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Ultrastructure of the placental region in a liverwort *Mannia androgyna.*

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Riassunto

Lo studio ultrastrutturale ha rivelato la presenza di *transfer cells* in entrambi i lati della placenta di *Mannia androgyna* (L.) Evans (Marchantiales, Hepaticae).

Le possibili modalità di traslocazione dei nutrienti dal gametofito allo sporofito vengono discusse in relazione alla insolita organizzazione della porzione gametofitica della placenta che è costituita da diversi strati di *transfer cells* le cui caratteristiche ultrastrutturali variano significativamente in funzione della distanza dal piede dello sporofito.

INTRODUCTION

A distinctive feature of the contact region between the sporophyte and gametophyte in the Bryophytes so far ultrastructurally investigated is the presence of cells specialized in solute transport over a short distance, characterized by extensive wall ingrowths and an amplified plasma membrane. These cells, present in analogous histological situations in a variety of higher plants, are generally known as transfer cells (GUNNING & PATE, 1969, 1974; PATE & GUNNING, 1972).

Recent physiological data (THOMAS et al., 1979) on the nutritional status of liverwort sporophytes emphasize the importance of the sporophyte-gametophyte junction for the life stra-

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tegy of the hepatics. Like their moss (Paolillo & Bazzaz, 1968; Proctor, 1977) and hornwort (Thomas et al., 1978) counterparts, the sporophytes of liverworts, although capable of photosynthesis, need additional nutrients from the gametophyte for their growth and development, besides water and mineral ions.

The present paper records the occurrence of transfer cells at the sporophyte-gametophyte interface in *Mannia androgyna* (L.) Evans, a member of Marchantiales, as main cellular specialization of the whole sporophyte foot-gametophyte vaginula complex.

Material and Methods

Talli of *Mannia androgyna* bearing archegoniophores were collected in March 1981 on Lattari Mountains in the Peninsula of Sorrento (Italy). Feet surrounded by gametophytic tissue were isolated from sporophytes that contained nearly ripened spores. The specimens were fixed with 4% glutaraldehyde + 2% paraformaldehyde in 0.05 M phosphate buffer at pH 7.38 for 2h at room temperature, post-fixed in 1% OsO_4 in some buffer at pH 6.33 for 2h, dehydrated and embedded in Spurr's (1969) resin.

Ultrathin sections, obtained by cutting with diamond Knives, were counterstained with uranile acetate and lead citrate and examined by a Siemens Elmiskop 1A electron microscope at the Centro di Studio di Microscopia Elettronica (Faculty of Sciences, University of Naples).

Results

At the investigated stage of the sporophyte development, the placental region of *Mannia androgyna* is easily recognized due to the presence of transfer cells. The placenta includes the epidermal continuous cell layer of the bulbous foot of the sporophyte and several cell layers of the surrounding gametophyte vaginula. An interplacental space separates the two generations.

The sporophyte transfer cells (Tab. I) exhibit finger-like protuberances mainly on the walls delimiting the interplacental space. The extensively developed and highly branched wall ingrowths form an extremely complex labyrinth trapping pockets of cytoplasm some of which enclose organelles like mitochondria, microbodies and chloroplasts (Tab. II).

The wall protoberances laid down on the primary cell wall appear as a specialization of the secondary wall (Tab. III, fig. 1). They consist of a fibrillar core surrounded by a peripheral clear zone of fairly constant thickness which is bordered by the plasma membrane.

The consistency of the fibrillar cores varies along the cell wall labyrinth (Tab. III, fig. 1). In proximity of the primary wall, where the protuberances appear fused, the microfibrillar material is wider and more loosely arranged than in the middle region of the labyrinth where the branched protuberances display the most compact and dense cores. Next to the cell interior, occasionally coalescing light interfacial zones embed several cores and contain numerous small membrane-bound vesicles apparently similar to those actively formed by the Golgi apparatuses.

The peculiar organization of the vacuolar system formed by a great number of small approached vesicles (Tab. I), the abundance of irregularly shaped chloroplasts (Tab. IV) as well as of mitochondria always provided with numerous cristae (Tab. I, II) are the most noticeable cytoplasmic specializations of the sporophyte transfer cells which also contain a large irregularly shaped nucleus, a rough endoplasmic reticulum (Tab. I), polysomes and active dictyosomes (Tab. III, fig. 3).

The chloroplasts (Tab. IV), always devoid of starch, have a poorly developed lamellar apparatus but contain abundant vesicles budding from the internal plastid envelope.

Lipid reservoirs are scarce (Tab. IV) and some plasmodesmata (Tab. III, fig. 2) connect the transfer cells to one another and with the internal cells of the foot.

The gametophytic side of the placenta, on the contrary, consists of several layers of transfer cells whose ultrastructure markedly varies with the distance from the sporophyte foot.

The cells closely abutting the foot are more or less severely damaged (Tab. I), only apparent from the outline of their flattened walls (Tab. V) or totally obliterated as the large lacunae (Tab. I) filled with various amorphous materials account for. The degeneration of the cytoplasmic contents appears to precede the lysis of the wall labyrinths in these cells which are no longer connected by plasmodesmata to the neighbouring vaginula cells.

Such cells (Tab. V) exhibit apparently intact but markedly dense cytoplasms containing the nucleus, mitochondria, chloroplasts, vacuoles, polysomes and enormous granules probably of lipids. A very highly developed labyrinth, often in incipient lysis, is apparent on the inner tangential walls of these cells. Their wall ingrowths, composed of coarsely textured dense cores, are extensively fused and no longer surrounded by the plasma membrane, except next to the cell interior where clear interfacial zones containing abundant vesicles appear to coalesce. The extension of the clear zone along the radial and outer tangential walls results in the distruption of the symplastic connections of these cells, among which likely schizogenous intercellular spaces also occour.

The wall labyrinth in the underlying transfer cells (Tab. VI) is formed by individual branched protuberances whose frequency and dimension decrease with the distance from the foot. Typically, their cytoplasms contain many mitochondria, rather developed chloroplasts always with vesicles, long stacked sheats of endoplasmic reticulum, and microbodies frequently associated with lipid granules.

Periphericallly, the gametophyte vaginula consists of closely approached and highly vacuolated cells devoid of wall protuberances.

Discussion

Ultrastructural studies on the sporophyte-gametophyte junction of Bryophytes revealed that the position of the transfer cells is rather variable in the placenta.

With the exception of the polytrichaceous mosses (Maier, 1967; Hébant, 1975) which lack gametophytic transfer cells and of the hornwort *Phaeoceros* (Gambardella et al., 1981) which, on the contrary, shows only gametophytic transfer cells, the most common arrangement observed in mosses (Eymé & Suire, 1967; Wiencke & Schulz, 1978; Browning & Gunning, 1979a; Lal & Chuahuan, 1981; Ligrone et al., 1982a) and liverworts (Kelley, 1969; Gambardella & de Lucia Sposito, 1983) consists of one layer of sharply outlined transfer cells on both the sporophytic and gametophytic sides of the placenta.

Multilayered gametophytic transfer cells, recordered in the archaic moss *Buxbaumia piperi* (Ligrone et al., 1982b) and in *Mannia androgyna* (this study) constitute a rather unfrequent arrangement of the bryophyte placenta.

Although the effectiveness of the bryophyte placental transfer cells in solute transport processes was experimentally proved (Browning & Gunning 1979b, c) it is still unclear what is the relation of the above mentioned variability of arrangement to any functional aspect.

In *Mannia androgyna* the occurrence of several layers of transfer cells on the gametophytic side of the placenta is unlikely related to an enhancement of the solute transport function towards the sporophyte foot.

In these cells, indeed, an obvious gradient of cellular damage can ben observed, that decrease with the distance from the sporophyte.

Therefore, only the transfer cells farther from the foot (Tab. VI) could be actually efficient in the translocatory function notwithstanding their exiguous wall-membrane apparatuses. Stacked endoplasmic reticulum and abundance of mitochondria observed in these cells are cytoplasmic specializations carachteristically accompanying the wall labyrinth of efficient transfer cells (Gunning & Pate, 1974).

On the contrary, nothing but a weak translocatory capability can be postulated for the transfer cells closer to the foot. Although exhibiting still intact cytoplasms, these cells, which are practically disjoined from the external transfer cell layer, as intercellular spaces and nearly brocken plasmodesmata account for, show wall labyrinths in which the surface amplification of the plasma membrane is largely lost because the extensive fusion of their ingrowths. Furthermore, the coalescence of the light interfacial zones in the wall labyrinths strongly suggests that the process of tertiary wall formation is in progress. This process, observed in ageing transfer cells of mossess (MAIER & MAIER, 1972; BROWNING & GUNNING, 1979a), results in wall labyrinth completely fused by deposition of light materials which are thought to minimize the water flow between the two generations.

A higher rate of labyrinth development in the gametophytic transfer cells than in the sporophytic ones is suggested by the observation that the wall labyrinth in the latter cells is formed by individual ingrowths with scarcely coalesced interfacial zones.

This might explain the multistratification of transfer cells observed in the vaginula. Before the older gametophyte transfer cells closer to the sporophyte foot reach the stage of tertiary wall labyrinth, the underlying cells in the vaginula develop their translocatory potentialities forming their wall-membrane apparatuses.

Furthermore, the observation that aged gametophytic transfer cells degenerate and obliterate suggests that some influence, mechanical and/or enzymatic, emanating from the sporophyte foot may be also involved.

Within the investigated Bryophytes, the less developed transfer cell chloroplasts are those found in the placenta of *Mannia*, expecially in the sporophytic side. For that reason a direct involvement of the photosynthesis in the translocation processes can be exluded. Indeed, the formation of the thylakoid system, by means of vesicles budding from the internal plastid envelope, is occurring when the nutrient request of the sporophyte capsule, containing nearly ripened spores, is presumably very low.

Lipid granules in the gametophyte transfer cells frequently in association with microbodies, likely with glyoxysomal activity (RICHARDSON, 1974), may provide a compact source of stored nutrients to be translocated into the sporophyte.

SUMMARY

Ultrastructural investigations revealed the presence of transfer cells on both sides of the placenta of *Mannia androgyna* (L.) Evans (Marchantiales, Hepaticae). The gametophyte-sporophyte translocation capabilities are discussed in relation to an unusual arrangement of the gametophytic side of the placenta where several layers of transfer cells exhibit noticeable ultrastructural variability with the distance from the soprophyte foot.

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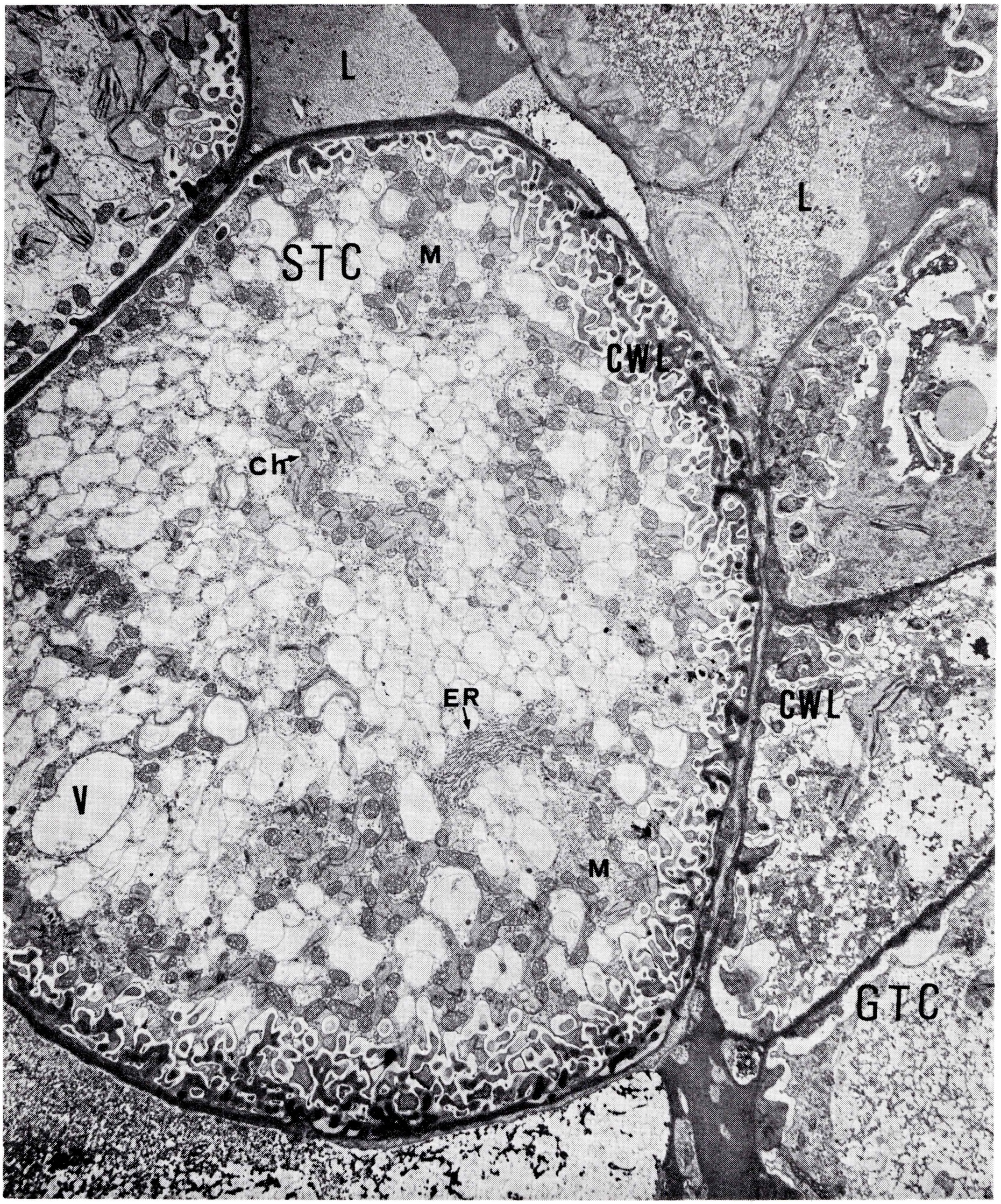
TAB. I - Transection of the sporophyte-gametophyte junction. An interplacental space separates the sporophyte (STC) and gametophyte (GTC) transfer cells whose labyrinths (CWL) are expecially developed on their opposite tangential walls. Generally, the gametophyte transfer cells closest to the foot are severely damaged, or totally destroyed as the large lacunae (L) containing amorphous materials account for. x 2.750 Ch = Chloroplast; ER = Endoplasmic reticulum; L = Lacuna; M = mitochondrion; V = Vacuole.

Table I



TAB. II - The sporophyte transfer cell wall ingrowths, composed of a fibrillar core and a peripheral light interfacial zone, form a complex labyrinth in which pockets of cytoplasm enclose cell organelles. Many mitochondria (M) accompains the wall-membrane apparatus. x 11.250 Ch = Chloroplast; L = Lacuna; Mb = Microbody.

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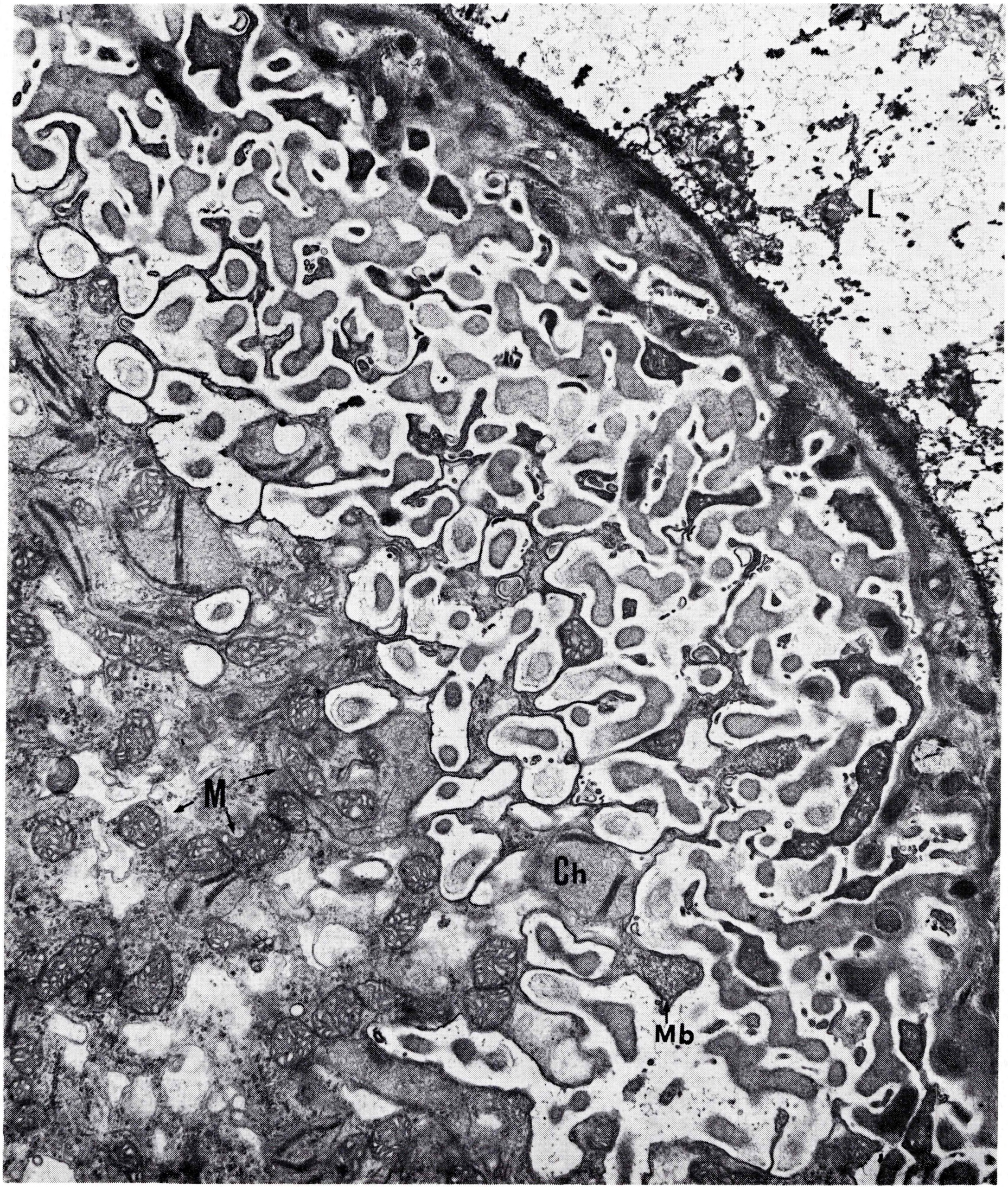
TABLE II

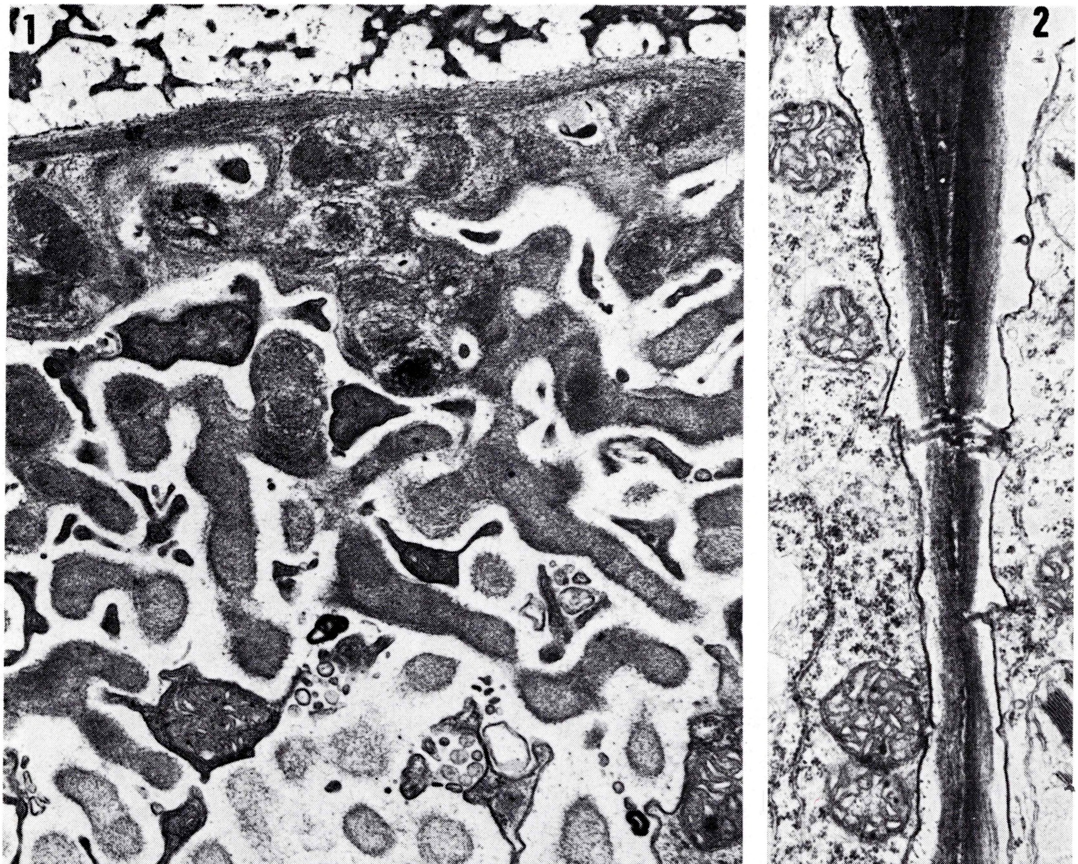


TAB. III - Fig. 1. - Sporophyte transfer cell wall ingrowths laid down on the primary cell wall. The fibrillar core of the protuberances are differently textured along the wall labyrinth. Sometimes the clear interfacial zone are coalesced next to the cell interior and contain numerous small membrane-bounded vesicles. W 19.500.

Fig. 2. - Plasmodesmata crossing the radial walls of sporophyte transfer cells. x 19.500.

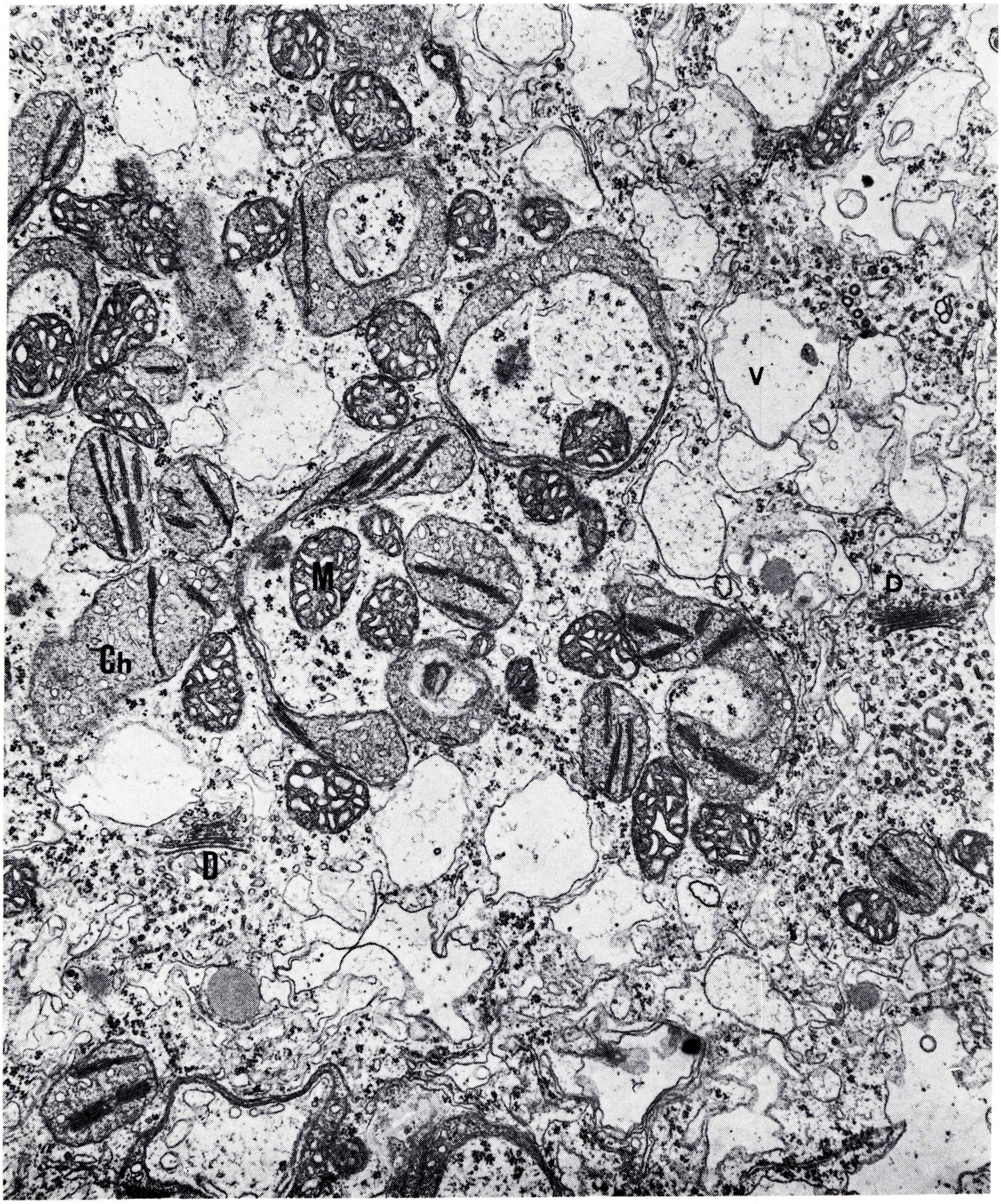
Fig. 3. - Dictyosomes and endoplasmic reticulum with associated polysomes in sporophyte transfer cells. x 32.000.

TABLE III



TAB. IV - Typical appearance of the sporophyte transfer cell cytoplasm rich in ribosomes, endoplasmic reticulum, vesicles produced by dictyosomes (D) small vacuoles (V) and mitochondria (M) always provided with numerous cristae. The irregularly shaped chloroplasts (Ch) contain a poorly developed lamellar apparatus and abundant vesicles budding from the internal plastid envelope. x 16.700.

TABLE IV



TAB. V - Gametophyte vaginula transfer cells. Fused ingrowths, composed of loosely textured cores and widely coalesced interfacial zones, form a labyrinth (CWL) more extensive than in the sporophyte transfer cells (STC). Notice the incipient lysis of the wall labyrinth in the cell marked by asterisk. Severely damaged vaginula cells (DVC) directely abutting the foot. x 5.600 — Ch = Chloroplast; IS = Intercellular space; LG = Lipid granule; M = Mitochondria; N = Nucleus; V = Vacuole.

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Table V

TAB. VI - Vaginula transfer cell farther from the foot. The wall labyrinth (CWL) is formed by individual ingrowths with compact cores and no-coalesced light interfacial zones. The chloroplasts (Ch) have a rather developed thylakoidal system. x 5.600 — ER = Endoplasmic reticulum; IS = Intercellular space; LG = Lipid granule; M = Mitochondria; Mb = Microbody; N = Nucleus; Pd = Plasmodesmata; V = Vacuole; Arrow indicates diretion of sporophyte.

TABLE VI