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Tapetal Ultrastructural Changes During Pollen Development. I. Studies on Antirrhinum Maius

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TAPETAL ULTRASTRUCTURAL CHANGES DURING POLLEN DEVELOPMENT. I.
STUDIES ON *ANTIRRHINUM MAIUS*

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INTRODUCTION

During the past years many authors (MARQUARDT *et al.* 1968; ECHLIN and GODWIN 1968*a*; MEPHAM and LANE 1969; HESLOP-HARRISON and DICKINSON 1969; RISUEÑO *et al.* 1969; ECHLIN 1971; CHRISTENSEN *et al.* 1972) made several observations on the tapetum ultstrastructure, investigating the possible relationships between the tapetum and the development of the pollen grains.

Nevertheless some problems concerning the meaning and origin of several vesicles typical of the tapetum (Ubish-bodies, orbicules, orbicular bodies, multivesicular-bodies) are still unsolved. Therefore we thought it interesting to study the subsequent developmental stages of the tapetal cells, and to extend these researches to systematically different plants having different types of tapetum.

In this paper we report our observations on the anthers of *Antirrhinum maius*.

MATERIALS AND METHODS

From the earliest stages of pollen mother cells up to the completely developed pollen grain, anthers of *Antirrhinum maius* in subsequent stages of development were fixed in cacodilate-buffered 3% glutaraldehyde pH 6.9, at +4°C for 2 h.

After washing in O. I M cacodilate buffer, the specimens were postfixed in cacodilate-buffered 1% osmium tetroxide for 2h, at +4°C, dehydrated in ethanol, and embedded in Araldite following the procedure of LUFT (1961). When in 75%

ethanol, the samples were impregnated with uranyl acetate in semisaturated solution.

Ultrathin sections were cut with an LKB Ultratome III, stained with lead citrate (REYNOLDS 1963) and examined in a Hitachi H11B electron microscope.

RESULTS

We report 4 stages representing subsequent steps of anther development. I STAGE: Sporogenous tissue.

In this early stage the tapetum consists of tightly juxtaposed cells, characterized by a very thin primary wall, like meristematic cells. Most cells (Fig. 1) have a dense cytoplasm, small vacuoles and a large nucleus with a conspicuous nucleolus.

The various organelles are scarcely differentiated: mitochondria have a
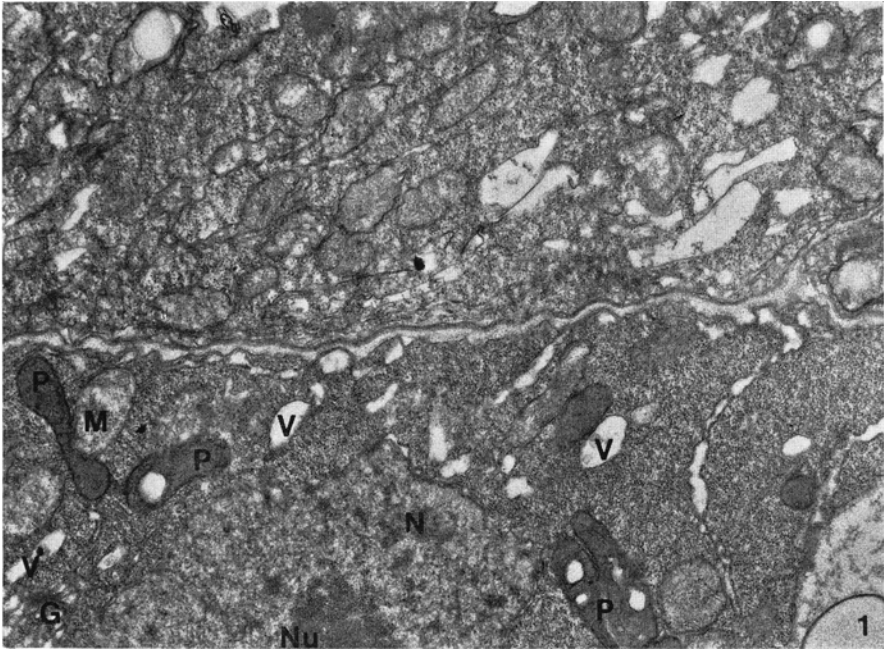
Fig. 1. — Electron micrograph of a tapetal cell (below) bounded by a thin primary wall. The cytoplasm is extremely rich in ribosomes and interrupted by several small vacoules (V). The nucleus (N) appears large and contains a central conspicuous nucleolus (Nu). Proplastids (P) are numerous, as well as mitochondria (M). Some Golgi bodies (G) are visible, surrounded by numerous vesicles. Membranes of the « rough » ER are seen in continuity with the plasma-lemma. A sporogenous cell is visible in the upper part of the figure. x 11,500.

Key to abbreviations:
cell wall = Cw; endoplasmic reticulum = ER; grey body resembling a spherosome = S; grey body = Grb; Golgi body = G; mitochondria = M; nucleolus = Nu; nucleus = N; proplastid = P; vacuole = V.

light matrix and small cristae. Plastids, present as proplastids, have a very dark matrix and contain a few short lamellae and small starch grains.

The Golgi apparatus shows dictyosomes consisting of parallel saccules surrounded by numerous vesicles. The endoplasmic reticulum (ER) often appears enlarged and of « rough » type. Frequently in the perinuclear space, in proximity of the plasmalemma and in the ER spherical bodies are seen, bounded by a single membrane and containing many ribosomes. They resemble portions of cytoplasm.

II STAGE: Pollen mother cells in meiotic division, characterized by a thick callose wall.

In this stage the tapetal cells are still well preserved (Fig. 2): their cytoplasm is generally rich in ribosomes. Mitochondria are numerous, large, with light matrix and few cristae. Proplastids have no starch grains, but more evident short lamellae.

The Golgi bodies are always frequent (Fig. 2) and show dictyosomes surrounded by a great amount of vesicles.

The « rough » ER often appears enlarged and delimits elongated cytoplasmic areas, concentrically disposed around the nucleus. Spherical bodies are still seen in connection with the ER: they are bounded by a single membrane, on which ribosomes are regularly ranged facing the body cavity (Fig. 3). Such formations are similar to those observed in the previous stage, but they are more numerous and more variable in size. Similar bodies accumulate at the cell periphery, just inside the plasma membrane, and in the perinuclear space (Fig. 4). Here the body limiting membrane is sometimes deprived of ribosomes or even interrupted on the side facing the nucleus.

Spherical « grey bodies » are also present: they are bounded by a thin membrane and recall spherosomes (FREY-WYSSLING et al. 1963) (Fig. 4).

Besides the above-described cells, there are others containing dark, small spherical bodies without any bounding membrane and generally located at the cell periphery (Fig. 5).

III STAGE: Meiosis just ended: tetrad stage. The four microspores are still joined together by a common callose matrix. Each microspore possesses its own more or less developed sporopollenin wall (Fig. 6).

This stage can be devided in two subsequent steps. At the beginning, the tapetal cells show a still evident cell wall and have a content similar to that of the previous stages (Fig. 7). The plasmalemma often appears characteristically convoluted; the Golgi apparatus is still conspicuous. Mitochondria are small, roundish and electrondense, with less evident cristae.

At the end of this stage (Fig. 8) the cell wall has disappeared, and the cell content is highly modified. Near the convoluted plasma membrane spherical bodies of various size are seen, which lack a bounding membrane
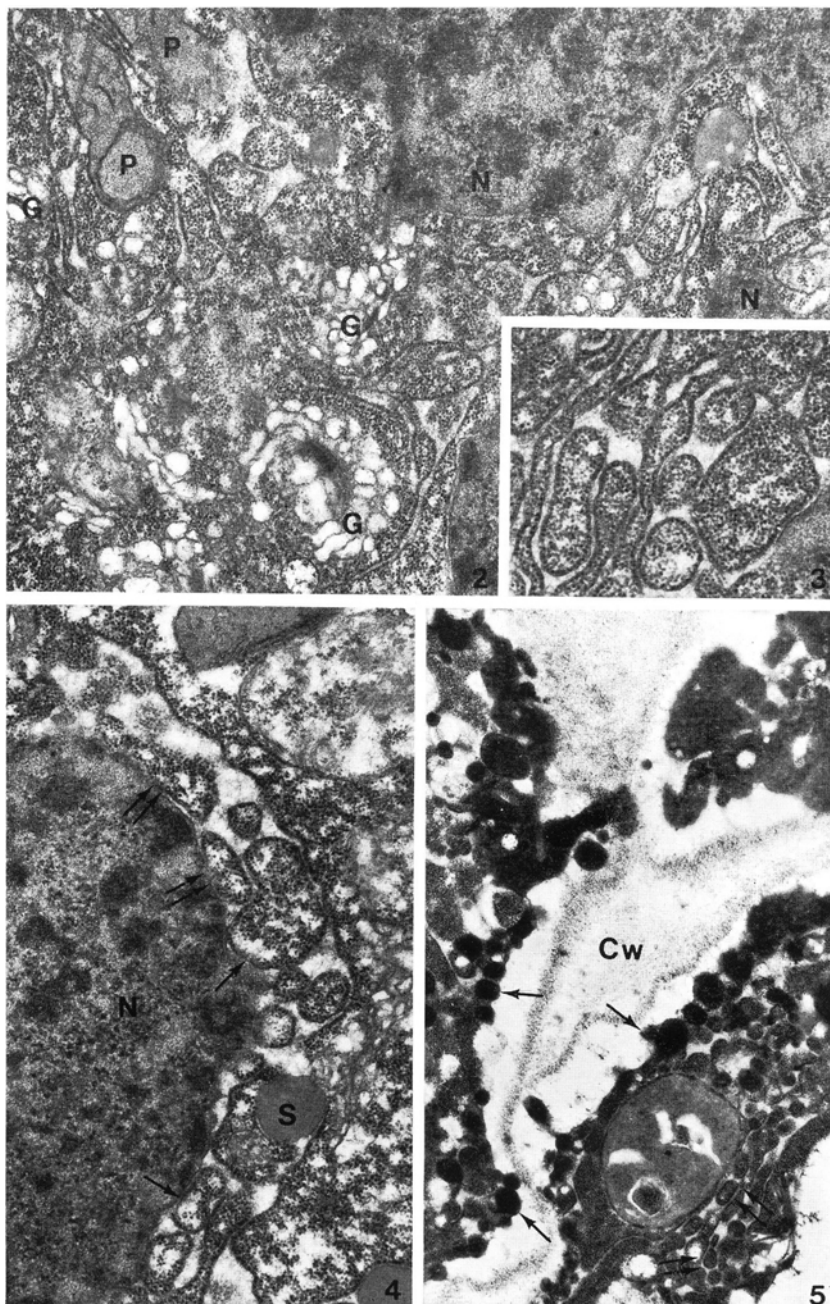
Fig. 2. — Electron micrograph of a tapetal cell portion showing a large amount of Golgi bodies (G), surrounded by a lot of vesicles. At this stage the cell contains two nuclei (N). Proplastids (P) appear still numerous. The « rough » ER is greatly developed and delimits elongated cytoplasmic areas. x 23,000.

Fig. 3. — Electron micrograph of spherical bodies in connection with the « rough » ER. The bounding membrane of the spherical bodies carries ribosomes regularly ranged on the inner face. Apparently these bodies contain portions of cytoplasm. x 27,000.

Fig. 4. — Electron micrograph of spherical bodies in the perinuclear space. The body limiting membrane is sometimes deprived of ribosomes (arrows) or even interrupted (double arrows) on the side facing the nucleus (N). Two spherical « grey » bodies (S), bounded by a thin membrane resemble spherosomes. x 24,500.

Fig. 5. — Electron micrograph of the tapetal cell periphery rich in small dark bodies (arrows), lacking a bounding membrane. The cell wall (Cw) appears quite thickened. Spherical bodies are connected with the « rough » ER (double arrow). x 13,000.
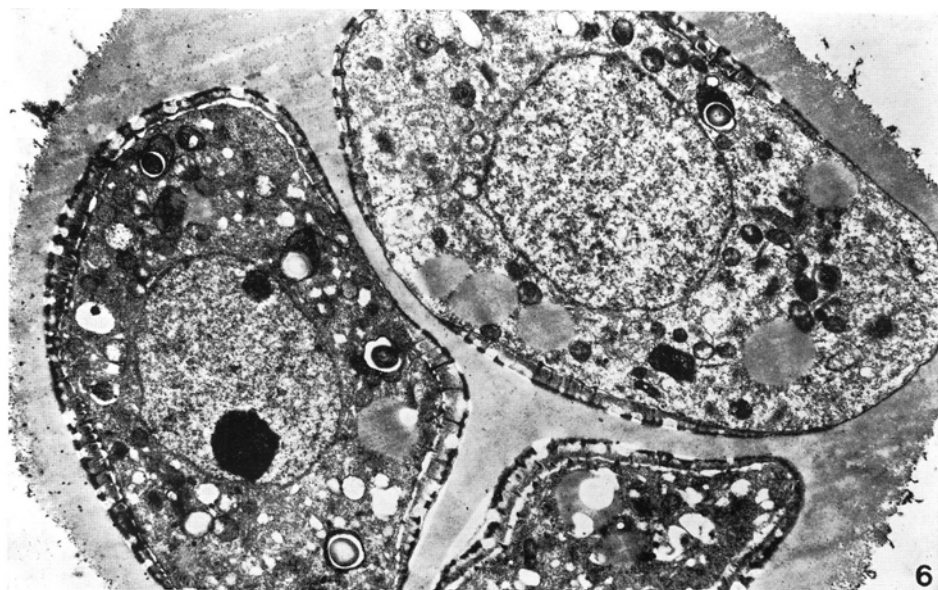
Fig. 6. — Tetrad: the microspores are joined together by a common callose matrix; they possess an own more or less developed spropollenin wall. x 7,500.

and sometimes are adjacent to light vesicles. Such bodies differ from those described in the previous stages, and still present at the periphery of degenerated cells.

The Golgi bodies are made only of vesicle clusters and the number of mitochondria seems reduced. In this stage the ergastoplasma has a characteristic disposition, being ranged in parallel, concentric rings (Fig. 9).

Proplastids are always numerous and characterized by a dense matrix containing well developed lamellae and large starch grains.

IV STAGE: Isolated pollen grains, each showing a more or less thick sporopollenin wall, in subsequent developmental stages.

In this stage the tapetum consists of cells with different kinds of structures. Many of them are still well preserved, with a cytoplasm rich in ribosomes and polysomes, normal ER, small dark mitochondria with visible cristae and many proplastids (Fig. 10). These are generally elongated, with electrondense matrix, and contain many vesicles. Subsequently such vesicles increase in number and fill the whole proplastid. Sometimes a close topographical relationship between the vesicles and the lamellar system was noticed (Fig. 10).

More or less electrondense bodies, generally lacking a bounding membrane, are also present. In the subsequent developmental stages such bodies increase in number and fill nearly the whole cell (Fig. 11). They
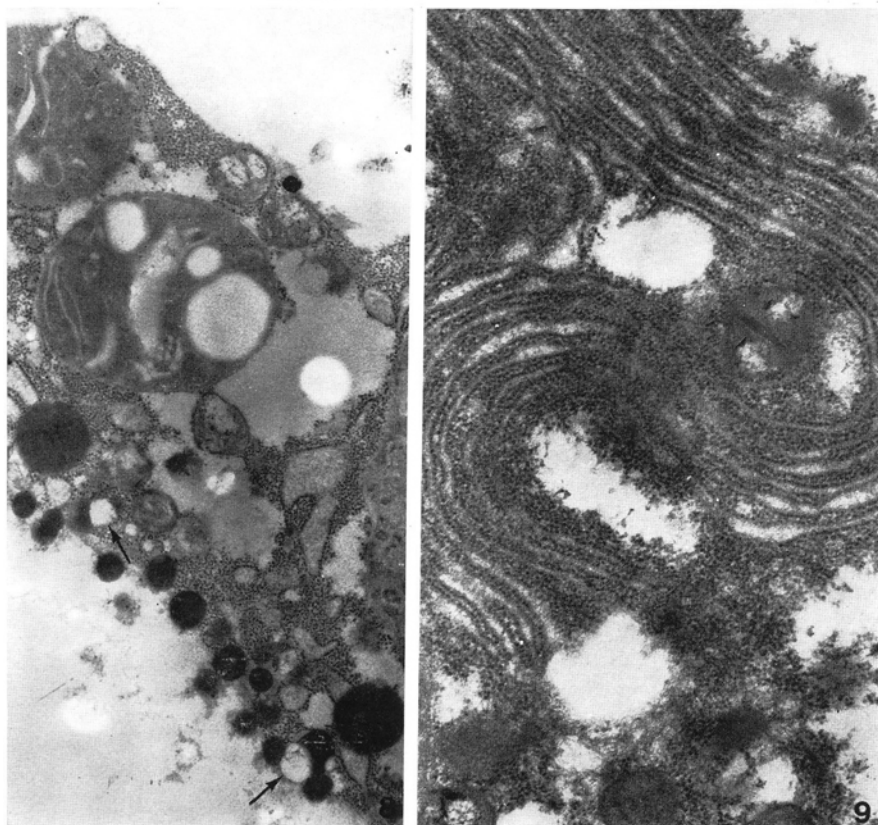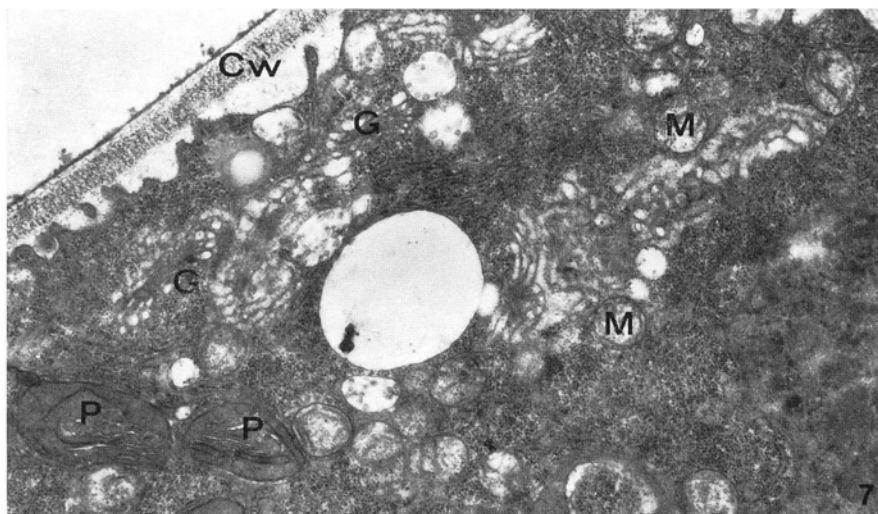
Fig. 7. — Electron micrograph of a tapetal cell at the early tetrad stage. The cell wall (Cw) is still evident and the plasmalemma appears highly concoluted. Golgi bodies (G) are conspicuous. Proplastids (P) are numerous as well as mitochondria (M), which appear roundish shaped. The « rough » ER appears well developed. x 19,000.

Fig. 8. — Electron micrograph of the tapetal cell periphery at the late tetrad stage. The cell wall has disappeared. Spherical bodies of various size appear along the plasmalemma. Some of them are contiguous with light vesicles (arrows). x 35,000.

Fig. 9. — Characteristic disposition of the tapetal ergastoplasma at the late tetrad stage. x 20,000.
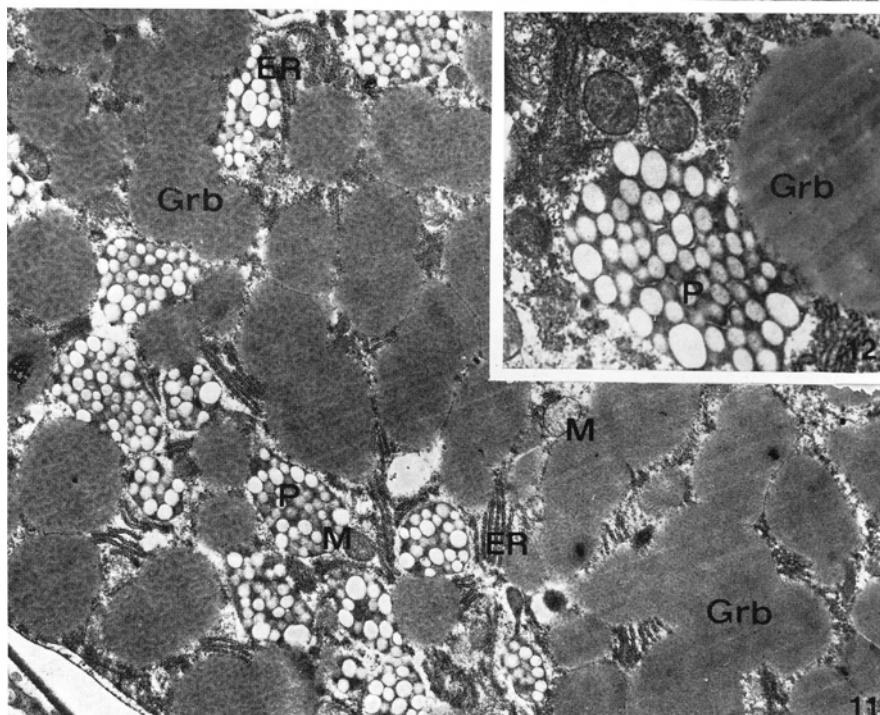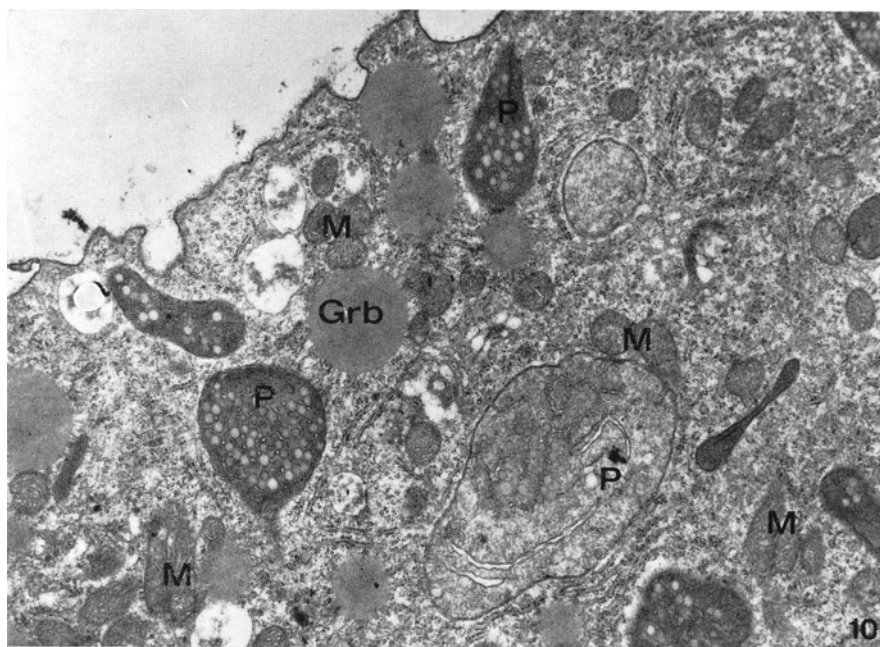
Fig. 10. — Electron micrograph of a tapetal cell now deprived of cell wall, delimited by the characteristically convoluted plasmalemma. Among the small dark mitochondria (M), large proplastids (P) are numerous. Their dark matrix contains many vesicles together with short lamellar portions. Several grey bodies (Grb) appear in the cytoplasm. x 15,500.

Fig. 11. — The number of grey bodies (Grb) appears extremely increased: proplastids (P) are now completely vesiculated. Small mitochondria (M) and ER fragments (ER) are still present. x 7,000.

Fig. 12. — Detail of Fig. 11, showing the close connection between a vesiculated proplastid (P) and a grey body (Grb). The proplastid is now deprived of limiting membrane. x 17,000.

appear simultaneously with the vesiculated proplastids, most of wich have lost their limiting membrane and show a close connection (Fig. 12) with the opaque bodies. Mitochondria, ribosome clusters and « rough » ER fragments are still visible. Many pollen grains are in close contact with the tapetal cells: in the contact areas the tapetal plasma membrane is typically convoluted and suggests a process of material extrusion towards the anther cavity (Fig. 13).

The final stage of tapetum development leads to the complete dissolution of the cell boundaries (Figs. 14 and 15), so that among the pollen grains ER fragments, mitochondria, large proplastids and many grey spherical bodies are visible. These bodies persist untill the end of anther maturation and represent the last tapetal residue.

DISCUSSION

From the early stages of development to the beginning of the tetrad formation, the tapetal cells are characterized by the presence of spherical bodies in the perinuclear space, ER and along the plasma membrane. These bodies are bounded by a single membrane studded with ribosomes, and seem to be in close connection with the cellular membrane systems. Some of them seem to originate from the ER, while others are in close contact with the nucleus.

In several areas those bodies appear connected with the nuclear content, while subsequently they become free. This phenomenon is not typical of *Antirrhinum maius*, but was observed also in other plants (unpublished data). On the basis of these observations we could assume that such bodies proliferate from the nucleus, and play a role in the nuclear-cytoplasmic information exchanges.

Similar bodies have been described by BARTH and VON RAHDEN (1967) in the perinuclear space of fertile cells of *Paeonia* anthers, up to the tetrad stage. In the pollen grain such bodies have been interpreted as related to information transmission for wall synthesis (BARTH and VON RAHDEN 1967). Also BELL (1972) pointed out phenomena of nuclear material extrusions, which could bring to the neoformation of organelles like plastids or mitochondria. Such extrusions wouldn't look like those we described in the perinuclear space of the tapetal cells of *Antirrhinum*, either for their meaning or origin. Besides, numerous studies on animal tissues (WATSON 1959; CLARK 1960; KESSEL and BEAMS 1963; KESSEL 1964; SZOLLOSI 1965) and particularly on germ cells report the presence of similar bodies in the perinuclear space. The Authors interpret such bodies as extrusions of nucleolar material, which probably transfers ribonucleoproteins or RNA from the nucleus to the cytoplasm.
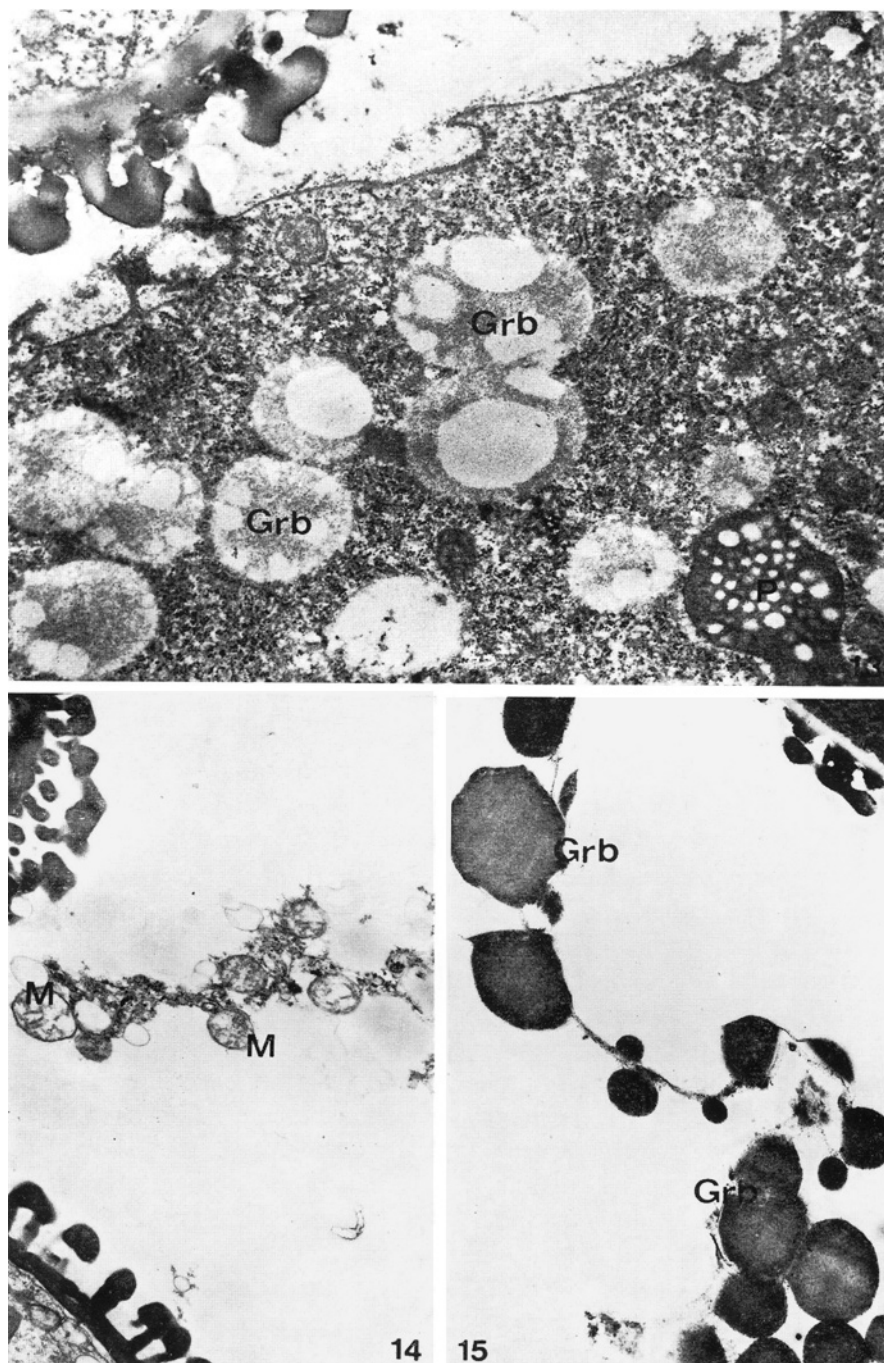
Fig. 13. — Close contact between a pollen grain and a tapetal cell: here the plasmalemma is typically convoluted and suggests a process of material extrusion towards the anther cavity. The cytoplasm contains numerous grey bodies (Grb) and vesiculated proplastids (P). x 19,000.
Fig. 14. — Electron micrograph of tapetal residues among the isolated pollen grains. Mitochondria (M) are still recognizable. x 15,500.
Fig. 15. — Grey spherical bodies (Grb) represent the last tapetal residue among the isolated pollen grains. x 11,500.

The apparently intense nucleo-cytoplasmic exchanges observed in *Antirrhinum* seem of great interest, because the bodies are present only in the stages when the sporogenous cells undergo mitosis and then meiosis.

Morphologically similar bodies are numerous also in the cytoplasm, particularly in connection with the ER. We agree with BARTH and MARQUARDT (1967) that these cellular features, which appear as isolated vesicles or cytoplasmic extrusion, are related to an enhanced ER membrane proliferation. They probably represent an enhanced ER activity.

The particularly high metabolic activity of the tapetum during the early stages of anther development is also denoted by the presence of a great number of intensely proliferating Golgi bodies. The activity of the Golgi apparatus ceases when meiosis ends, so that in tapetal cells in the tetrad stage the number of dictyosomes is markedly decreased, and also the cytoplasmic bodies have nearly disappeared. An enhanced Golgi activity, though not so conspicuous as in *Antirrhinum*, has been noticed in other plants (*Pelargonium*, unpublished data). In their study on the tapetal periplasmodium in *Tradescantia*, MEPHAM and LANE (1969) suggest that the dictyosome vesicles, gathered in « multivesicular bodies », can be related to an enzyme production, which would cause the early lysis of the tapetal wall towards the anther loculus. On the contrary in *Antirrhinum maius*, which has a typical secretory tapetum, the dictyosome activity does not coincide with the wall changes of the tapetal cells, but is contemporaneous with the conspicuous callose deposition around the sporogenous cells.

This observation is in agreement with those of ECHLIN and GODWIN (1968b) on the pollen mother cells of *Helleborus feotidus*. Only future autoradiographic studies will be able to establish whether during this stage the tapetal Golgi apparatus contributes to callose formation around the tetrads. Just after meiosis, when the young tetrads are surrounded by a thick common callose matrix, another kind of bodies appears in the tapetum: these bodies are spherical, electrondense and have no bounding membrane. They are localized at the periphery of the tapetal cells, just under the plasma membrane, which appears typically convoluted, while the pectocellulosic wall is completely dissolved. Clusters of similar bodies have already been described by several authors (ECHLIN and GODWIN 1968a; MARQUARDT *et al.* 1968; HESLOP-HARRISON and DICKINSON 1969; RISUEÑO *et al.* 1969; ECHLIN 1971; CHRISTENSEN *et al.* 1972) specially in the tapetal cell portion facing the anther loculus.

In *Sorghum* CHRISTENSEN (1972) speaks of « pro orbicules » turning into « orbicules », in connection with the sporopollenin deposition; in *Lilium* HESLOP-HARRISON (1969) repeatedly emphasizes the presence of « pro orbicular bodies » turning into « orbicular bodies »; these are vesicles

giving lipid reaction, equivalent to the « orbicules » and still involved in sporopollenin deposition. They gather on the outer surface of the tapetal cell, along the portion facing the anther loculus and build a nearly continuous wall, which can favour pollen dissemination (avoiding its adherence to the tapetum). On the basis of their morphology and the time of their appearance also the bodies we observed in *Antirrhinum maius* could be interpreted as « orbicules » or « orbicular bodies ».

Transfer of orbicular bodies to the pollen grains was not observed. Nevertheless when the tetrads dissolved and the pollen grains became free in the anther loculus, we frequently observed that the pollen grains were tightly appressed to the tapetal cells. In these areas the tapetal plasma membrane was always much convoluted. Most probably an exchange of material was going on between tapetum and pollen, but the characteristics and meaning of such exchanges remain obscure.

When the pollen grains have already differentiated, just before the tapetal cells undergo the final degeneration, a great amount of spherical « bodies » appears in these cells.

HESLOP-HARRISON (1968*a*, 1968*b*, 1969) emphasized the presence of similar bodies in *Lilium* tapetal cells at the end of anther maturation. The Author states that such bodies probably contain lipid substances which may support the carotenoids and form the « Pollenkitt ». We think that also in *Antirrhinum maius* such bodies can be related to the Pollenkitt formation, because they represent the last tapetal residue after its degeneration and appear randomly dispersed among the pollen grains. The following observations seem of particular interest: a) the low electron density of the vesicles at the beginning of the final stage, as compared with their greatly enhanced density at the end of this stage; in agreement with HESLOP-HARRISON (1968*a*, 1968*b*, 1969), we might suppose that during this stage a carotenoid increase occurs in these vesicles. b) The progressive intense vacuolation of the hole plastidial content and the apparent relationship between the disappearance of the plastid lamellar system and the appearance of the vesicles. c) The tight relationship between the degenerating plastids and the developing vesicles after the plastidial membrane has disappeared.

On the ground of these observations we might suppose that during this stage the increasing number and size of the spherical bodies can be at least partially related to the plastid changes, or that the latter can be responsible for the carotenoid increase in the « grey » bodies. When these vesicles move toward the anther cavity, we might think that they contribute to pollen grain differentiation at least as regards carotenoid deposition.

Mitochondria, too, undergo conspicuous changes: during the early stages of anther maturation they show an electron transparent matrix and

lack well-developed cristae, like the mitocondria of meristematic cells. On the contrary, after meiosis has completed, mitochondria have reduced size, an electrondense matrix and cristae which become particularly conspicuous in the final stages, just when the plastids degenerate. They maintain these characteristics until the complete tapetal dissolution.

Further studies will be needed to clarify which enzymatical or biochemical modifications are involved in these changes.

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SUMMARY

Anther sections of *Antirrhinum maius* in various stages of development have been examined with the electron microscope and the ultrastructural modifications of the tapetal cells have been followed during the development of the pollen grains. Characteristic of the early stages of development is the presence of a great number of intensely proliferating Golgi bodies and of spherical bodies, located in the perinuclear space in proximity of the plasmalemma and in the endoplasmic reticulum. They are bounded by a single membrane on which ribosomes are regularly ranged facing the body cavity. Probably the bodies located in the perinuclear space play a role in the nuclear-cytoplasmic information exchanges, while those in connection with the endoplasmic reticulum seem to be simply related to an enhanced endoplasmic reticulum activity. In the late tetrad stage a second body population appears. These bodies are spherical, electrondense and without bounding membrane. They are located at the cell periphery towards the anther cavity near the plasma membrane; probably they are connected with the sporopollenin deposition.

In the final stage of pollen development a large amount of grey bodies, generally lacking a bounding membrane, appears inside the tapetal cells, togheter with numerous proplastids, showing a dark and highly vesiculated matrix. Of particular interest seems the frequent close connection between the vesiculated proplastids and the grey bodies. It is supposed that the vesiculated proplastids contribute to the carotenoid increase in the grey bodies; probably the latter are responsible for the « Pollenkitt » formation around the differentiated pollen grains.

RIASSUNTO

Sono state esaminate al microscopio elettronico sezioni di antere di *Antirrhinum maius* in vari stadi di sviluppo e sono state studiate le modificazioni ultrastrutturali delle cellule del tappeto durante la maturazione del granulo pollinico. Negli stadi di sviluppo più precoci è caratteristica la presenza di apparati di Golgi in intensa proliferazione e di « bodies » sferoidali presenti nello spazio perinucleare, in prossimità del plasmalemma e del reticolo endoplasmico. Tali « bodies » sono delimitati da una membrana semplice recante ribosomi regolarmente allineati verso la cavità della vescicola stessa. Probabilmente i « bodies » situati nello spazio perinucleare rivestono grande importanza negli scambi di informazioni tra nucleo e citoplasma; i « bodies » in relazione con il reticolo endoplasmico sembrerebbero invece semplicemente collegati ad una elevata attività del reticolo stesso.

Nello stadio di tetrade avanzata, compare una seconda popolazione di « bodies »: sferoidali, elettrondensi, non delimitati da membrana. Tali « bodies » sono collocati alla periferia della cellula verso la cavità dell'antera subito sotto il plasmalemma: sarebbero in relazione alla deposizione di sporopollenina.