Ultrastructural Changes Associated With Chilling of Tomato Fruit

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ABSTRACT

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Ultrastructural modifications of tomato fruit organelles were correlated with low-temperature injury. Chilling of fruit for 10 days at 2 C interfered with conversion of chloroplasts to chromoplasts. After 15 days, mitochondria and plastids swelled and degenerated and after 21 days, organelles were barely discernible. Fruit stored at 7 C underwent similar changes, but those stored at 13 and 18 C ripened without breakdown. Fruit warmed after low temperature storage of up to 15 days usually recovered, as evidenced by reversible ultrastructural changes in mitochondria and plastids.

The susceptibility of tomato fruit to low-temperature injury is well documented (4, 5). Typical macroscopic symptoms include pitting and subsequent infection of pitted areas after removal of fruit to ripening temperatures (5). Even without such symptoms fruit may fail to ripen; or their locules may remain green and seeds turn brown. Exposure to chilling temperatures must be relatively lengthy before cells are irreparably damaged. Pitting of the fruit surface requires 10-17 days at temperatures below 10 C (50 F). However, after as few as 7 days, storage life may be reduced by increased susceptibility of the damaged tissue to decay-causing microorganisms (5).

When the temperature of chilling-sensitive fruit is lowered to the critical temperature (below 10 C), membrane lipids may solidify, contract, and break, thus destroying selective membrane permeability. A phase transition that increases the activation energies of membrane-bound enzyme systems leads to a suppression of their reaction rates and thus establishes an imbalance within nonmembrane-bound enzyme systems (4).

The effects of such enzymatic changes should be observable at the ultrastructural level. Indeed, mitochondrial structural changes have been reported for sweet potatoes stored at chilling temperatures for 14 days (8, 9).

MATERIALS AND METHODS

Tomatoes (cultivar Walter) used in this study were either grown at Beltsville, Maryland, or in Florida. Fruit maturity was determined spectrophotometrically at an OD of 510-600 nm (7), and only mature-green fruits of uniform density were selected for the study.

Fruit were stored at 2, 7, 13, and 18 C for 7, 10, 15, and 21 days, then transferred to 18 C for ripening. Tissue samples were removed from fruit at the time of transfer from storage and at intervals during ripening.

A fruit from each storage treatment was sampled immediately after its OD was recorded (7). Segments 0.5-1 mm square were removed from the epidermis and from areas of the pericarp containing vascular bundles. All tissues were removed from the side of the fruit. All treatments were replicated three times.

Tissues were immersed immediately in 4% glutaraldehyde (buffered at pH 7.2 with 0.01 M Na-cacodylate) and fixed for 4-6 hours at 0 C. They were rinsed with three changes of cold buffer, postfixed overnight in buffered 2% OsO_4 and rinsed. After dehydration through the standard ethanol-propylene oxide series, the tissues were embedded in Spurr plastic. Tissues were sectioned with a diamond knife, stained with lead citrate, and examined with a Philips EM 200 electron microscope.

RESULTS AND DISCUSSION

One of the most obvious changes in mature-green tomatoes chilled at 2 or 7 C was their failure to ripen properly; they turned dull green or pale pink during storage. Ultrastructural observations indicated that the failure to ripen was due, in part, to interruption in the conversion of chloroplasts to chromoplasts. The chloroplasts of mature-green fruit have, as do most green tissues, a well-developed lamellar network. As fruit begin to ripen, plastid conversion to chromoplasts occurs (1, 2, 6). Present results indicate similar changes in tissue chilled at 2 and 7 C; I have chosen, therefore, to report changes observed following chilling at 2C.

Organelles showing degeneration accompanying low-temperature storage are compared with those of nonchilled fruit in Fig. 1. The healthy plastid (P) of fruit stored at 18 C for 21 days (NC) shows development of lycopene crystals and membrane proliferation that corresponds to conversion of plastids to chromoplasts and is associated with carotenoid production (2). The adjacent mitochondria have a well developed network of cristae. Ribosomes are numerous and distinct throughout the cytoplasm and along the surfaces of the rough endoplasmic reticulum. This cell, typical of mature fruit

pericarp (1), is highly vacuolated, and the cytoplasm is limited to a thin peripheral layer.

The fruit chilled for 10 days at 2 C (Fig. 1, C-10) has plastids (P) that have begun to swell. They have begun to lose their grana, and the intergranal lamellae are distended. Numerous peripheral vesicles that have developed just beneath the inner membranes of the plastid envelopes indicate the incipient senescence of the plastids. An associated reduction in the number and size of osmiophilic granules is also apparent. Mitochondria are also swollen, and the cristae have begun to lyse. Numerous small vacuoles have begun to develop in the cytoplasm. Baker (1) correlated the appearance of numerous small cytoplasmic vacuoles, resembling autophagic vacuoles, in the cells of ripening tomato fruit, with other changes occurring within the senescing fruit.

Plastids of a fruit stored for 15 days at 2 C (Fig. 1, C-15) are obviously degenerating, and many, having lost all grana, have developed large vacuoles. Some plastids that have remained intact, but have lost much of their thylakoid membrane network, resemble etioplasts. These plastids contain few osmiophilic globules. Harris and Spurr (2), conversely, noted that osmiophilic globules increased during fruit ripening. Mitochondria are swollen and cristae appear to have developed into numerous vesicles. Many mitochondria, like chilled ones previously examined (8, 9) are very electron-transparent. The nucleus also appears electron-transparent, and the chromatin is aggregated into dense clumps spread randomly throughout the nucleoplasm. Numerous small vacuoles have developed in the cytoplasm, and few ribosomes are discernible among cytoplasmic contents. Endoplasmic reticulum, when evident, appears distended and no longer contains the clear channels present in that of nonchilled tissue.

Very few organelles are distinguishable in a fruit stored for 21 days at 2 C (Fig. 1, C-21). Many plastids have been reduced to what appears to be double-membrane vacuoles containing a few osmiophilic bodies. Others appear rounded, with granular stroma and few thylakoid membranes. Mitochondria are also swollen and have lost most of their matrices. Nuclei have been reduced to double-membrane sacs with a few strands of chromatin adhering to the inner membrane. Many small vacuoles fill the cytoplasm, and few ribosomes can be distinguished in the cytoplasm. This cell appears to be in an advanced stage of lysis, incapable of repair after removal of the fruit to 18 C for ripening.

After 21 days of chilling a few cells in the vascular parenchyma contained apparently viable organelles, but most cells in the pericarp appeared as in Fig. 1, C-21. Cells of fruit chilled up to 15 days recovered, as some of these fruit ultimately ripened. However, when chilled for 21 days, cells appeared to be in advanced stages of senescence and although independent organelles may yet have been able to carry on vital functions, the cells could not and ultimately the fruit decayed.

Figure 2 compares the organelles of fruit not chilled (NC), chilled for 15 days (C-15), and chilled for 15 days, then removed to 18 C for 7 days (C-15 + 7). Although organelles in the chilled fruit (C-15) appear disorganized and in an advanced state of senescence, those of a pericarp cell that was chilled and rewarmed (C-15 + 7) resemble a fruit chilled for only 10 days (Fig. 1, C-10). The plastids, however, are swollen and have failed to develop into chromoplasts; the mitochondria are also swollen, with few visible cristae. The cytoplasm appears less dense than that of the control tissue and there are few ribosomes.

Numerous cristae appeared in the mitochondria of some cells in fruit that had been chilled and rewarmed, which indicated a high rate of respiration. However, when distinguishable, the endoplasmic reticulum appeared distended, with few ribosomes attached.

Cursory examination of Fig. 3 might indicate that the chilled and rewarmed nucleus (C-15 + 7) is nearly normal. It is granular, however, and the chromatin tends to be distributed throughout the nucleoplasm in aggregates rather than uniformly, as in the nonchilled (NC) cell. Membrane damage was evident along the plasmalemma which had been pulled away from the cell wall, mitochondria were swollen, and plastids were small with few membrane proliferations.

Golgi apparatus were evident in some chilled cells. Figure 4 shows, however, that they are not as distinct as those in nonchilled fruit. The inner mitochondrial membrane in the chilled cells has stained more densely than the outer, and the endoplasmic reticulum appears disrupted.

The swelling of vascular parenchyma walls (Fig. 5) is readily apparent in fruit chilled 15 days (C-15). This swelling may lead to increased wall permeability, as chilled walls appear more electron-transparent than nonchilled walls. Pericarp cell walls (C-21) also swelled, but not as extensively as vascular parenchyma.

Ultrastructural evidence of damage to chilled tomato fruit is readily apparent and distinct from changes associated with ripening. Changes in chloroplasts, mitochondria, and endoplasmic reticulum suggest that changes in their functions may have occurred; however, these changes do not appear similar to those associated with ripening (7). Cell wall changes concurrent with organelle changes may also be responsible for some senescent changes by allowing increased leakage from the vascular tissues (3).

Although the structural changes due to chilling-induced degeneration of plastid structure partly resemble those due to conversion of chloroplasts to chromoplasts (1, 2), synthesis of lycopene and carotenoids appears to be inhibited once degenerative changes have begun. Evidence is presented that shows that plastids can recover after short periods of low-temperature storage if the fruit is removed to ripening temperatures; however, prolonged storage reduces this ability. If the membrane damage caused by the solidification of lipids at low temperature (4) becomes severe enough, the cell may exhaust its synthetic capabilities in attempting membrane repair. An increased number of polysomes together with an abundance of rough endoplasmic reticulum in many cells of fruit chilled for 10 days and rewarmed suggests such repair activity. Reports have also shown that respiratory activity increases after chilling (see 4). However, after 15 days of chilling, the endoplasmic reticulum was distended, and few polysomes were evident.

The apparent programmed sequence of degeneration observed in chilled plastids might be arrested by light and/or hormone treatment. Hopefully, we may soon be able to correlate cellular changes in chilled tissues with light transmittance of the whole fruit. Then, whether and

to what extent fruit have been chilled in market channels can be determined nondestructively.

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Fig. 1. Comparison of cell sections from pericarp tissues of chilled and nonchilled tomato fruit. Nonchilled fruit (NC) ripened at 18 C for 21 days; chilled fruit stored at 2 C for 10 (C-10), 15 (C-15), and 21 (C-21) days, respectively. Nucleus (N) and plastids (P) in the nonchilled tissue (NC) are prominent. Numerous osmiophilic granules (OG) and membrane proliferation in plastids indicate conversion to chromoplasts. Swollen chloroplasts (P) of tissue chilled for 10 days (C-10) contain few osmiophilic granules and numerous small peripheral vesicles (arrows). Mitochondria (M) are also swollen and contain few visible cristae. Plastids in tissue chilled for 15 days (C-15) are also swollen and have lost most of their thylakoid membrane network, nuclear chromatin (arrows) is granular and clumped through the nucleoplasm; mitochondria (M) are swollen and contain vesicles. Tissue chilled 21 days (C-21) has many small vacuoles (V) throughout the cytoplasm, and many organelles appear as double-membrane-bound vacuoles. (W = Cell Wall, Bar = 1 μm).

Fig. 2. Effects of rewarming on organelles chilled at 2 C. Plastics (P) in the nonchilled pericarp cell (NC) stored at 18 C for 21 days show the typical structure of chromoplasts in ripening fruit; the plastids contain numerous osmophilic granules (OG) and developing lycopene crystals (L). Organelles of a pericarp cell chilled 15 days (C-15) appear disorganized and membrane damage is apparent. Organelles in the rewarmed cell (C-15+7), however, appear nearly normal, except that membranes are dilated (arrows), plastids are numerous, and mitochondria are somewhat swollen. (W = Cell Wall, Bar = 1 μm).

Fig. 3. Senescent changes in an epidermal cell associated with chilling at 2 C. The nonchilled cell (NC) has a prominent nucleus (N), and chromoplasts (P) with membrane proliferation and numerous osmiophilic granules (OG) that are typical of those in a ripened fruit. The chilled and rewarmed cell (C-15 + 7) has a granular nucleus, and plastids contain few membranes or osmiophilic granules; the plasmalemma is pulled from the cell wall (arrows), and the mitochondria (M) are swollen. (V = Vacuole, W = Cell Wall, LR = Lycopene Remnant, Bar = 1 μm).

Fig. 4. Abnormal membranes in tissue chilled at 2 C and rewarmed. Nonchilled (NC) pericarp tissue contains normal mitochondria (M), Golgi apparatus (G) and extensive rough endoplasmic reticulum (ER). In chilled tissue (C-15 + 7), Golgi apparatus, although present appear abnormal; endoplasmic reticulum is distended; and the inner mitochondrial membranes are more prominent than the outer. (Bar = 500 nm).

Fig. 5. Cell wall modifications associated with chilling injury at 2 C. The nonchilled (NC) vascular parenchyma cell wall (W) appears dense and has absorbed little stain, whereas microfibrils of the chilled vascular parenchyma wall (C-15) (arrows) are swollen and have stained readily. The chilled pericarp cell wall (C-21) is also electron opaque, and cellular contents (arrows) appear to have leaked between the plasmalemma (Pl) and cell wall. (Bar = 500 nm).

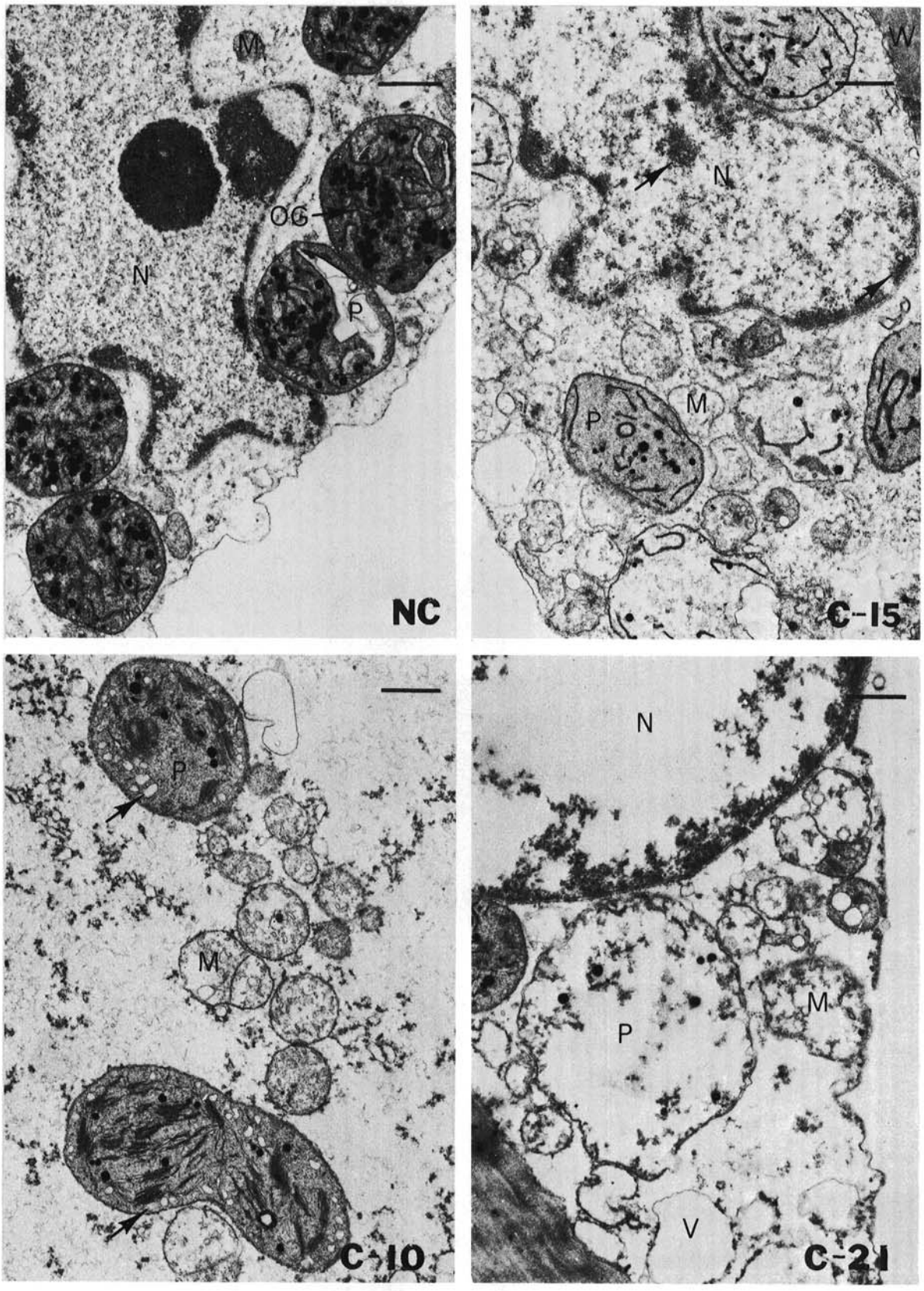

Figure 1

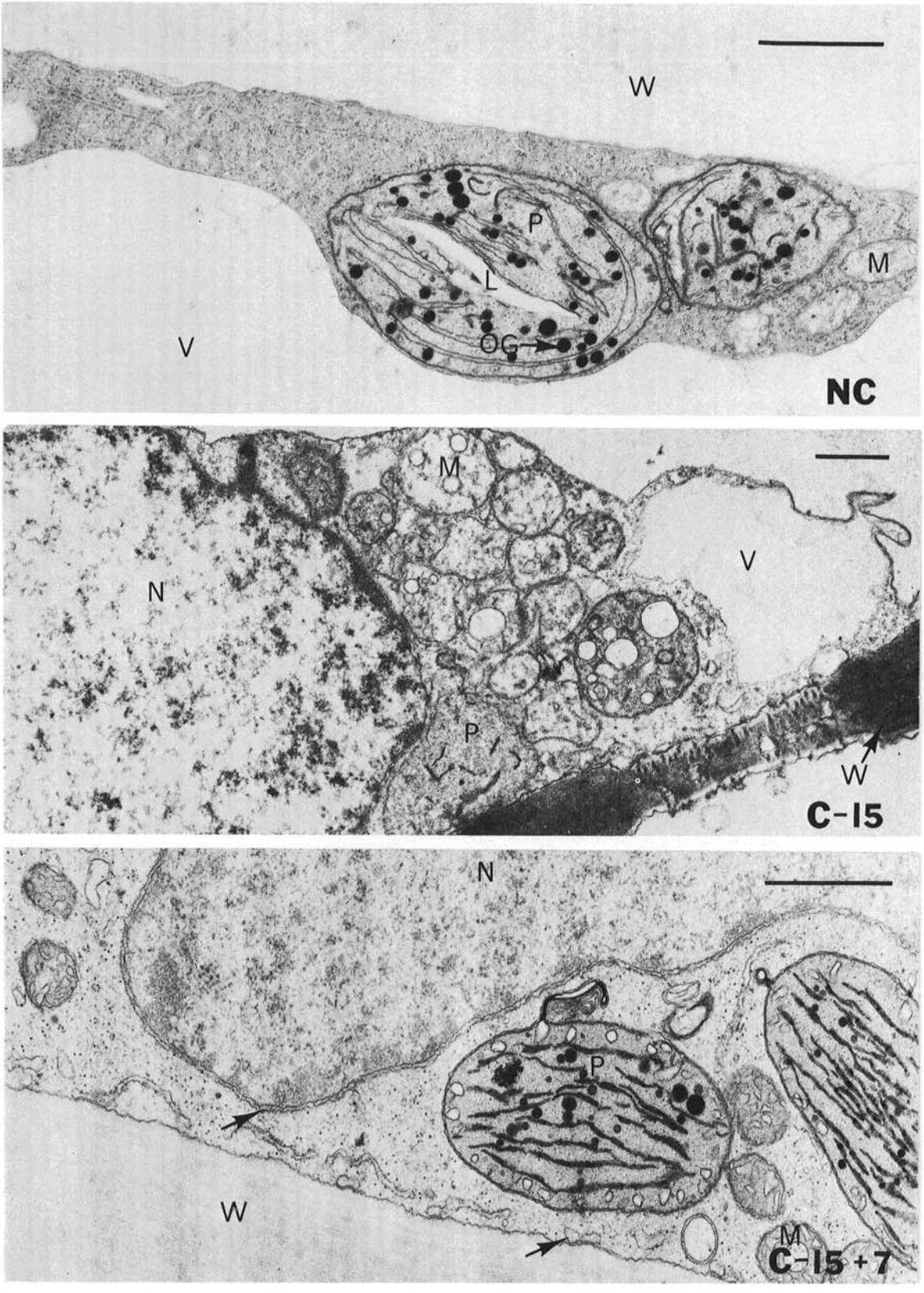

Figure 2

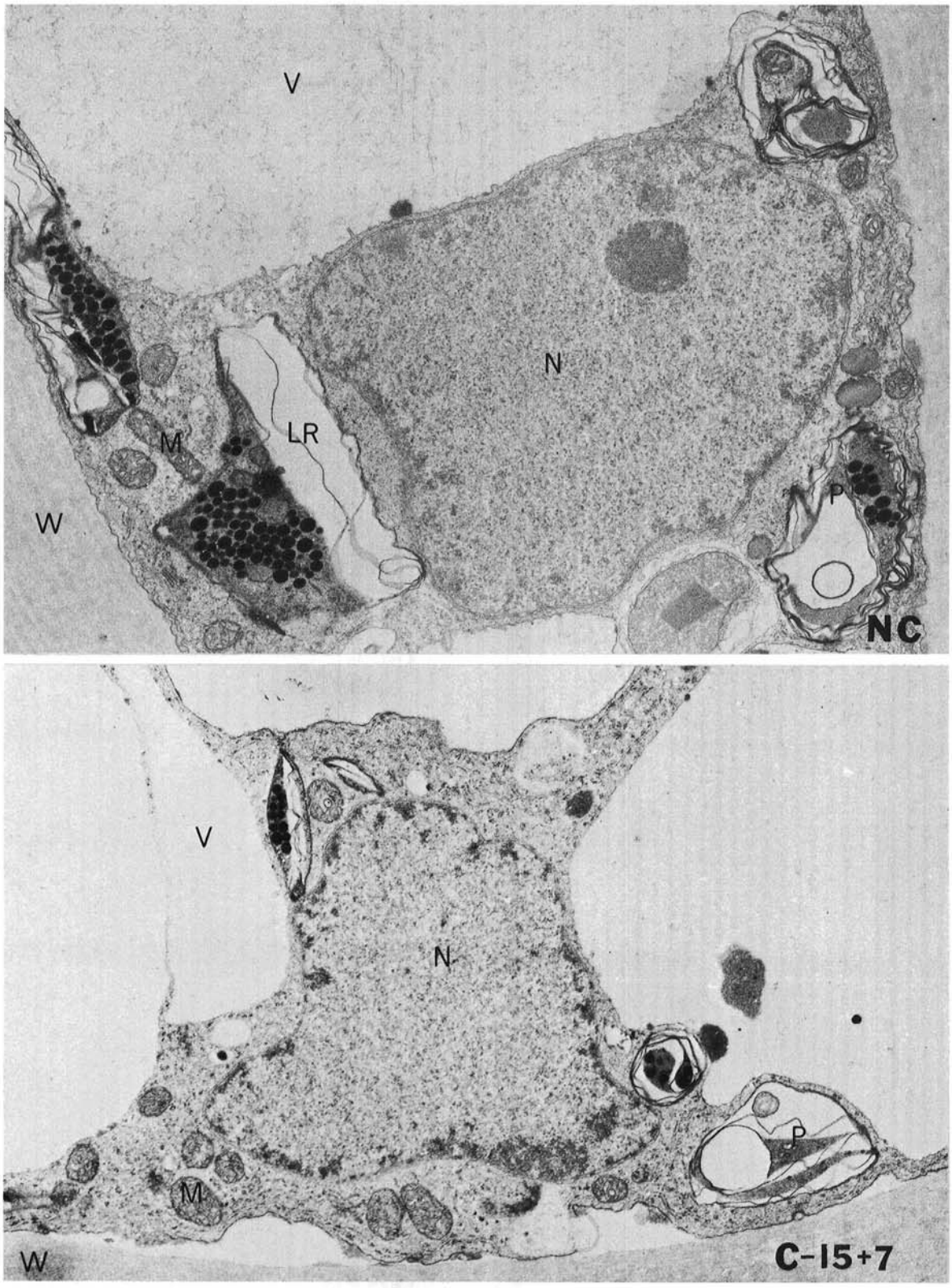

Figure 3

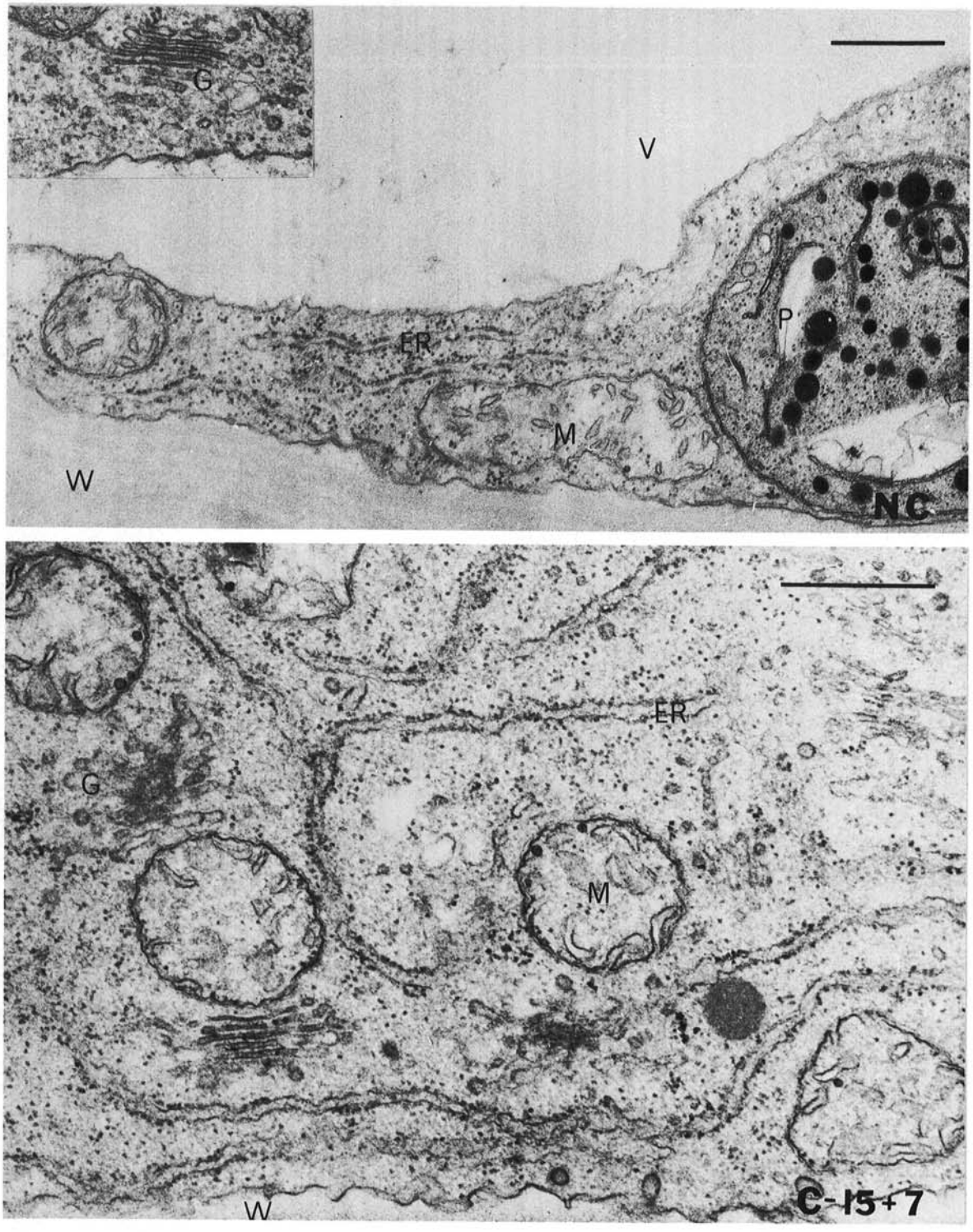

Figure 4

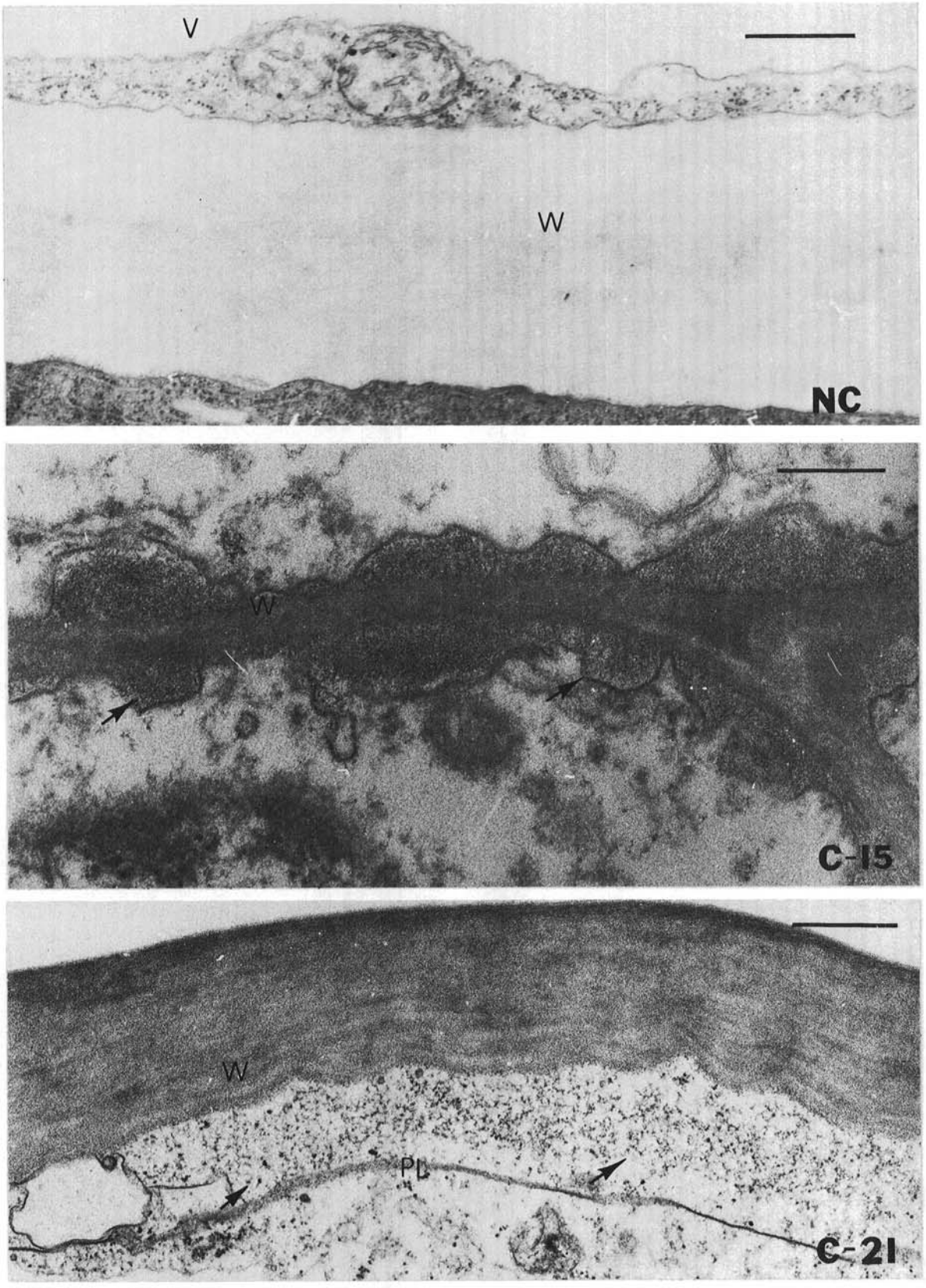

Figure 5