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Ultrastructure of Meiocyte and Callose Walls in *Fuchsia* Megasporogenesis

Ultrastruktura mejocytu i ściany kalozowej w megasporogenezie *Fuchsia*

Ультраструктура мейоцита и калозовой стены в мегаспрогенезе у *Fuchsia*

In the monosporic type of embryo sac development in angiosperms two events are clearly distinguishable: megasporogenesis and megagametophytogenesis. Usually one cell situated in a determined place of the ovule ceases to divide mitotically, grows out and eventually enters meiosis. During premeiotic growth an extensive endoplasmic reticulum is built up both in monosporic as well as in tetrasporic types of megasporogenesis (8, 9, 11, 14, 20, 21). A meiocyte attains its final dimensions when the first meiotic prophase is in progress. At this stage, callose appears in the walls of the meiocytes of the monosporic type of development (16, 19). In the meiocyte cell wall of many species callose is distributed according to a certain pattern which seems to reflect the polarized state of the cell, and, later, of the megaspore tetrad (13, 16, 17).

At the first meiotic division the originally dense cytoplasm of the meiocyte undergoes gradual vacuolization. Deformation of plastids and of mitochondria has been observed in various phases of female gametophyte development (2, 5, 6, 12, 24).

MATERIAL AND METHODS

Fuchsia hybrida (Onagraceae) ovules fixed in a mixture of ethyl alcohol and acetic acid (3 : 1) were hydrolized in 1n HCl for 5—10 minutes, then rinsed in water. Squash preparations of the ovules were made in a solution of aniline blue by the Arens method specific for callose

which gives off a yellow fluorescence in a fluorescence microscope (7).

The ovules were fixed for electron microscopy in 3% glutharaldehyde with O_sO_4 postfixation. The specimens were embedded in Epon 812. Lead citrate stained preparations were examined under a Tesla electron microscope.

RESULTS

Preliminary observations have shown, that callose walls occurring in megasporogenesis in *Fuchsia hybrida* display a pattern of polarization typical of *Onagraceae* (17). Intensity of fluorization and thickness of callose walls in this species are exceptionally great in comparison with those in others. The callose wall appearing in the meiocyte shows a very strong fluorescence at the chalazal apex of the cell and in the submicropylar region, whereas fluorescence is much weaker or nonexistent at the micropylar apex (Fig.1a—h). In further development the micropylar apex remains entirely without fluorescence typical of callose.

Developed megaspore tetrads show certain variation in shape and size. Some tetrads are very narrow and have strongly elongated chalazal megaspore (Fig. 1i, j). Such tetrads usually do not display a clear polarized pattern of callose distribution in the cell walls. Variation of tetrad characters may be explained by the hybrid origin of the plant.

The wall

The early callose wall of the meiocyte is not fully transparent to electrons. The picture (Fig. 2) shows such a callose wall which is already several times thicker than the adjacent primary walls of the nuclear cells. It is probable, that particularly intensive building in of callose takes place at the region of the cell wall where a large callose projection is growing (Fig. 3). At the basal part of the projection there is a gathering of numerous bodies or vesicles with optically dense contents. These vesicles seem to merge into the callose wall. The presence of several plastids in the vicinity of the wall is also noticeable. One of the plastids appears to be attached to be attached to the cell wall.

In a somewhat later stage the meiocyte cell wall becomes much thicker and more transparent to electrons (Fig. 4). In some other pictures, however, meiocyte walls of almost equal thickness are stained dark (Figs. 7 and 8).

The three inactive megaspores degenerate during the development of the embryo sac from the active megaspore. Callose gradually disappears, and the cells undergo complete crushing and resorption. In the picture (Fig. 9) two megaspores with degenerated protoplasm are visible.

The walls separating the megaspores and the wall between the megaspore and the embryo sac reveal their fibrillar structure. This structure was invisible in megasporogenesis when the walls gave off strong callose fluorescence. Now the two degenerating megaspores are separated by five distinct layers differing in the arrangement of fibrillar material. The middle layer 1—2 μ thick, being undoubtedly a middle lamella, contains fibrills distributed in a loose netlike pattern. The cell walls of the megaspores extend on both sides of the lamella. The outside layer of each megaspore wall is formed of densly packed more or less parallel fibrills. The inside layer obviously differs in having fibrills arranged in a loose irregular net. The internal surface of the cell wall is irregular, uneven with many protrusions and cavities reaching the middle lamella.

The embryo sac is separated from the degenerating megaspore by three layers, two of which constitute the megaspore cell wall and the third the middle lamella. This middle lamella, as it is visible in the left part of the picture (Fig. 9), is continuous with the middle lamella situated between two degenerating megaspores. Beside the fibrillar material there are agglomerations of osmiophilic substance dispersed in the middle lamella.

The cytoplasm

The cytoplasm of meiocyte with the callose wall has numerous mitochondria, mostly of circular profiles, some dictyosomes and plastids; many plastids contain starch grains. The endoplasmic reticulum, fairly extensive in the early stage, diminisehes later on. There are also deformed plastids in the shape of so-called cup bodies (Fig. 2, 5, 6, 8).

Vacuoles of various size, profile outline and contents occur in meiocyte cytoplasm. Some vacuoles of crescent profile partially surround the cytoplasmic regions which are enclosed in cytoplasmic membranes (Fig. 7). Other vacuoles of circular profiles contain small cytoplasmic inclusions bounded in a membrane or they contain groups of vesicles. Optically empty vacuoles and some with flaky remnants are also present (Figs. 4. 8).

DISCUSSION

During the first meiotic prophase begins the formation of meiocyte callose wall, which may be easily followed throughout the entire megasporogenesis in a fluorescence microscope. When the development of the embryo sac begins, callose fluorescence disappears at first from the cell wall of an active megaspore and then from the cell wall of degene-

rating, inactive megaspores. It is well known, that the formation of thick special callose walls is characteristic of microsporogenesis. Small vesicles produced by the endoplasmic reticulum cisterns take part in the building up of this wall (10).

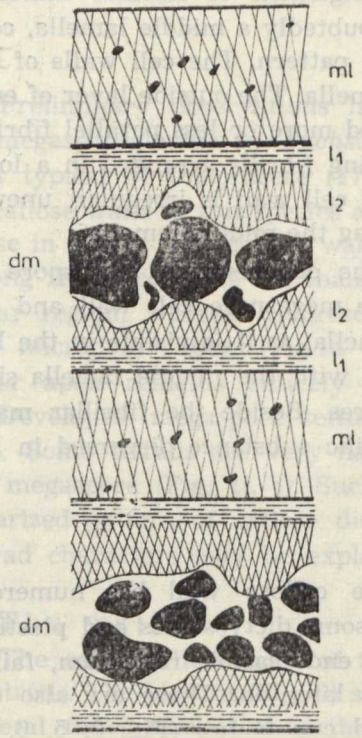

Fig. 10. Diagramic reconstruction of the cell wall between the inactive megaspores and the embryo sac; degenerating megaspores (dm), embryo sac (es), middle lamella (ml), outer layer of the megaspore cell wall (l_1), inner layer of the megaspore cell wall (l_2); the walls and the middle lamella are composed of callose and noncallose fibrillar material

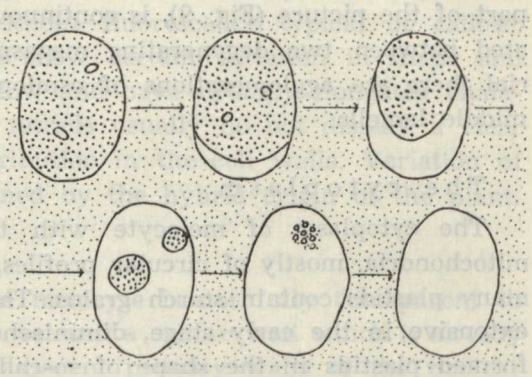

Fig. 11. Diagramic representation of successive stages in formation of vacuoles in meiocyte cytoplasm; the meiocyte is already surrounded by a callose wall

During *Oenothera* megasporogenesis ER vesicles and dictyosome vesicles participate in laying down of callose walls (13). It seems, that in *Fuchsia hybrida* some vesicles merge with the growing callose wall. The origin of these vesicles, however, cannot be presumed because the organelles producing vesicles are not present near the site of the parietal grouping of vesicles.

Callose walls of a meiocyte and megaspores have a slightly nonhomogeneous appearance when seen in the electron microscope. Particularly the outer layer of the cell wall contains fibrillar components in its structure (13). Nonhomogeneity of the megaspore cell walls shows up especially well after callose has been hydrolized during the natural degeneration of inactive megaspores. The remaining cell walls display then distinct fibrillar structures, which presumably were uncovered after callose hydrolysis. There are two layers in the megaspore cell wall differing in the arrangement of the fibrillar components: the outer layer, where parallel fibrills are densly packed and the inner layer with a loose netlike arrangement of fibrillar structures (Fig. 10).

A conspicuous middle lamella is situated between the megaspore cell walls. The middle lamellas set up during meiosis are much thicker than those formed after mitotic divisions. Callose appears very early in their development. A cell plate arising in meiotic cytokinesis already contains a large amount of callose which fluoresces strongly before the complete separation of dyad cells has been reached (Fig. 1d). On both sides of the middle lamella thick callose walls are laid down. In each wall there is an outer layer with a great amount of noncallose fibrillar structures.

In the meiocyte enveloped in the callose wall an extensive vacuolization of the cytoplasm is carried out. At least, part of this vacuolization is due to the autolytic processes in which some parts of the cytoplasmic material, in the form of cytoplasmic inclusions surrounded by membranes, undergo dissolution. A reconstruction of the course of autolysis is presented diagramatically (Fig. 11). Similar autolytic or autophagic processes were described in microsporogenesis (1), in *Physomyces* sporangiophores (23), in pine oospore (4), in lily embryo sac (15), and in many somatic cells (3).

The plastid population in meiocyte consists of apparently normal plastids, some containing starch grains and many plastids in the shape of cup bodies. According to some authors, the degeneraton of plastids might lead to the complete elimination of these organelles from the cells in the generative line. See discussion by B e l l (2) and D i e r s (5). Data presented seem to show that the period of extensive vacuolization of meiocytes coincides with the occurrence of deformed plastids. It may be pointed out, however, that prophiles of mitochondria remain unchanged. Some of the deformed plastids and also some mitochondria may undergo a hydrolytic process in the cytoplasm where autophagic vacuoles are presumably formed and active.

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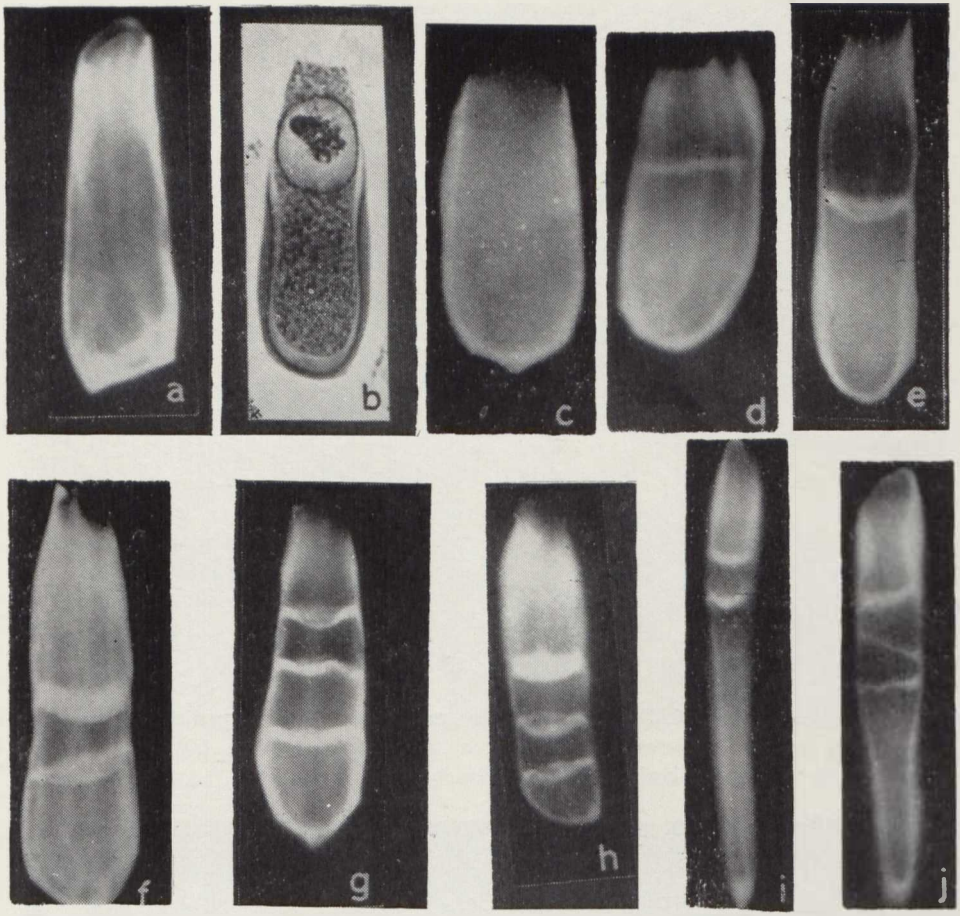

Fig. 1. Megasporogenesis in *Fuchsia hybrida*; fluorescence of callose cell walls after aniline blue treatment: a — megasporocyte, b — megasporocyte in a light microscope, c — megasporocyte with nonfluorescing micropylar apex, d — cell plate before completion in the meiocyte at the first telophase, e — dyad, f — triad, g — megaspore tetrad, h — megaspore tetrad, micropylar megaspore larger than in the preceding picture, i — a typical triad with a long chalazal cell, j — a typical tetrad with an enlarged chalazal megaspore (×600)

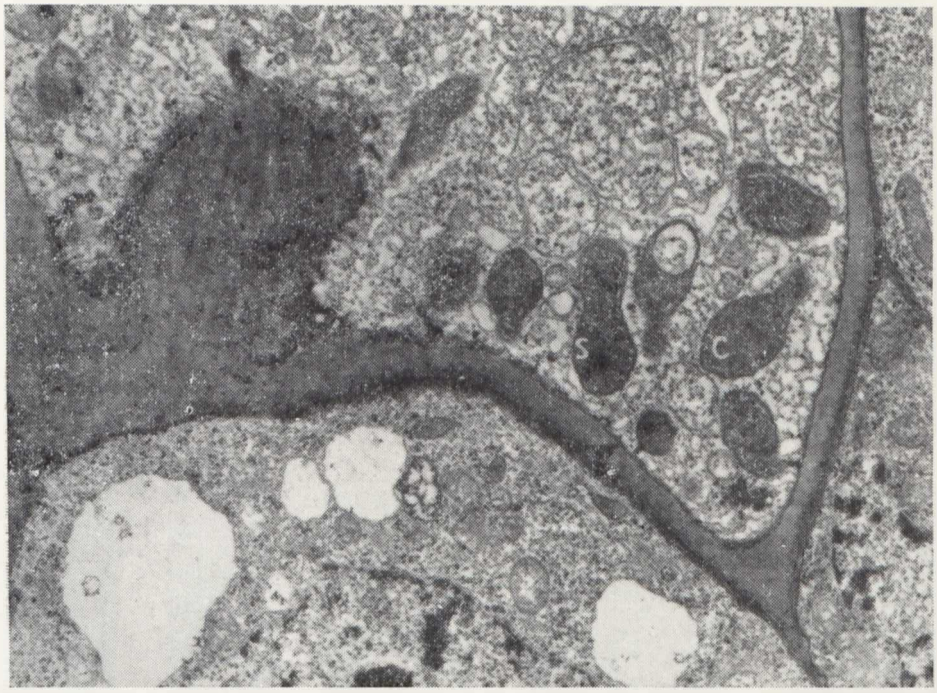

Fig. 2. Chalazal part of *Fuchsia hybrida* meiocyte; Callose projection on the callose wall; optically dense plastids, some with starch grains (s), a plastid of a cup body shape (c), mitochondria (m), ×8000

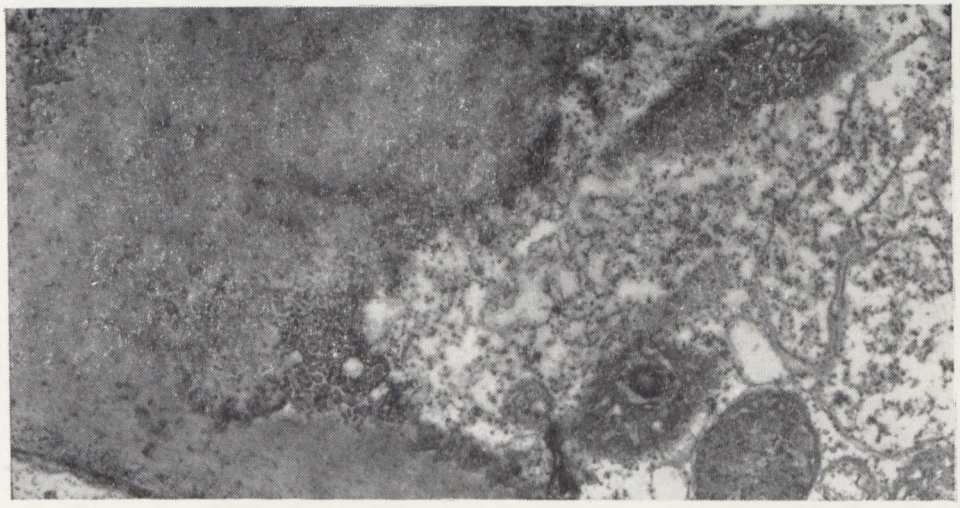

Fig. 3. A magnified part of fig; at the base of callose wall projection a gathering of optically dense vesicles is visible (×25 000)

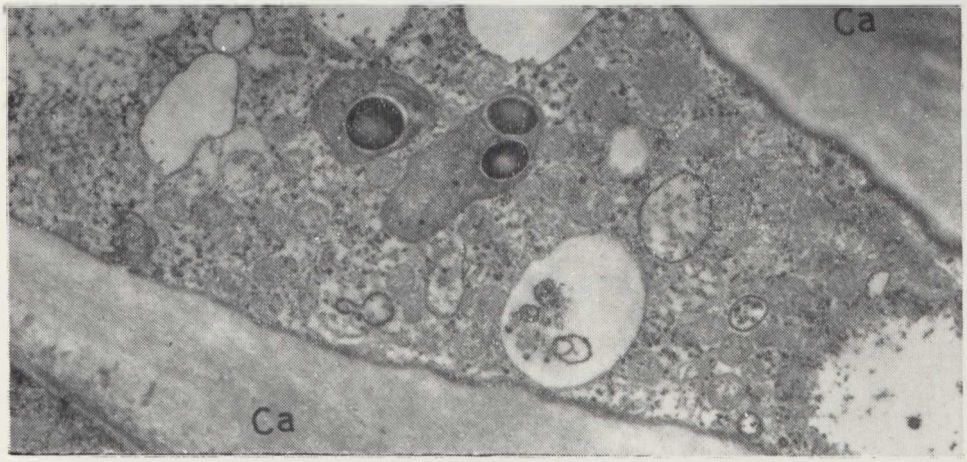

Fig. 4. Meiocyte of *Fuchsia hybrida*, thick callose wall (ca); plastids with starch grains and mitochondria in cytoplasm (×8000)

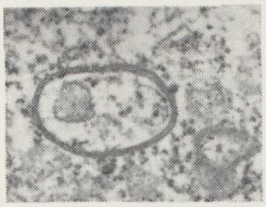

Fig. 5. A section through the deformed part of a cup body plastid (×5000)

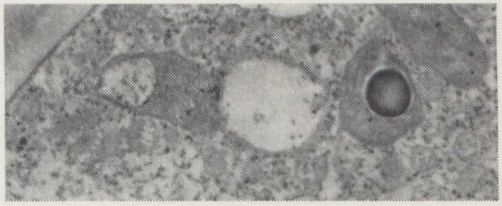

Fig. 6. Plastid with a starch grain and a cup body plastid (×5000)

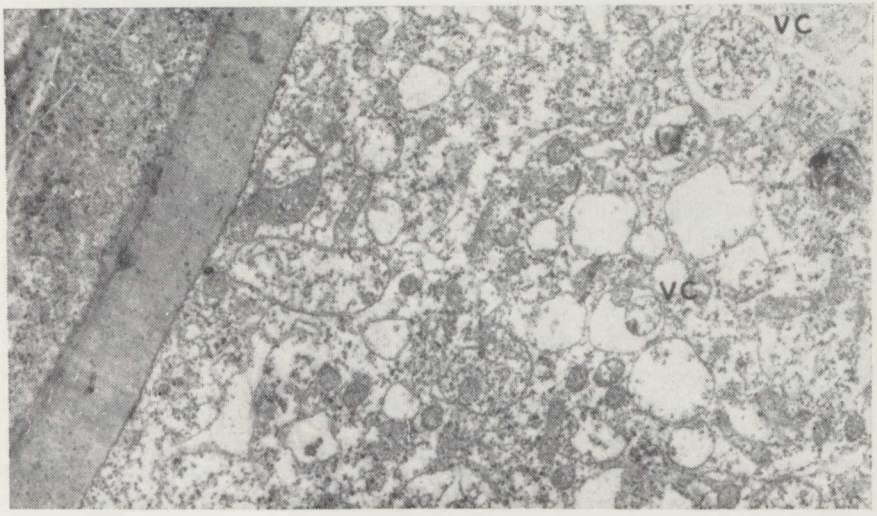

Fig. 7. Vacuolized cytoplasm of the meiocyte, with some deformed plastids and multivesicular bodies; cytoplasmic inclusions partiaally or completely surrounded by vacuoles (vc), ×8000

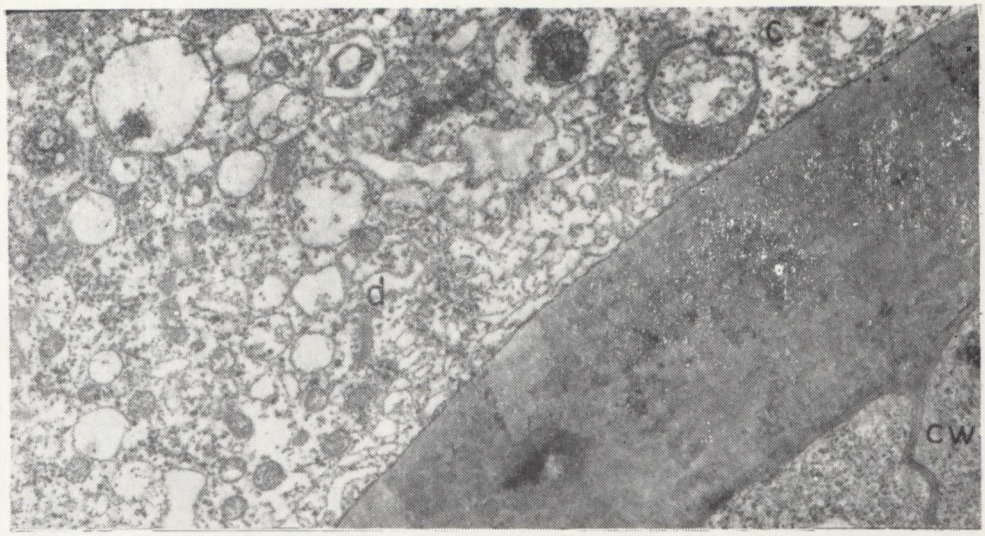

Fig. 8. Meiocyte cytoplasm with a large number of vacuoles, some dictyosomes (d), multivesicular bodies, deformed plastids (c); thick callose wall with adjacent thin cell walls of the nucellar cells (×8000)

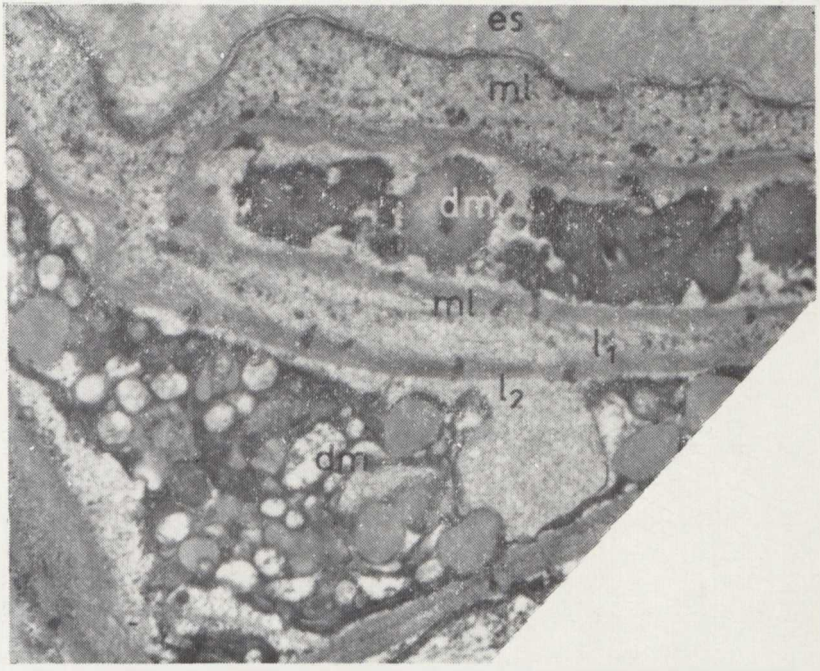

Fig. 9. Two degenerating inactive megaspores (dm) at the chalazal end of the embryo sac (es); middle lamella (ml) between the megaspore and the embryo sac, fibrillar material (l_1) in the outer layer of the megaspore cell wall, netlike fibrillar material (l_2) in the inner layer of the megaspore cell wall; inside the lower megaspore a projection of the inner cell wall layer is visible (×8000)

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STRESZCZENIE

W megasporogenezie u *Fuchsia hybrida* wokół mejocytu i megaspor wytwarzają się grube ściany kalozowe. Podczas degeneracji nieaktywnych megaspor kaloza ulega hydrolizie, a w ścianach ujawnia się pozostały materiał ściany w formie fibrylarnej. W zewnętrznej warstwie ściany zajdują się równolegle, gęsto ułożone fibryle, w warstwie wewnętrznej materiał fibrylarny tworzy luźną, nieregularną sieć.

РЕЗЮМЕ

В мегаспрогенезе у *Fuchsia hybrida* вокруг мейоцита и мегаспор образуются толстые калозовые стены. Во время дегенерации неактивных мегаспор калоза подвергается гидролизу, а в стенах появляется остальной материал стены в фибрилярной форме. Во внешнем слое стены находятся густо уложенные параллельные фибрили, во внутреннем слое фибрилярный материал образует свободную нерегулярную сеть.