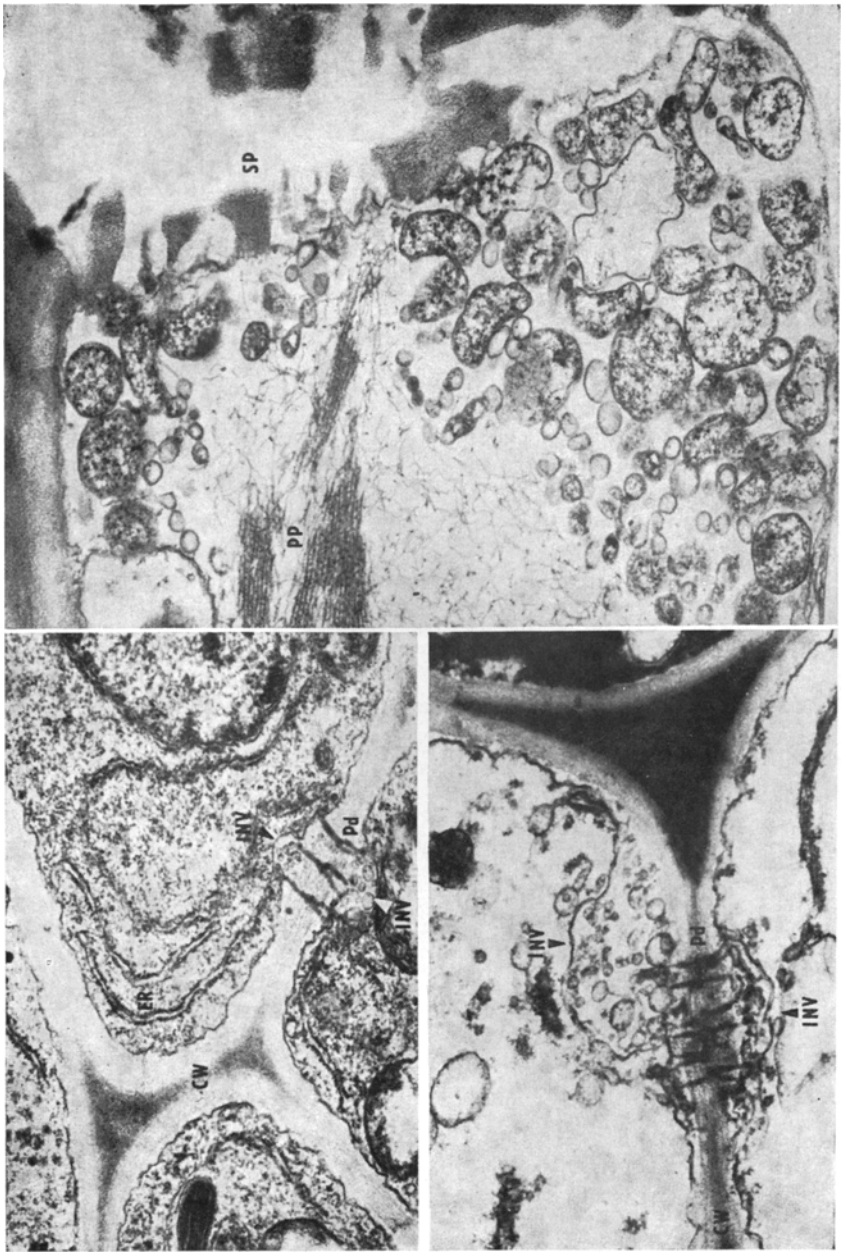

Fig. 13. Invaginations situated opposite in two adjacent cells and near plasmodesmata (19 000× and 22 000×). Fig. 14. MLO in a sieve tube (at the sieve plate) which contain slimy fibrils (P-protein) (23 500×).

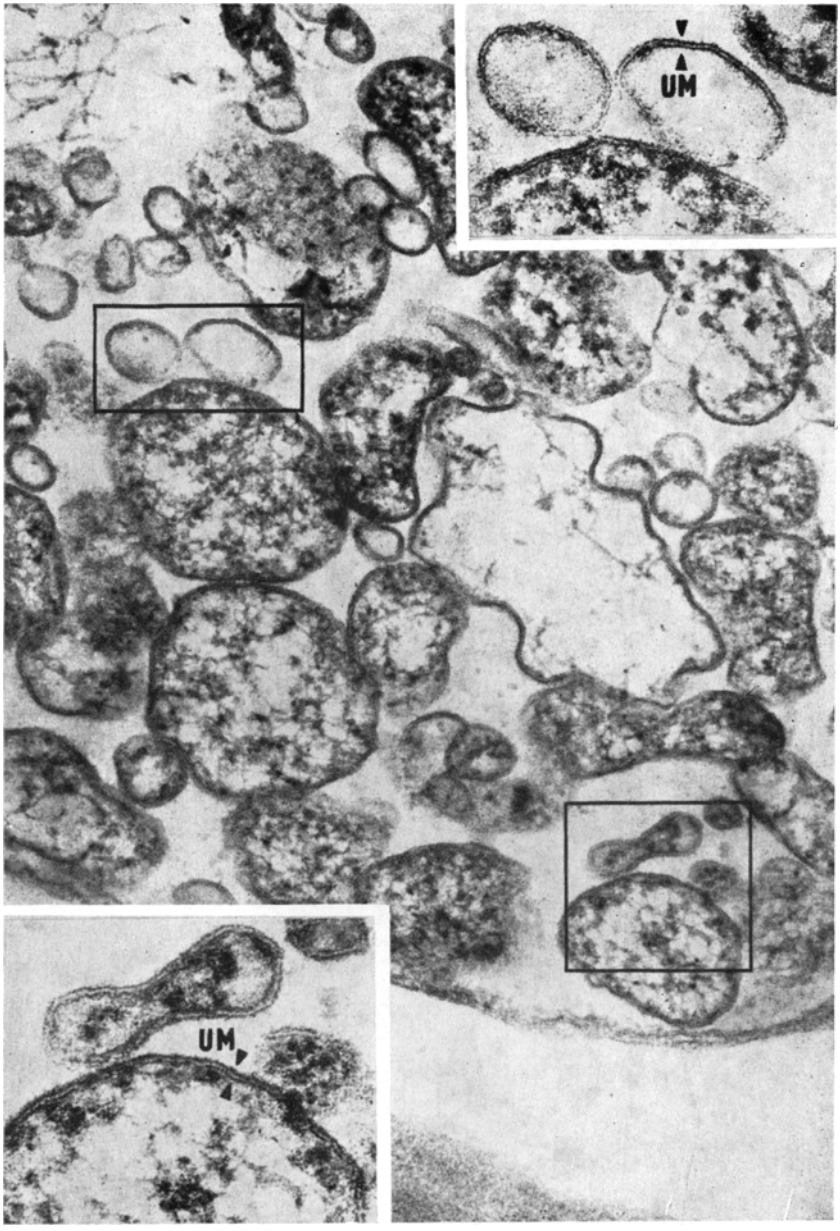

Fig. 15. Details of Fig. 14; note the unit membrane of MLO (52 500×, 89 000×, 110 000×).