NECTAR PRODUCTION IN *ABUTILON*

II.* SUBMICROSCOPIC STRUCTURE OF THE NECTARY

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Abstract

The nectary hair cells of *Abutilon* are particularly rich in mitochondria and endoplasmic reticulum. The vacuoles are initially small but enlarge and coalesce during nectar production when invaginations of the plasmalemma are often present at the tips and at the cross-walls of the hairs. The presence of these invaginations suggests that nectar may be transported through the nectary by a process resembling pinocytosis and exocytosis. Entry of nectar into the hair is restricted to a route through the protoplast of the stalk cell by a cutinized sheath in the outer walls of this cell.

Pretreatment of the nectaries in anaerobic conditions or low temperature results in changes in the structure of the endoplasmic reticulum.

I. INTRODUCTION

In a recent review Schnepf (1969) points out that there are considerable differences between nectaries in detailed organization but little indication of any widespread special structure involved in nectar production other than structures characteristic of metabolically active cells.

In this paper we describe electron microscope studies which extend the light microscope observations of the structure of *Abutilon* nectaries (Findlay and Mercer 1971). Our aim was to establish whether nectar production could be related to structural features of the nectary cells and to this end the structure of the nectary cells before, during, and after the secreting phase were compared. The conclusions we reached have been briefly reported elsewhere (Mercer and Rathgeber 1962) and are in general agreement with the conclusions of Schnepf (1969).

II. MATERIALS AND METHODS

Nectaries of *Abutilon* (Findlay and Mercer 1971) were fixed in 2% potassium permanganate in veronal buffer or water. Often sodium chloride was added to the fixative to increase its osmotic pressure to values more nearly isotonic with the tissue. The nectaries were cut into pieces less than 0·5 mm thick in at least two dimensions and fixed for 3 hr at room temperature. The pieces were soaked in 2% aqueous uranyl nitrate overnight and then dehydrated and embedded in Araldite according to the method of Mercer and Birbeck (1961). The tissue was soaked in the Araldite for at least 3 hr as penetration into the nectary hairs was slow.

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Some material was fixed for 2–6 hr in 2% osmium tetroxide in veronal buffer with sucrose to increase osmotic pressure of the fixative. Embedding was in methacrylate.

Sections were cut with glass or diamond knives on a Porter–Blum or LKB microtome and examined with a Siemens Elmiskop I electron microscope.

III. Observations

The general structure of the *Abutilon* nectary is shown in Figure 2 of Findlay and Mercer (1971).

The following descriptions are based on permanganate-fixed material except where otherwise stated since osmium tetroxide fixation was in general unsatisfactory.

(*a*) *Structure of the Nectary Shortly before the Beginning of Nectar Secretion*

Figure 1 shows the tip of a nectary hair just before the beginning of nectar secretion.

The cuticle surrounding the hair generally became separated from the cell wall at the hair tips during preparation of this material, suggesting that the binding between the cell wall and the cuticle is weak or absent. In fresh material the cuticle and the cell wall do not become separated until the beginning of nectar secretion (Findlay and Mercer 1971). Pores in the cuticle have been described in Findlay and Mercer (1971).

A conspicuous feature of the cytoplasm of the nectary hair cells (Figs. 1 and 2) is the large number of mitochondria, which are not only more frequent but also more densely packed with crystae than those in the root meristem cells of *Abutilon*. The endoplasmic reticulum is seen as small circular cross-sections throughout the cytoplasm, as elongated profiles particularly near the surface of the cell, and as groups of a number of endoplasmic membranes lying parallel to one another. The irregular shape of the vacuoles is probably a fixation artefact as the vacuoles appear rounded in fresh material and rapid changes in the shape of the vacuoles were visible if they were observed during fixation. The density of the contents of the vacuoles varies from one vacuole to another even in the one cell.

The quality of fixation of the nectary tissue, basal cells, and stalk cells is inferior to that of the hair cells. Better results were obtained with pretreatment and fixation in the cold, which has little effect except on the endoplasmic reticulum in the hair cells [Section III(*d*)].

The nectary tissue cells are more highly vacuolated than the nectary hair cells but they nevertheless contain a higher proportion of cytoplasm than the surrounding parenchyma cells. The cytoplasm contains numerous organelles (Fig. 3). The plastids often contain starch grains and those from cells near the base of the nectary may contain a few chloroplast lamellae. Crystals, presumably of calcium oxalate, are present in many of the cells. Small intercellular spaces are present between the cells which in fresh material are filled with air, not liquid as reported by Lüttge (1961). The cell walls are thicker than in the nectary hair cells and contain pits with numerous plasmodesmata.

The phloem traces which penetrate the nectary tissue consist of sieve elements and companion cells of approximately equal size. They are slightly elongated and narrower than the nectary tissue cells. Sieve pores up to 0·25 μm in section have been

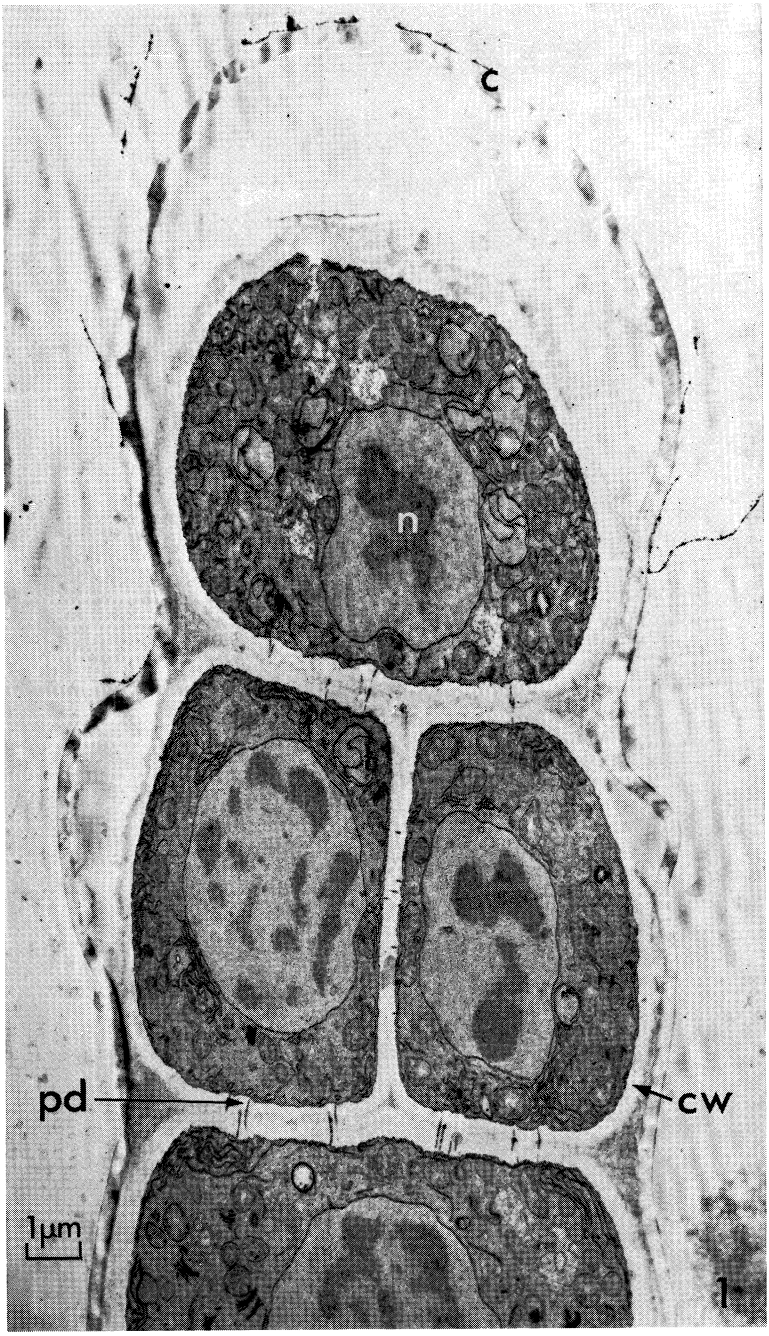


Fig. 1.—Longitudinal section through the tip of a nectary hair shortly before the beginning of nectar secretion (corolla just unfolding). The cuticle (*c*) shows damage due to sectioning. The spaces between cell walls and cuticle are due to shrinkage during preparation. Plasmodesmata (*pd*) cross the thin cell walls (*cw*) between cells. Much of each cell is occupied by the nucleus (*n*). The cytoplasm contains numerous mitochondria, abundant endoplasmic reticulum, several small vacuoles, leucoplasts containing a few dense globules, moderate numbers of dictyosomes which show no marked dilation of the cisternae nor any associated vesicles, some spherosomes, and lipid bodies. Permanganate fixation; osmotic pressure (O.P.) = 15 atm.

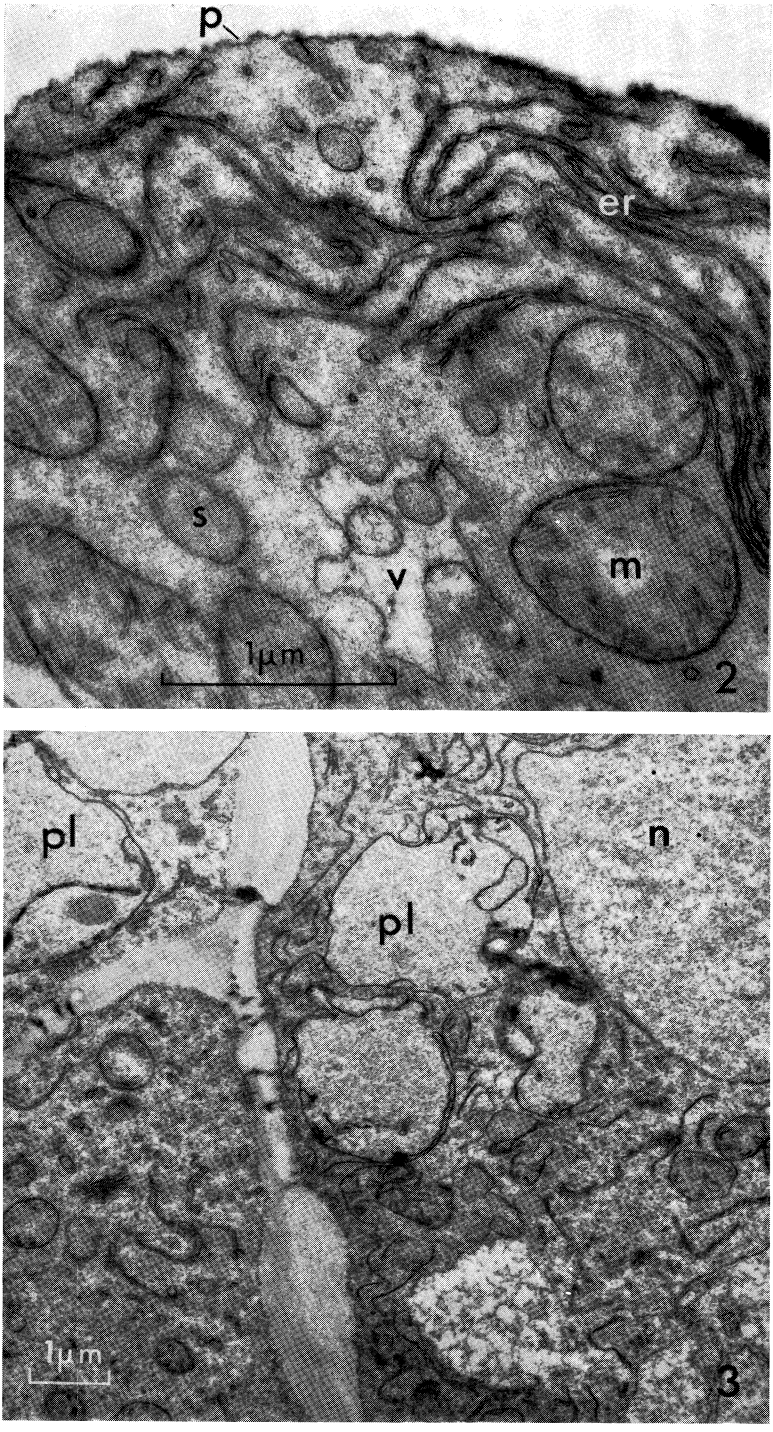


Fig. 2.—Apical part of a tip cell before the beginning of nectar secretion (corolla half its final length). Mitochondrion (*m*), small vacuole (*v*), endoplasmic reticulum (*er*), spherosome (*s*), plasmalemma (*p*). Permanganate fixation; O.P. = 15 atm.

observed in the sieve elements. The companion cells are of a greater general electron-density than the surrounding nectary tissue cells. Much of the cell is filled by the nucleus, and the cytoplasm is densely packed with organelles. The dictyosomes are more numerous than in the nectary cells and often have cisternae with dilated edges.

The stalk cell (Fig. 4) as in many other glandular hairs (Schrödter 1925; Schnepf 1969) has a modified lateral wall. The whole thickness of this wall appears electron-dense and is presumably cutinized. The wall surface adjoining the protoplast shows various degrees of roughness. The tonoplast of the stalk cell was usually damaged during fixation. There are no obvious structural differences to the protoplasts of the nectary hair cells other than that they appear to contain slightly fewer organelles. This has been noted in other stalk cells (Schnepf 1969) and also in structurally somewhat similar endodermal cells (Falk and Sitte 1960).

The basal cells are similar in structure to the nectary tissue cells. They sometimes contain dictyosomes in somewhat larger numbers than the other nectary cells. The edges of the cisternae of these dictyosomes are slightly dilated, and small vesicles are associated with these dictyosomes but cannot be differentiated from tubular cross-sections of endoplasmic reticulum except by their position.

(*b*) *Structure of Nectary Hair Cells during Nectar Secretion*

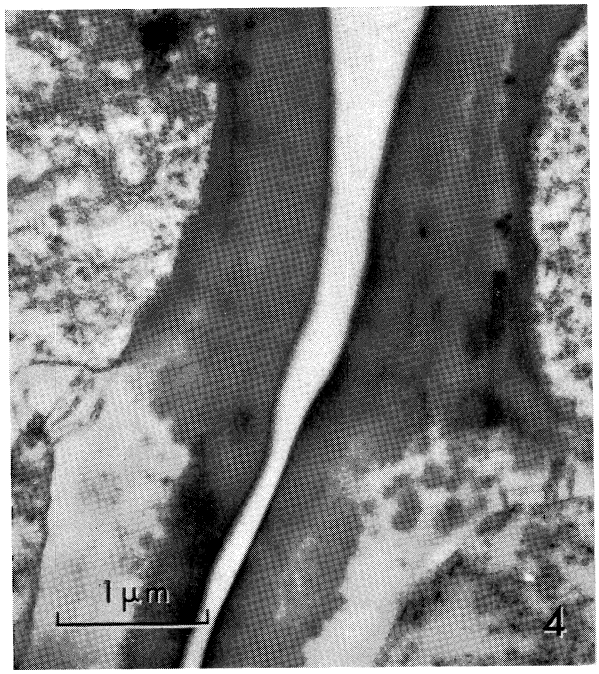
Some changes occur in the nectary hair cells after nectar secretion begins.

The small vacuoles present at the beginning of nectar secretion increase in size and coalesce. By the end of nectar secretion a single large vacuole is present which is often annular in shape. The timing of the changes in the vacuoles varies from flower to flower and does not appear to be closely related to the stage of nectar secretion. These changes in the vacuoles are readily observed in fresh material.

In the hair cells of secreting nectaries, invaginations of the plasmalemma with finely granular contents may be present (Figs. 5 and 6). These invaginations occur at the tip of the hairs and to a lesser extent on either side of the cross-walls of the nectary hair cells. They are variable in size and shape and several are often grouped together. At the cross-walls they are often associated with plasmodesmata. Resolution of the bounding membrane of the invaginations is seldom good but where it is clearest it appears similar to the plasmalemma. Vesicles, which may be sections through invaginations, are often found close to the invaginations. Since the cell wall in permanganate-fixed material appears very pale it is possible that the invaginations are cell wall extensions such as are found in many septal nectaries (Schnepf 1964). However, no extensions were found in osmium tetroxide-fixed material in which the cell wall stains densely.

Multivesiculate vesicles similar to those recorded in onion root tip cells (Porter and Machado 1960) and salt glands of *Tamarix* (Thomson and Liu 1967) occur in small numbers in secreting nectary hair cells. The endoplasmic reticulum can occur as long elements stretching almost from wall to wall in addition to the forms found in younger nectaries.

Fig. 3.—Parts of three nectary tissue cells at the beginning of nectar secretion. The plastids (*pl*) in one of the cells contain starch grains. The cell wall shows pits with plasmodesmata. This section includes little of the vacuoles. Nucleus (*n*). Pretreatment for 3 hr and fixation in permanganate (O.P. = 15 atm) in the cold (0–4°C).



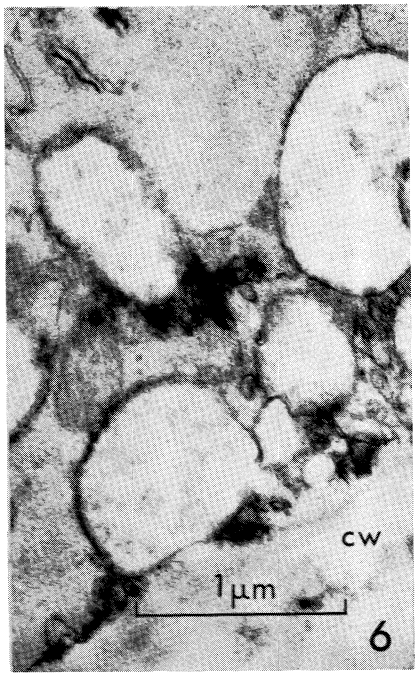


Fig. 4.—Parts of two stalk cells showing cutinized lateral walls and adjoining basal cells. Osmium tetroxide fixation; O.P. = 15 atm.

Fig. 5.—An invagination in the plasmalemma at the tip of a tip cell from a secreting nectary. Permanganate fixation; O.P. = 20 atm.

(*c*) *Structure of Nectary Hairs which have Ceased Nectar Secretion*

In nectaries which have just or almost ceased nectar secretion the vacuoles are moderately large. Invaginations of the plasmalemma still occur but are filled with numerous small vesicles (Fig. 7) and are similar in appearance to those called lomasomes (Moore and McAlear 1961; Esau, Cheadle, and Gill 1966). A few small multivesiculate invaginations may also be found in still active nectaries. Myelin figures and other peculiar configurations of membranes are present in the cytoplasm about the time nectar secretion ceases.

(*d*) *Effects of Pretreatment in Nitrogen or in the Cold*

In nectaries under anaerobic conditions or at temperatures close to 0°C nectar secretion is inhibited (Findlay, Reed, and Mercer 1971). It was thought desirable to compare the appearance of any invaginations in such pretreated nectaries with control nectaries. However, it was found that both the treated and control nectaries, like other nectaries which had been isolated for several hours, showed very few invaginations even though they secreted at appreciable rates. However, other changes appeared in the treated nectaries.

Nectaries kept in an atmosphere of nitrogen developed numerous collections of parallel or concentric lamellae of endoplasmic reticulum within 9 hr and these disappeared within 3 hr in air. Similar proliferation of endoplasmic reticulum under anaerobic conditions has been found in root meristems by Wrischer (1960) and Gavaudan, Poussel, and Guyot (1960) and in yeast grown anaerobically (Linnane, Vitols, and Nowland 1962).

Pretreatment of nectaries at temperatures of about 4°C for several hours before fixation resulted in a fragmented appearance of the endoplasmic reticulum both in nectary hair and tissue cells. The basal cells contained an exceptionally large number of small membranous profiles which could be either fragmented elements of endoplasmic reticulum or Golgi vesicles.

IV. Discussion

The most obvious feature of the *Abutilon* nectary cells is their high content of cytoplasm, and the abundance of mitochondria and endoplasmic reticulum compared with most other plant cells. This has been found in other nectaries and glands (Schnepf 1969). The apparent lack of connection of the dictyosomes with nectar secretion is also in agreement with the findings in other nectaries (Schnepf 1969).

The occurrence of the invaginations during nectar secretion only and their position suggest that they are involved in nectar secretion. Eymé (1966) found invaginations in the secreting nectaries of *Diplotaxis* and *Helleborus* and suggested that they may be responsible for the transport of nectar by pinocytosis. On the other hand, Schnepf (1969) found similar structures in *Passiflora* nectaries but only in those which had ceased secreting. Invaginations have not been found in the nectaries of several other species studied. In *Abutilon* the invaginations may represent either an increase in surface area of the plasmalemma and cells, as suggested for *Cicer*

Fig. 6.—Invaginations and vesicles adjoining the cross-wall (*cw*) in a tip cell from a secreting nectary. Permanganate fixation; O.P. = 20 atm.

Fig. 7.—Invaginations containing numerous small vesicles in a tip cell from a slightly wilted flower. Permanganate fixation; O.P. = 10 atm.

by Schnepf (1965), or a transport of solution by pinocytosis and exocytosis. However, these interpretations cannot be more than tentative. The invaginations did not appear in all material from secreting *Abutilon* nectaries nor in all hairs from material which showed some of these structures. It is possible that they often disappear during fixation as the fixative penetrates the hairs slowly but on the other hand they may be the result of mechanical damage, possibly as a result of sudden osmotic pressure changes. Similar invaginations are sometimes produced as a result of mechanical damage to root meristem cells (Mollenhauer, Whaley, and Leech 1960).

V. Acknowledgments

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VI. References

Esau, K., Cheadle, V. I., and Gill, R. H. (1966).—Cytology of differentiating tracheary elements. II. Structures associated with cell surfaces. *Am. J. Bot.* **53**, 765.

Eymé, J. (1966).—Infrastructure des cellules nectarigènes de *Diplotaxis erucoides* D.C., *Helleborus niger* L. et *Helleborus foetidus* L. *C.r. hebd. Séanc. Acad. Sci., Paris* D **262**, 1629.

Falk, H., and Sitte, P. (1960).—Untersuchungen am Caspary-Streifen. Proc. Eur. Reg. Conf. Electron Microsc. Delft. Vol. 2, p. 1063.

Findlay, N., and Mercer, F. V. (1971).—Nectar production in *Abutilon*. I. Movement of nectar through the cuticle. *Aust. J. biol. Sci.* **24**, 647.

Findlay, N., Reed, M. L., and Mercer, F. V. (1971).—Nectar production in *Abutilon*. III. Sugar secretion. *Aust. J. biol. Sci.* **24**, 665.

Gavaudan, P., Poussel, H., and Guyot, M. (1960).—Production expérimentale dans la cellule végétale anoxique de "nebenkerns" ergastoplasmiques. *C.r. hebd. Séanc. Acad. Sci., Paris* **250**, 4029.

Linnane, A. W., Vitols, E., and Nowland, P. G. (1962).—Studies on the origin of yeast mitochondria. *J. Cell Biol.* **13**, 345.

Lüttge, U. (1961).—Über die Zusammensetzung des Nektars und den Mechanismus seiner Sekretion. I. *Planta* **56**, 189.

Mercer, E. H., and Birbeck, M. S. C. (1961).—"Electron Microscopy. A Handbook for Biologists." (Blackwell Press: Oxford.)

Mercer, F. V., and Rathgeber, N. (1962).—Nectar secretion and cell membranes. Proc. 5th. Int. Conf. Electron Microsc., Philadelphia. WW-11.

Mollenhauer, H. H., Whaley, W. G., and Leech, J. H. (1960).—Cell ultrastructure responses to mechanical injury. *J. Ultrastruct. Res.* **4**, 473.

Moore, R. T., and McAlear, J. H. (1961).—Fine structure of *Mycota*. V. Lomasomes, previously uncharacterized hyphal structures. *Mycologia* **53**, 194.

Porter, K. R., and Machado, R. D. (1960).—Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis of cells of onion root tip. *J. biophys. biochem. Cytol.* **7**, 167.

Schnepf, E. (1964).—Zur Cytologie und Physiologie pflanzlicher Drüsen. 4. Teil. Licht- und electronenmikroskopische Untersuchungen an Septalnektarien. *Protoplasma* **58**, 137.

Schnepf, E. (1965).—Licht- und elektronenmikroskopische Beobachtungen an den Trichom-Hydrathoden von *Cicer arietinum*. *Z. PflPhysiol.* **53**, 245.

Schnepf, E. (1969).—Sekretion und Exkretion bei Pflanzen. *Protoplasmatologia* **8**, 8.

Schrödter, K. (1925).—Zur physiologischen Anatomie der Mittelzelle drüsiger Gebilde. *Flora, Jena* (N.S.) **20**, 19.

Thomson, W. W., and Liu, L. L. (1967).—Ultrastructural features of the salt gland of *Tamarix aphylla* L. *Planta* **73**, 201.

Wrischer, M. (1960).—Veränderungen des endoplasmatischen Reticulums pflanzlicher Zellen, verursacht durch Sauerstoffmangel. *Naturwissenschaften* **47**, 521.