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Ultrastructure of Plant Cells Doubly Infected with Potyviruses and Other Unrelated Viruses

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Abstract

Ultrastructure of plant cells doubly infected with a potyvirus and the other unrelated viruses was investigated. Five potyviruses and five other viruses were used to make ten combinations of double infections on systemic host plants, and infected leaves were examined by electron microscope. In double infection, the two viruses in every combination were detected in the same cells and were observed at the same sites as those in single infection. Virus-specific inclusions such as virus aggregates or cytoplasmic inclusion bodies were consistently observed in doubly infected cells. Particles of cucumber mosaic virus and two tobamoviruses were closely associated with potyvirus-induced inclusion bodies of the tube and scroll types. No significant differences were found in cytopathological effects and structure of inclusion bodies between single and double infections.

Introduction

Mixed infections of plant viruses have considerable effects on virus ecology and disease epidemiology but have been relatively little studied[10]. Most of the available information are concerned with the investigation on physiological changes by host-virus interactions. Few reports have been indicated different cellular modifications after mixed infection[1,2,5]. Recently, electron microscopic observation of doubly infected cucumber leaf cells have revealed the characteristic association of zucchini yellow mosaic virus inclusion bodies with particles of cucumber mosaic virus[12]. The phenomena suggested some modified interactions of two viruses and the host cells.

The present study describes cytopathological effects occurring in plant cells doubly infected with two unrelated viruses in order to characterize their interactions. Part of this work has been reported eleswhere[11].

Materials and Methods

Viruses and plants : Strains and isolates of five potyviruses and five other viruses used are listed in Table 1. The potyviruses were selected from the two major subgroups according to their types of inclusion bodies[3,9]. WMV-2, ZYMV and PVY induce tube and scroll type of pinwheel, while BYMV and TuMV induce inclusion bodies containing laminated aggregates. Ten virus-host combinations employed are shown in Table 2.

Inoculation : All plants were mechanically inoculated. For single infection, using as a control, plants were inoculated with single virus inoculum prepared from crude sap of infected leaves. For double infection, each half leaf of test plants was inoculated with different virus inocula. Inoculated plants were kept in a greenhouse of 20-30 C for symptom development.

Table 1. Viruses used.

Group	Virus	Strain or Isolate[a)]
Potyvirus	Watermelon mosaic virus-2 (WMV-2)	W-T
	Zucchini yellow mosaic (ZYMV)	Z-T
	Potato virus Y (PVY)	PL-Y
	Bean yellow mosaic (BYMV)	P-242
	Turnip mosaic (TuMV)	D-80-T
Tobamovirus	Tobacco mosaic (TMV)	OM
	Cucumber green mottle mosaic (CGMMV)	CV3-I
Cucumovirus	Cucumber mosaic (CMV)	Pepo, 71-P-16
Nepovirus	Tobacco ringspot (TRSV) b)	
Non-grouped	Broad bean wilt (BBWV)	P-82-T

a) Maintained in the laboratory as refer to Terami (1985)[13)]
b) Supplied by Dr. F. Fukumoto, National Agriculture Research Center, Tsukuba Science City, Ibaraki, Japan.

Table 2. Virus-host combinations investigated.

Combination no.	Viruses	Host plant	Growth stage of plant inoculated[a)]
1	BYMV + CMV (71-P-16)	Broad bean	10
2	BYMV + BBWV	do.	10
3	TuMV + CMV (Pepo)	Turnip	25
4	TuMV + BBWV	do.	25
5	WMV-2 + CMV (Pepo)	Cucumber	10
6	WMV-2 + TRSV	do.	10
7	ZYMV + CMV (Pepo)	do.	10
8	ZYMV + CGMMV	do.	10
9	PVY + CMV (Pepo)	Tobacco, cv. Xanthi	35
10	PVY + TMV	do.	35

a) Days after sowing.

Electron microscopy : Samples were collected from newly developed leaves showing obvious symptoms. Infected leaf tissues were cut into pieces, fixed in 5 % glutaraldehyde and then in 1 % osmium tetroxide. They were dehydrated in a graded ethanol series and embedded in Spurr's resin. Ultrathin sections were cut by glass knives on LKB 8000 microtome, and stained with 2% uranyl acetate and 1% lead citrate. A Hitachi H-300 electron microscope was used for investigation.

Results

Single infections

In CMV infected cucumber, chloroplasts were severely affected and deformed in shape with abnormal thylakoid system (Fig.1). Crystalline aggregates of CMV particle

were often observed in the vacuole. CGMMV-infected cells showed accumulation of the modified vesiculated mitochondria (Fig.2). In broad bean plant cells infected with BBWV, crystalline aggregates of virus particle in tubular form were detected together with the membrane inclusion. However, no such crystal of BBWV particles was detected in turnip plant infected with the same virus, and the structure of virus-induced membrane inclusion was different from the structure found in infected broad bean. Potyvirus-induced cytoplasmic inclusion bodies were readily observed in their respective forms (Figs 3, 4). Particles of potyviruses were usually seen singly or in mass in the cytoplasm. Chloroplast is the organelle found to be severely affected by the infection of TMV in tobacco (Fig. 5), BYMV in broad bean, and TuMV in turnip plants (Fig. 6A).

Mixed infections

ZYMV and CMV; WMV-2 and CMV : These two combinations revealed similar appearance of infected cells. CMV and ZYMV or CMV and WMV-2 occurred interspersing each other in the cytoplasm of the same cells as a single particle or in masses (Fig.7). CMV was abundantly seen in the cytoplasm of doubly infected leaf cells and was much more than in single infected ones. Deformed chloroplasts appeared to have electron dense materials embedded in the stroma, together with deep protrusion of cytoplasm and sometimes with virus aggregate (Fig.8). Moreover, CMV particles were also associated closely with WMV-2-induced pinwheel inclusion either inside the tube and scrolls (Fig. 9A) or between the pinwheel arms (Fig.9B). CMV was easily distinguished from WMV-2 particles by its larger size and distinct internal core area of the virion (Figs.9B, 9C).

PVY and CMV : The general appearance of mixedly infected cells were similar to those of ZYMV or WMV-2 and CMV. PVY and CMV were readily found in the same cell but no aggregate of CMV particles was observed in the cytoplasm. CMV particles were closely associated with PVY-induced inclusions (Fig.10). Cytopathological effects on doubly infected cells resembled those of CMV single infection.

WMV-2 and TRSV : WMV-2 particles and its pinwheel inclusions were less seen in doubly infected plant compared with the single infection. Particles of TRSV were hardly recognized if they existed singly in the cytoplasm. Some of TRSV masses was observed in the vacuole of epidermis and phloem cells while WMV-2 particles and inclusions were localized in the cytoplasm. There appeared to have necrotic cells or zones in corresponding with the necrotic symptoms of doubly infected leaves, but no virus particles could be detected in those cells. The association of TRSV particles with WMV-2 inclusion bodies was not observed.

ZYMV and CGMMV : Particles of these two viruses could be detected in the same cells due to the presence of their inclusions (Fig.11). However, it was difficult to identify the particles which scattered singly in the cytoplasm or vacuole. It was more likely that many rod-shaped particles which embedded in tubular inclusions of ZYMV were CGMMV particles rather than ZYMV particles themselves because of the greater internal core similar to the particles in crystalline array of CGMMV (Fig.12). In addition, transverse section of pinwheels strongly indicated that CGMMV particles were closely associated with ZYMV inclusions (small block in Fig.12). Doubly infected cells had vesiculated mitochondria and abnormal chloroplasts which contained large starch granules and lipid globules but less thylakoid system. Cylindrical inclusions induced by ZYMV were ob-

served in the form of tube and scroll much more than in pinwheel form. The latter was always found in vacuole and very less in number than those in ZYMV infected cells.

PVY and TMV : Cellular modifications of doubly infected cells were similar to those of TMV single infection except for the presence of PVY particles and inclusion bodies (Fig.13). Cytoplasmic matrices were densely filled with particles of the two viruses, their inclusion bodies as well as degraded cell organelles. Again, TMV particles were often associated closely with the pinwheel inclusions (Fig.14A). PVY cylindrical inclusions were observed as frequently as they were in single infection. Doubly infected cells showed severely affected nucleus and chloroplast (Fig.14B) like those in TMV single infection.

BYMV and CMV : The two viruses were detected in the same cells, together with BYMV-induced laminated inclusions (Fig.15). CMV particles were observed in the cytoplasm singly or in crystalline array not associating with BYMV inclusion bodies (Fig.16).

BYMV and BBWV : BBWV in doubly infected cells appeared in tubular form of crystalline array as found in single infection and were observed in the same cells with BYMV particles (Fig.17). Membrane inclusion of BBWV were often found in the cytoplasm together with dense body inclusion of BYMV (Fig.18). Severely affected chloroplasts became round and lost the thylakoid system.

TuMV and CMV; TuMV and BBWV : Cytopathology of infected cells of these two combinations were similar to those of TuMV single infection. The chloroplast elongated in shape and contained degenerated grana (See Fig.6A). CMV particles appeared in aggregated masses or in crystal together with TuMV inclusions (Fig.19). BBWV particles were observed as aggregated mass in the vacuole or singly in the cytoplasm. Vesiculate membrane inclusions of BBWV were detected together with TuMV-induced tubular inclusions (Fig.20). The association of CMV or BBWV particles with TuMV inclusion was not observed.

Discussion

It is apparent that the paired viruses used in this study can multiply together in the same cells as recognizable either by virus particles or by the inclusion bodies. These particles appeared in the forms and sites as they were in singly infected cells with some exception. In the cases of ZYMV or WMV-2 mixedly infected with CMV in cucumber plant, doubly infected cells contained very much CMV particles and some of them arranged in unusual form never found in CMV single infection. CMV and ZYMV particles sometimes co-aggregated but did not show any specific arrangement. Neither did the paired viruses in other combinations. The modified arrangement of CMV particles has been reported in CMV-infected tobacco which was challenged by TMV[5] but no association of CMV and TMV virions was noticed. There was only a report by Carr and Kim (1983a)[1] demonstrating a specific arrangement of two unrelated viruses in the same cell.

An association of other kinds of virus particles with potyvirus-induced inclusion bodies was the striking feature demonstrated firstly by the present work. Particles of

CMV, CGMMV and TMV were associated closely with tube and scroll inclusions induced by different potyviruses. There had been some reports illustrating ultrastructure of plant cells doubly infected with potyvirus and another virus in various groups[4, 6, 7, 8, 14]. The electron micrographs of those literatures revealed two kinds of virus occurred in the same cells, but not showing any relationship between potyvirus inclusions and the different viruses.

Cytopathology of cells doubly infected with potyvirus and the other viruses generally resembled their respective single infections. However, the modification of cells usually has a prominent characteristic of infection by one of the two viruses. For instance, mixed infections of CMV and ZYMV, CMV and WMV-2, TMV and PVY, or TuMV and CMV revealed severely deformed chloroplasts similar to those in single infection of CMV, TMV and TuMV, respectively. This observation corresponds very well with the external symptoms typical of CMV, TMV and TuMV, respectively. Through the present investigation, no differences in cellular modifications were found between doubly and singly infected cells. As a conclusion, the virus specific inclusions are proved here to be distinctive and usable to the diagnosis for a certain virus disease even in mixed infection.

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Explanation of Plates

Plate I Bars represent 500 nm

Fig. 1 A mesophyll cell of cucumber leaf infected with CMV showing deformation of chloroplasts. The electron-dense materials (Dm) embedded in grana and stroma, with large starch grain deposition.

Fig. 2 Vesiculated mitochondria (M) typical of CGMMV (arrows)-infected cucumber cells accumulated near the cell wall (Cw).

Fig. 3 Part of a mesophyll cell of broad bean infected with BYMV. The cytoplasm filled with laminated inclusions (LI) and dense body (DB). Arrows = BYMV particles, Ch = Chloroplast, M = Mitochondria.

Fig. 4 PVY-induced pinwheel inclusion (PI) in the cytoplasm of PVY-infected tobacco leaf cell.

Fig. 5 A mesophyll cell of TMV-infected tobacco leaf containing abnormal chloroplasts with numerous vesicles (Vc) and degraded thylakoid system (Th). Protrusion of cytoplasm into the chloroplast often coupled with appearance of isometric lipid-like granules (arrows).

Fig. 6 Deformation of the chloroplast (Ch) (6A), and laminated inclusions (LI) (6B) in TuMV infected turnip plant cell. V = TuMV particles.

Plate II Bars represent 250 nm

Fig. 7 CMV particles forming round aggregates (arrows), or scattering with ZYMV particles (Z) in the cytoplasm of a mesophyll cell of infected cucumber. Vu = Vacuole.

Fig. 8 A deformed chloroplast together with a CMV + ZYMV aggregates (V) observed in doubly infected cucumber cell. Th = Thylakoid, L = Lipid globule.

Fig. 9 Association of CMV particles (C) with WMV-2-induced pinwheel inclusions (9A) in a cucumber mesophyll cell. Note the different size and feature between CMV particles (9B) and WMV-2 (9C) (arrows) in the pinwheel.

Fig. 10 A tobacco cell doubly infected with PVY and CMV. CMV particles may scatter in the cytoplasm or associate with the inclusions with PVY particles.

Plate III Bars represent 250 nm

Fig. 11 ZYMV inclusion bodies (TI), and crystal of CGMMV (G) including CGMMV specific amorphous materials (Am) in the cytoplasm of doubly infected cucumber cell.

Fig. 12 CGMMV particles associated with ZYMV- induced pinwheels. Note the central

core of particles recognizable for CGMMV, and a threadlike structure connecting virus particles and the pinwheel (Small frame up-left).

Fig. 13 Tobacco mesophyll cells doubly infected with PVY and TMV containing malformed chloroplasts (Ch), PVY-induced inclusions (PI) and crystals of TMV particles (T).

Fig. 14 PVY inclusions associated with TMV particles (14A) and TMV-affected chloroplast (14B) in doubly infected tobacco leaf cell.

Plate IV Bars represent 250 nm

Figs. 15, 16 A broad bean leaf cell doubly infected with BYMV and CMV. CMV particles (C) scatter together with BYMV-induced laminated inclusion s(LI) (Fig. 15) or form small crystals in cytoplasm. Dense bodies (DB) induced by BYMV infection are observed nearby (Fig. 16).

Figs. 17, 18 BYMV (BY) and BBWV (BB) doubly infected cells of broad bean showing two virus particles and degraded chloroplasts (Ch) (Fig. 17). Specific inclusions (LI, DB) of BYMV infection and BBWV-induced membrane inclusions (MI) appear together in the cytoplasm (Fig. 18).

Fig. 19 CMV particles (C) and laminated inclusions (LI) in TuMV and CMV infected turnip plant cell.

Fig. 20 TuMV-induced inclusions (TI) and membrane inclusion (MI) present together with BBWV particles (arrows) in the cytoplasm of turnip plant cell.

Plate I

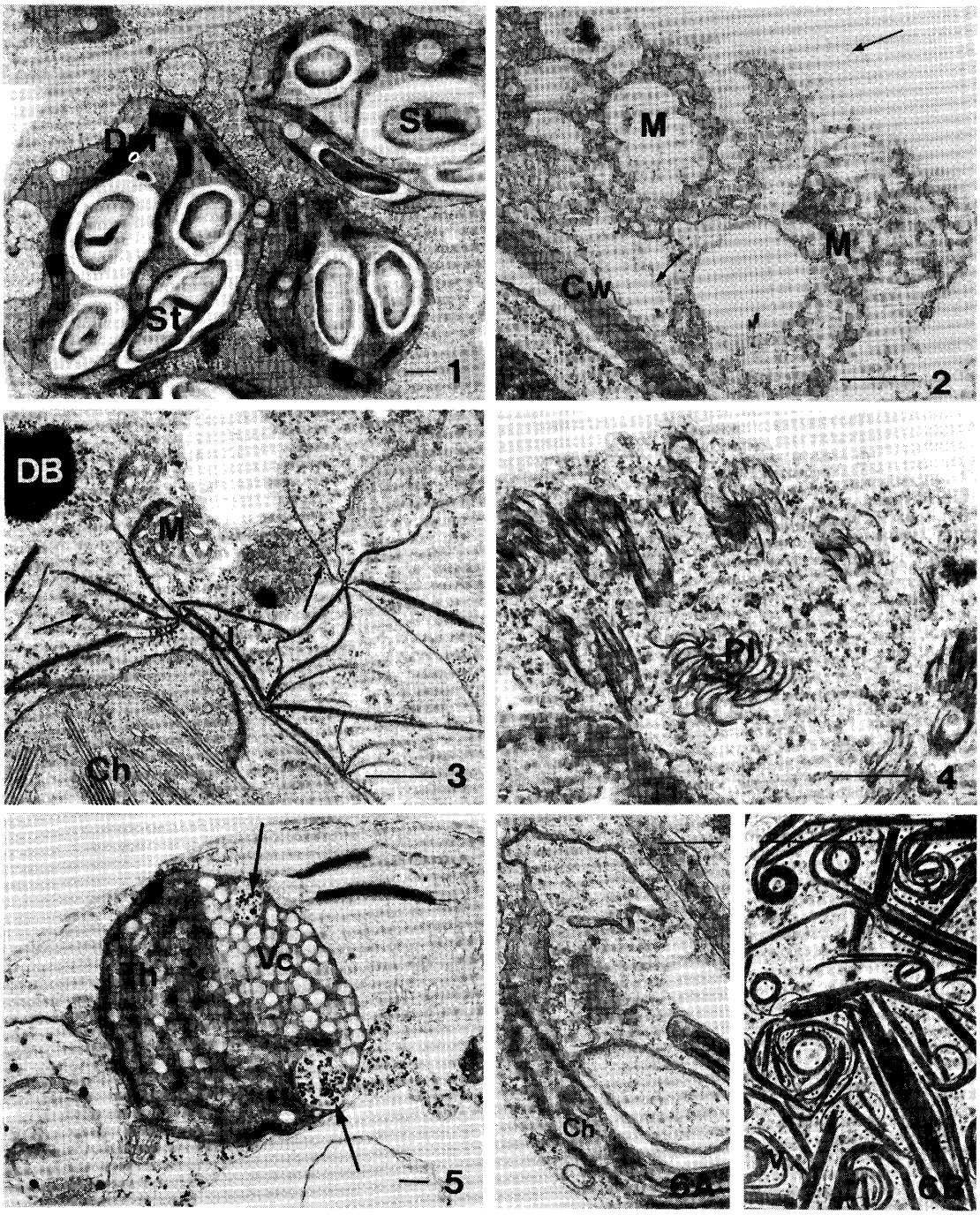


Plate II

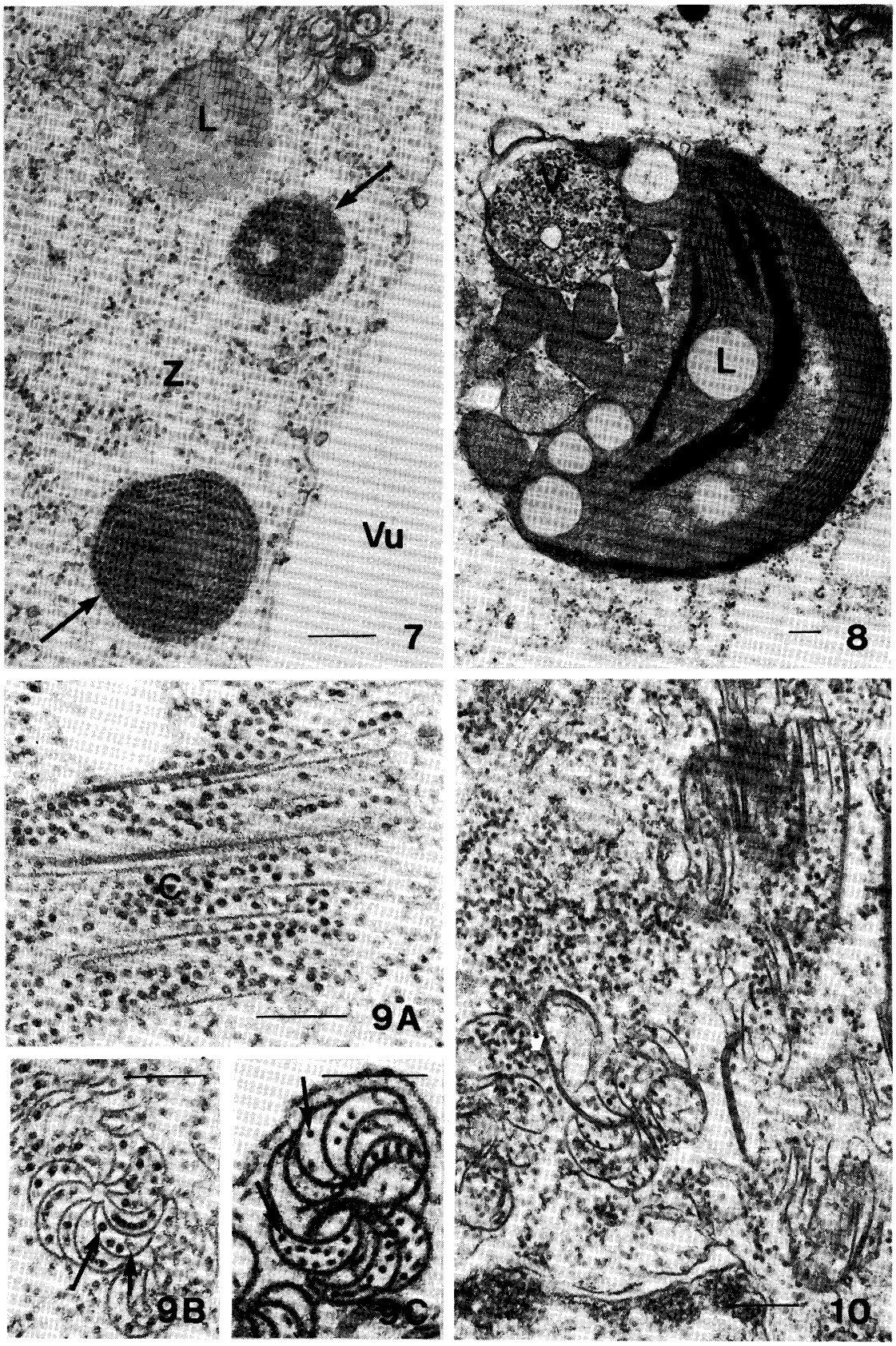
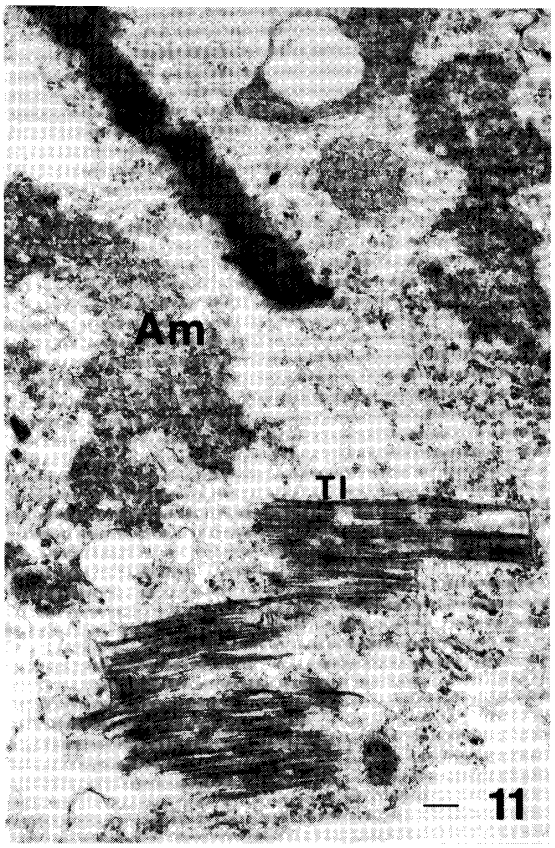
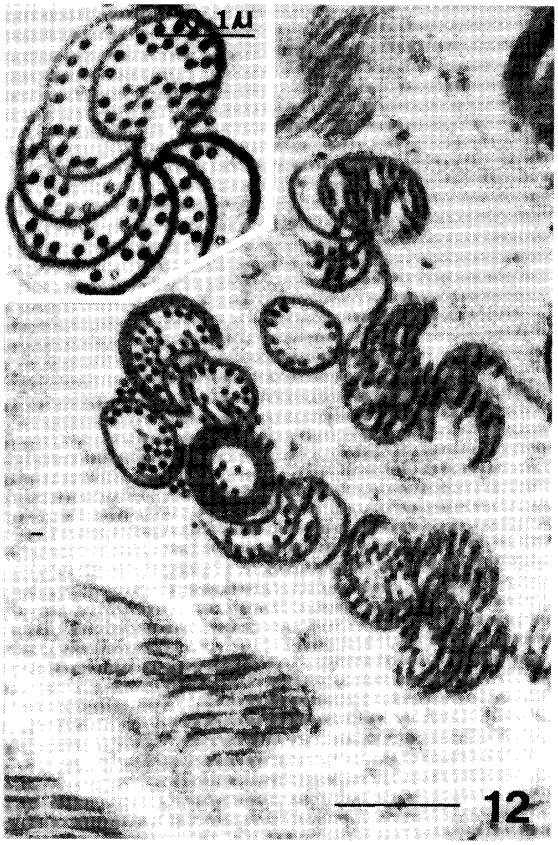
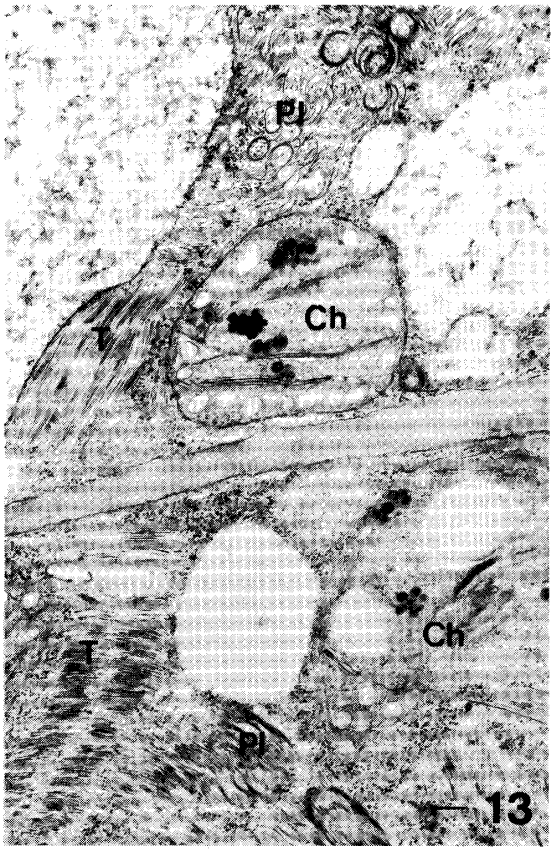
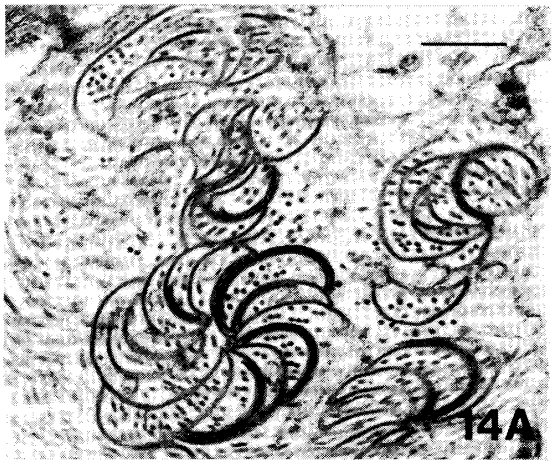


Plate III









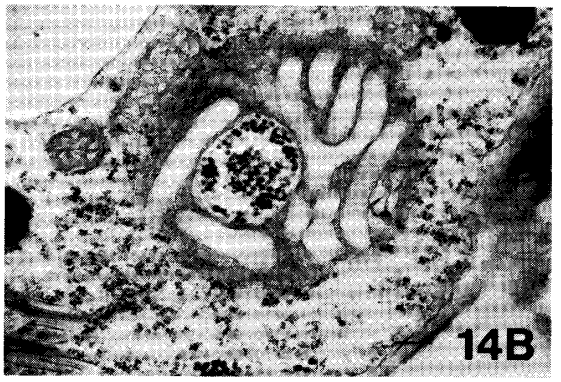


Plate IV