ULTRASTRUCTURE AND MECHANISM OF SECRETION IN EXTRAFLORAL NECTARIES OF RICINUS COMMUNIS L.

FLÓRA KÁLMÁN and S. GULYÁS

Department of Botany, Attila József University, Szeged

(Received October 12, 1973)

Abstract

Electronmicroscopic examinations of the cells of the extrafloral nectary of *Ricinus communis* L. were used to study the question of whether the nectar transport is a granulocrine process linked to a cell-component, or the result of molecular transport.

It could be concluded from the results that the fluid transport is a function of the lomasomes: there are many in the grandular tissue cells of the functioning nectary, and there are many mitochondria around them. The cell wall accumulating role of the lomasomes is not probable, since they can be found only in functioning glandular tissue cells, and significant wall-building processes can not be observed in the period of secretion.

To date the study of the relation between the fine structure and function of the nectaries has been dealt with by many research workers, e.g. MERCER and RATHGEBER (1962), WHRISCHER (1962), SCHNEPF (1964a, b), EYMÉ (1966a, b, 1967), VASILIEV (1969), FAHN and RACHMILEVITZ (1970), FINDLAY and MERCER (1971), VASILIEV (1971) and RACHMILEVITZ and FAHN (1973).

In spite of this, there are at present a fair number of conceptions as to the mechanism of nectar secretion. Nor has the important question been clarified as to the nature of the cell organelle to which the fermentative transformation of the sugars, for example, and the nectar transport are linked.

In our work we have studied the ultrastructure of the extrafloral nectary to establish whether the transport is a granulocrine process related to a cell component, or whether the phenomenon of molecular transport occurs.

Studies to date indicate the presence of well-developed ER in the nectarogenous cells. MERCER and RATHGEBER (1962), SCHNEPF (1964a, b), and FAHN and RACHMILEVITZ (1970, 1973) have suggested the possibility that vesicles of reticular origin might take part in the transport of the sugary fluid.

EYMÉ (1966a, b, 1967) and FINDLAY and MERCER (1971) have described multivesicular structures from the secreting nectaries. EYMÉ conceived that the sugar is secreted as a fluid via vesicles of Golgi origin.

A very recent paper (RACHMILEVITZ and FAHN 1973) takes into account the previous findings and concludes that the sugary fluid is secreted by vesicles of ER origin.

Another type of conception is transport by molecular means. As a result of his observations, SCHNEPF (1964a, b) reached the conclusion that the plasmalemma is the site of localization of enzymes and carriers which assist the transport of sugar molecules across the membrane.

According to VASILIEV (1969), the protoplasm of the cells does not take part directly in the transport of the bulk of the nectar. Part of the phloem fluid proceeds passively in the cell wall, and part by means of an active transport mechanism in the protoplasm.

Materials and Methods

Segments from the individualt issue areas from the extrafloral nectary of *Ricinus communis L.* in various stages of ontogenetic development were fixed in 3% glutaraldehyde. After the 3-hour fixation, the tissue pieces were washed in phosphate buffer and postfixed in 1% buffered OsO_4. The material was dehydrated in ethanol and embedded in Durcupan ACM.

The ultrathin sections were stained with uranyl acetate and lead citrate, and examined with TESLA 242 D and JEM 100 B electronmicroscopes.

For the light-microscopic examinations the nectaries were embedded in celloidin.

Observations

The automorphic extrafloral nectaries of ricinus develop at the two ends of the leaf-stalk, in the vicinity of the base and leaf-plate. Viewed from above they resemble an elongated disc. *Ricinus* is one of those rare plants in which the nectaries also appear on the cotyledon.

The glands vary as to number and site of occurrence. They generally occur singly on the lower part of the leaf-stalk, whereas one or two large glands may develop near the plate.

Our histogenetic examinations show that the nectary is formed completely before the final development of the leaf-plate and leaf-stalk.

From a structural aspect the nectary consists of two important tissue regions: glandular tissue and parenchyma.

Thick cuticle covers the epidermal cells over the nectary. The epidermis covering the glandular tissue consists of column-shaped cells. Among these it was also possible in places to observe ducts covered by cuticle. The ducts are interconnected with the small intercellulars between the epidermis and glandular tissue, where the nectar is preliminarily collected after secretion from the glandular tissue.

The small-cell glandular tissue is surrounded by parenchyma, in which mainly calcium oxalate and starch accumulate. Vascular bundles consisting of yxlem and phloem elements run into the parenchyma from the leaf-stalk in the direction of the glandular tissue. Before they reach the vicinity of the glandular tissue they are richly ramified. The tracheal and tracheidal lines finally continue into xylem parenchyma. The terminal branches of the phloem part run directly up to the glandular tissue. From here the phloem fluid is transferred to the glandular tissue cells by cells of a "transfusion" nature.

Ultrastructure of nectary cells in the stage of secretion

In the study of the fine structure we paid the greatest attention to the structure of the glandular tissue cells, since it is to this tissue region that the transformation of the phloem fluid and the secretion of the nectar are attributed.
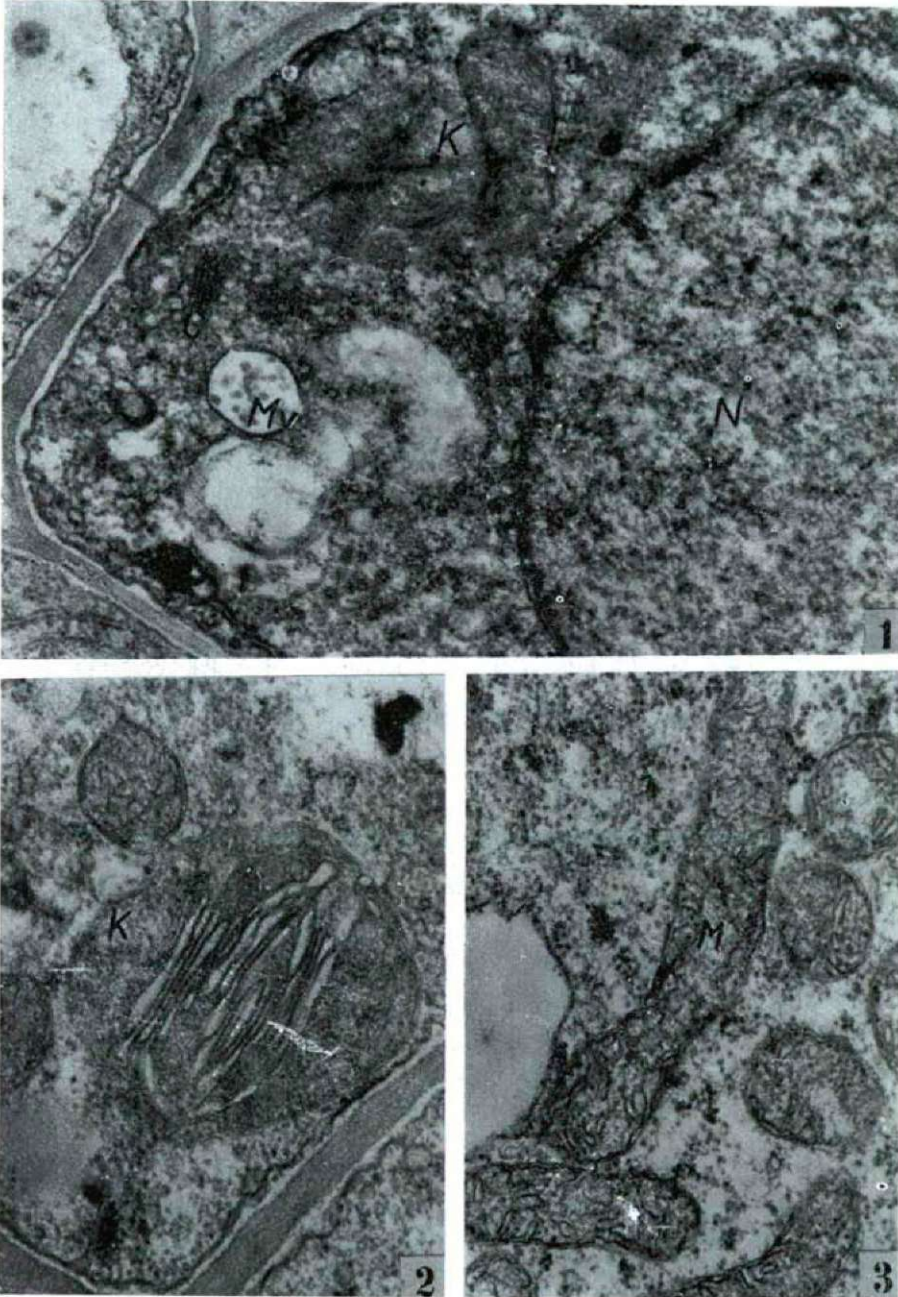
Fig. 1. 1. Cell detail from glandular tissue. The closeness of the multivesicular bodies to the Golgi
apparatus points to their possible origin (x27 000).
2. Chloroplast cross-section from the phase of maximum secretion (x34 000).
3. The number of mitochondria correlated with the secretory activity is very high (x34 000).

Light-microscopically, this consists of isodiametric cells. At the beginning of secretion the cytokar ratio in these cells is 1:1, while later the amount of cytoplasm dominates.

The ricinus nectary cells are only relatively rich in granular ER, in contrast with the floral nectaries, where hyperactive agranular or granular ER has been observed by the above-mentioned authors. In ricinus a real lacunar system develops at several places between the membranes of the reticulum, the cisternae are distended, and the isolation of smaller vesicles is common.

Mono- and polyribosomes can be seen in large numbers in the cytoplasm.

There are few chloroplasts; they vary in size in the range 3—4 µ and are ovate, but are frequently deformed. The lipoprotein lamellar system is condensed in the central part of the granular stroma. The grains comprise 2—5 grana vesicles. The strach grains (8—10) can be found near the poles of the chloroplasts. The weakly developed lamellar system is indicative of minimal photosynthetic activity (Fig. 1,2).

The mitochondria are very varied in shape. Besides the elongated form, it is also possible to observe U, Y and ramified forms too. Their length is generally 1.8—2.4 µ, and there are very many of them in the secretory glands (Fig. 1,3).

The Golgi apparatuses are localized near the vacuole-system of the cells, and sometimes in the vicinity of the multivesicular bodies. Similarly as indicated by the observations of FAHN (1970), their number is not indicative of high activity. They may participate in the secretion, but because of their low occurrence their role in the transport processes is difficult to conceive (Fig. 1,1., Fig. 2,3).

Multivesicular bodies were also observed in the ageing, and no longer secreting nectaries. The diameters of these is 0.5—0.7 µ; their role in the secretion has not yet been elucidated. Their closeness to the Golgi apparatus points to their possible origin. A large number of vesicles of dense content can be seen within the membrane (Fig. 1,1).

The wall of the glandular tissue cells consists exclusively of cellulose. According to the literature data published so far, this tissue is without intercellular. Micro-photographs reveal that on the meeting of cells (in a functioning gland) intercellulars with loose structure are found in all cases. In the course of the gradual ageing of the nectary this spongy structure becomes increasingly broken up, and lacunae develop in it.

In the peripheral and central parts of the cytoplasm a strikingly high number of large-volume lomasomes were observed (Figs. 2—3). On the sectional surface of one cell there are 5—6 lomasomes, occupying a very large volume compared to the amount of the cytoplasm. This observation holds for all glandular tissue cells. It should be noted that lomasomes were not observed in non-functioning glands. These organelles are present in the part of the cytoplasm near to the nucleus, just as the vicinity of the plasmalemma. Taking into consideration a picture (Fig. 2,1) showing vesicles crossing the plasmalemma, it seems justified to assume that in the present case the lomasomes take part not in the accumulation of the wall material (as is beginning to receive general acceptance), but in the nectar transport. The vesicles of the lomasomes contain dense material. The closeness of these organelles to the mitochondria and ER is striking. It can be seen in Fig. 3,3 that the ER closely adheres to the external membrane of the lomasome. Between the larger vesicles, which probably contain phloem fluid, small vesicles can be observed, similar in size to the diameter of the reticulum. All this permits the conclusion that those fermentative processes which result in a sugar composition characteristic of ricinus
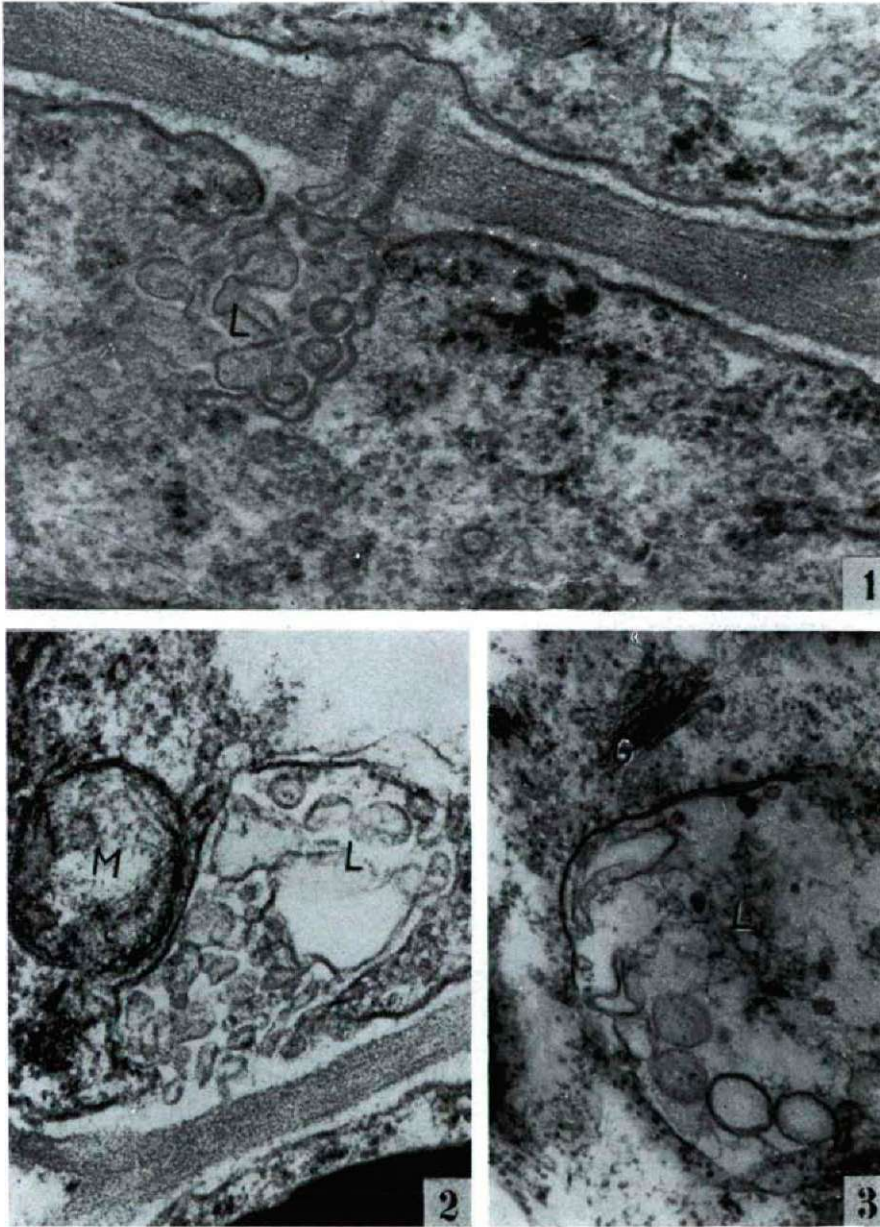
Fig. 2,1. The vesicles of the nectar-transporting lomasomes cross the plasmalemma (x74 000).
2. The energy necessary for the fermentative processes in the lomasomes is provided by the mitochondria (x39 000).
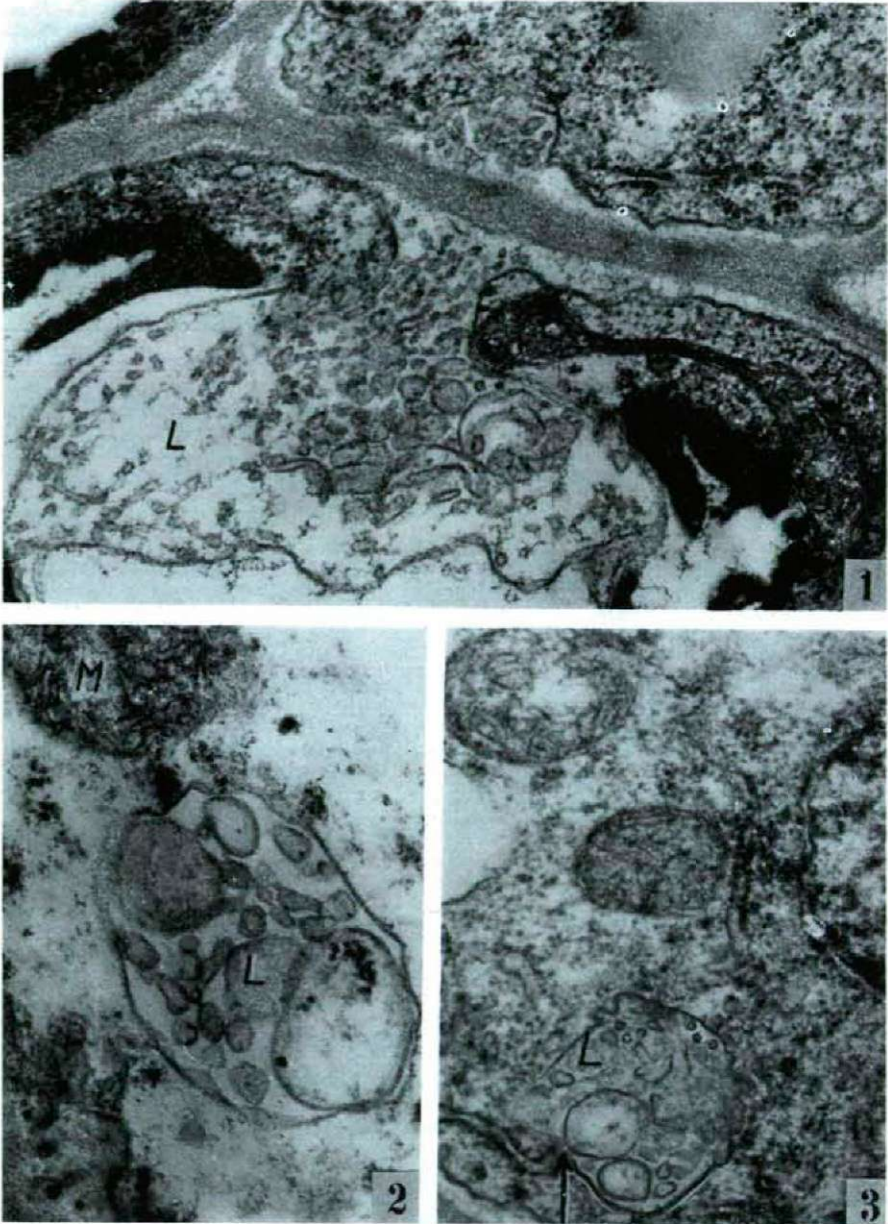3. Lomasome and Golgi apparatus (x35 000).

Fig. 3,1. The lomasomes take part not in the accumulation of the wall material in the cells, but in the nectar transport. For a possible minimal wall-material accumulation the participation of the lomasomes with such a significant volume is not considered necessary (x39 000).

2. 43 000x.

3. Vesicles similar in size to the ER diameter between the vesicles of the lomasome. A close connection of the ER can be observed (arrow) (x35 000).

nectar from the phloem fluid, take place within the lomasome. The energy necessary for the processes is provided by the mitochondria in the vicinity of the reaction.

The secretory surface of the nectary:

The ultrastructure of the column-shaped epidermal cells covering the gland agrees essentially with the fine structure characteristic of the glandular tissue. The only difference appears in the vacuolization. Numerous small vacuoles can be seen in the peripheral cytoplasm, while the central ones are fused into one central vacuole (Fig. 4,4).

The surface of the columnar epidermal cells is covered by very thick (5 μ) cuticle. Light-microscopic examinations showed that ducts bounded by cuticle also occur between the columnar epidermal cells, and we assume that the bulk of the nectar may reach the surface via these.

Electronmicroscopic examinations supported the above findings. The ducts run to the surface and there broaden out like a funnel. Their content is osmiophilic, and this can be observed continuously under the cuticle covering the epidermal cells.

Ultrastructure of nectaries in period following secretion

A considerable change occurs at the organelle level in the structure of the glandular tissue cells in the period following secretion. Deep cavitation proceeds on the surface of the nucleus, its shape is extended and it exhibits varied forms. Chromatin may accumulate in aggregates in the karyoplasm (Fig. 4,1).

Vacuolization of the cytoplasm is enhanced. The presence of autophage vacuoles is also indicated by ZIEGLER (1968) and VASILIEV (1971). Cytoplasm, ER and ribosomes can be observed in the autophage vacuole shown in Fig. 4,1. The amount of ER decreases, and the membranes are frequently fragmented.

The originally ovate chloroplasts flatten out, but their lamellar system is substantially more developed than in the previous stage, while the number of grains increases by a factor of 3. Moniliform lipoid drops are secreted below the peristromium (Fig. 4,1). The gland now obtains the greater part of the carbohydrates necessary for its vital processes not via the sieve elements, but by producing them during the photoreactions (Fig. 4,3).

The appearance of the peroxysomes is striking (Fig. 4,2), which indicates that the functions are changed in the nectarogenous cells.

The Golgi apparatus does not exhibit a numerical decrease, while in addition to the cisternae vesicles with a dark content can be seen.

The cell wall is not thickened even in the aged state. Lacunae develop from the loose-structured intercellulars of the secretory glandular tissue.

There are fewer mitochondria and chloroplasts in the parenchyma cells of the nectary. The cytoplasm forms a thin layer along the cell wall. The major part of the volume of the cells is comprised of the coherent vacuole system. The cell wall layers separate from each other in several places. By the end of the secretion period the plasm of the parenchyma cells, originally fulfilling a storing role, is contracted, its place being taken over by the ever larger vacuoles.
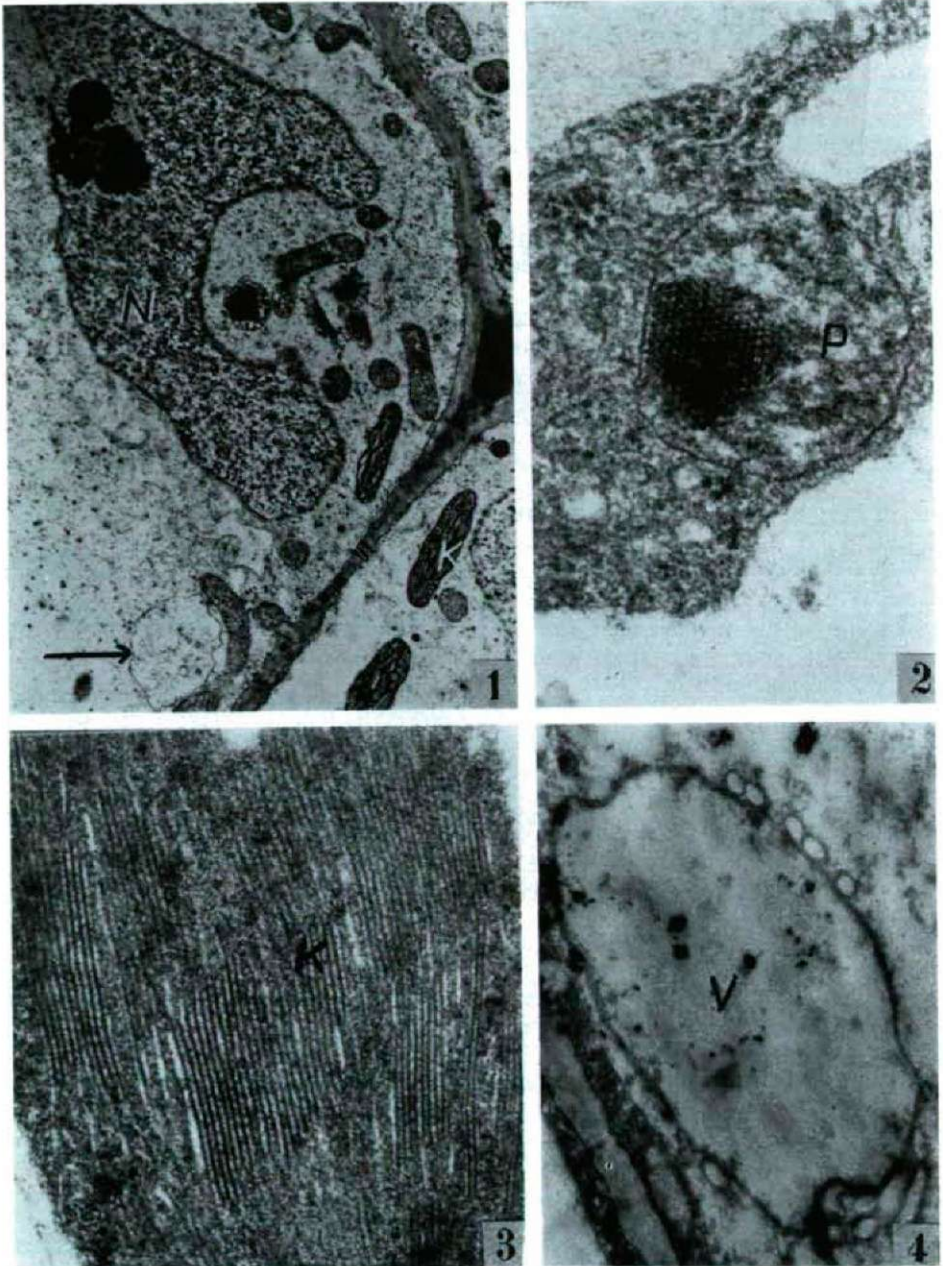
Fig. 4,1. Glandular tissue cells in the period following secretion. Autophage vacuoles appear in the cytoplasm (x20 000).
   2. Peroxysome from grandular tissue cell (x56 000).
   3. The lamellar system of the chloroplasts is more developed (x60 000).
   4. Detail from columnar epidermal cell, with vacuole (x20 000).

Discussion

By examining the ultrastructure of the extrafloral nectaries of ricinus, we tried to find an answer to the question of whether the nectar transport is a granulocrine process linked to a cell component, or proceeds by molecular transport.

There is, practically unanimous agreement (MERCER and RATHGEBER, 1962; WHRISCHER, 1962; SCHNEPF, 1964a, b; EYMÉ, 1966a, b; 1967; VASILIEV, 1969, 1971; FAHN and RACHMILEVITZ, 1970, 1973; FINDLAY and MERCER, 1971) that the most highly developed organelles in the cells of the various nectaries are the mitochondria and ER.

In physiological examinations, ZIEGLER (1955) found that the respiration of the nectaries is very intensive in the period of secretion. This was confirmed by electronmicroscopic examinations. These observations led MERCER and RATHGEBER (1962), SCHNEPF (1964a, b) and FAHN and RACHMILEVITZ (1970, 1973) to conclude that the ER somehow fulfils an important synthetic and transport function. These authors generally studied floral glands. In such glands, however, it is necessary to reckon with many factors which are not involved in the functioning of extrafloral glands. Thus, the development processes of the seed and ovary, the steroid synthesis assumed by VASILIEV, and possibly the enzyme demands of these processes may be interrelated with the high development of the ER.

The functioning of the extrafloral glands can be regarded as somewhat more simple.

EYMÉ (1966) studied the floral nectary of *Ficaria,* and found that one of the most characteristic features of the ultrastructure is the presence of the membrane vesicles. He assumed that these vesicles of Golgi origin ensure the entry of the fluid into the nectarogenous cells by means of characteristic pinocytosis, move towards the plasmalemma, and secrete their content there. The observations of MERCER and RATHGEBER (1962) and FAHN and RACHMILEVITZ (1970, 1973) indicate that the nectar is secreted with the aid of the ER vesicles. In 1964, SCHNEPF assumed sugar transport by monomolecular means.

VASILIEV (1969) concluded from his studies that the protoplasm of the cells does not take part directly in the transport of the bulk of the nectar. He discounts the observations and conceptions of EYMÉ; the described high Golgi activity is valid only for the Ranunculaceae family, and the vesicles more distant from the Golgi cisternae are not of Golgi origin, but cross-sections of the ER. According to VASILIEV (1969), in the floral nectary of *Acer* the bulk of the phloem fluid bypasses the protoplasm of the cells and moves passively in the cell wall towards the secretion surface. A certain proportion of the quantity of nectar, however, does progress in the plasma of the secretory cells by means of active transport. And since the nectary cells absorb certain substances at the time of active secretion, from the fluid migrating along the cell wall, the protoplasm reabsorbs the necessary materials.

The presence of multivesicular bodies in the nectarogenous cells is mentioned by EYMÉ (1966), FINDLAY and MERCER (1971) and FAHN and RACHMILEVITZ (1970, 1973), who also point to their transport role.

In contrast with some of the above authors, we consider that the fluid transport is carried out by the lomasomes.

Very many authors have dealt with the lomasomes since the report of GILBART (1961), and their origin has been reviewed by MARCHANT and ROBARDS (1968). At

present their function has not been completely clarified: they may participate in the secretion, cell wall formation, haustorial absorption, glycogen synthesis, membrane proliferation, cytoplasm degeneration, response to stress, etc. (BRACKER, 1967; HUGHES and BISALPUTRA, 1970).

The phloem fluid, with a sugar content of 10—25%, passes the pores of the cribriform plate into the "transfusion" type cells, where the fluid is probably enclosed by cytoplasmatic membrane, and as lomasomes the phloem fluid containing vesicles traverse the plasmodesm to reach the adjacent glandular tissue cells.

These organelles, in relatively high numbers and forming a very large proportion of the amount of cytoplasm, appear in the glandular tissue cells. Their transport function is proved by Figs. 2—3.

The vesicles of the lomasomes contain the untransformed phloem fluid, in which the materials for reabsorption are also present. We assume that the fermentative transformation proceeds within this formation, whereby the mainly saccharose-containing fluid is converted to a sugary fluid with a composition charcteristic of the nectar of ricinus. The energy requirements of the process are provided by the mitochondria in reaction-proximity to the lomasomes (Fig. 2,2; Fig. 3, 1—2). The close connection of the ER and the lomasomes can similarly be observed (Fig. 3,3). Among the larger vesicles with a dense content, smaller vesicles, similar in size to the diameter of the ER and presumable of ER origin, are also present; these carry or transport the enzymes necessary for the carbohydrate metabolism to the site of reaction.

The lomasomes can not have a cell wall accumulating effect, since in the period of secretion (ca. 10 days) no substantial wall-forming processes take place. Moreover, for a possible minimal cell-wall accumulating process neither is it considered necessary for the lomasomes to take part with such an appreciable volume, and to migrate in those cells in which the main function in the given period is the fermentative transformation and the transport.

The presence of lomasomes was not observed in cells not carrying out secretion. The appearance of the peroxysomes is striking; this indicates that the function of the nectarogenous cells is changed. The lamellar system of the chloroplasts is developed (Fig. 4,3) and the gland now acquires the bulk of the carbohydrates required for the vital processes of the cell in the course of photoreactions.

References

BARBIER, E. (1956): Investigation on nectar concentration. — XVI. Int. Beokcep. Congr. Prelim. Sci. Meet.

BARTON, R. (1965): An unusual organelle in peripheral cytoplas mof Chara cells. — Nature 205, 21. — Electron microscope studies on surface activity in cells of Chara vulgaris. — Planta (Berlin) 66, 95—105.

COLE, K. and SHU-CHANG LIN (1970): Plasmalemmasomes in sporelings of the brown alga Petalonia debilis. — Canad. J. Bot. 48, 265—268.

CRAWLEY, J. C. W. (1965): A cytoplasmic organelle associated with the cell walls of Chara and Nitella. — Nature 205, 200—201.

DAUMANN, E. (1930): Das Blütennektarium von Magnolia und die Fütterkörper in der Blüte von Calycantus. — Planta 11, 108—116.

ECHLIN, P., GODWIN, H., CHAPMAN, B. and ANGOLD, R. (1966): The fine structure of polyvesicular bodies associated with the cell walls in the developing anthers of Ipomoea purpurea L. (Roth). —Sixth Int. Congr. Electron Microsc., Kyoto. pp. 317—318.

EDWARDS, M. R. (1962): Plasmalemma and plasmalemmasomes of *Listeria* monocytogenes. — Eighth Int. Congr. Microbiol., Montreal. p. 31.

ESAU, K., CHEADLE, V. I. and GILL, R. H. (1966): Cytology of differentiating tracheary elements, II. Structures associated with cell surfaces. — Amer. J. Bot. *53*, 765—771.

EYMÉ, J. (1966a): Infrastructure des cellules nectarigénes de *Diplotaxis erucoides* D. C., *Helleborus niger* L. et *H. foetidus* L. C. R. — Acad. Sci. (Paris) *262*, 1629—1632.

EYMÉ, J. (1966b): Infrastructure des constituents cellulaires des tissues excréteurs de nectaries floraux. — J. Microsc. *5*, 46a.

EYMÉ, J. (1967): Nouvelles observation sur l'infrastructure de tissues nectarigénes floraux. — Le Botaniste *50*, 169—183.

FAHN, A. and RACHMILEVITZ, T. (1970): Ultrastructure and nectar secretion in *Lonicera japonica*. —In New Research in Plant Anatomy. Suppl. J. Linn. Soc. pp. 51—56. Ed. N. K. B. ROBSON, D. F. CUTLER and M. GREGORY. Academic Press, London.

FINDLAY, N. and MERCER, F. V. (1971): Nectar production in *Abutilon*. Submicroscopic structure of the nectary. — Aust. J. biol. Sci. *24*, 657—664.

GIRBARDT, M. (1961): Licht- und Elektronenmikroskopische Untersuchungen an *Polystictus versicolor*. II. Die Feinstructur von Grundplasma und Mitochondrien. — Arch. Mikrobiol. *39*, 351—359.

GULYÁS, S. (1968): Szerkezet és produkció kapcsolata a Labiatae nektáriumokban. (Connection of structure and production in the Labiatae nectaries.) — Thesis for degree of C. Sc.

HELDER, R. J. (1958): The excretion of carbohydrates (nectaries). — Encycl. of Plant Physiol.

HUGHES, G. C. and BISALPUTRA, A. (1970): Ultrastructure of hyphomycetes. Conidium ontogeny in *Peziza ostracoderma*. — Canad. J. Bot. *48*, 361—366.

KÁLMÁN, F. (1973): Ricinus nektáriumok hisztogenezise, fotomorfogenezise és sejtjeinek ultrastruktúrája. (Histogenesis, photomorphogenesis and cell ultrastructure of ricinus nectaries.) — Degree Thesis.

MAHLBERG, P., OLSON, K. and WALKINSHAW, C. (1971): Origin and development of Plasma Membrane Derived Invaginations in *Vinca rosea* L. — Amer. J. Bot. *58*, 407—416.

MARCHANT, R. and ROBARDS, A. W. (1968): Membrane systems associated with the plasmalemma of plant cells. — Ann. Bot. (London) *32*, 457—471.

MATILE, P. (1956): Über den Stoffwechsel und die Auxin-abhängigkeit der Nektarsecretion. — Ber. Schweuv iz Bot.

MOORE, R. T. and MCALEAR, J. H. (1961): Fine structure of mycota. 5. Lomasomes — previously uncharacterized hyphal structures. — Mycologia *53*, 194—200.

RACHMILEVITZ, T. and FAHN, A. (1973): Ultrastructure of Nectaries of *Vinca rosea* L., *Vinca major* L. and *Citrus sinensis* OSBECK cv. Valencia and its Relation to the Mechanism of Nectar Secretion. — Annals of Botany *37*, 1—9.

SCHNEPF, E. (1964a): Zur Cytologie und Physiologie pflanzlicher Drüsen. 4. Teil: Licht- und elektronenmikroskopische Untersuchungen an Septalnektarien. — Protplasma *58*, 137—171.

SCHNEPF, E. (1964b): Zur Cytologie und Physiologie pflanzlicher Drüsen. 5. Teil: Elektronenmikroskopische Untersuchungen an Cyanthialnektarien von *Euphorbia pulcherrima* in verschiedenen Funktion-zuständen. — Ibid 193—219.

VASILIEV, A. E. (1969): Submicroscopicheskaya morfologiya kletok nektarnikov. — Botanicheskii Zhur. *54*, 1015—1031.

VASILIEV, A. E. (1971): Noviye danniye po ultrastrukture kletok nektarnikov tsvatka. — Bot. Zhur. *56*, 1292—1305.

WYKES, G. R. (1952): The influence of variations in the supply of carbohydrate on the processes of nectar secretion. — New Phyt. *51*, 294—300.

Address of the authors:
FLÓRA KÁLMÁN
Dr. S. GULYÁS
Department of Botany,
A. J. University, H—6701 Szeged,
P. O. Box 428, Hungary