THE ULTRASTRUCTURE OF THE SYNERGIDS OF PETUNIA

J. L. VAN WENT

Botanisch Laboratorium, Universteit, Nijmegen

SUMMARY

The synergids of *Petunia* show a number of changes in size, shape and ultrastructure during anthesis. Before anthesis, the immature synergids are small cells with a rather simple ultrastructure. During anthesis, they greatly enlarge.

Between the tops of the synergids a filiform apparatus is formed. The numbers of mitochondria and dictyosomes increase, whereas the number of plastids does not. The mature synergids contain an extensive rough ER. From the ultrastructural data, the author concludes that the metabolic activity in the synergids increases sharply during anthesis. The mature synergids are proposed to produce and secrete substances which direct the growth of the pollen tube.

1. INTRODUCTION

The synergids have been studied in a great number of species since their discovery (*cf.* MAHESHWARI 1950). In most species, they are elongated cells with a polar distribution of the cytoplasm. The micropylar parts of the cells are frequently occupied by a filiform apparatus. However, in his light microscopical study of *Petunia* COOPER (1946) described the presence of a cavity between the tops of the synergids.

Little was known about the ultrastructure of the synergids until 1964. A few reports have appeared since (VAN DER PLUIJM 1964; JENSEN 1965; DIBOLL & LARSON 1966; SCHULZ & JENSEN 1968), which show that the synergids are highly differentiated cells with a complex ultrastructure.

Many hypotheses have been postulated on the function of the synergids (VAZART 1958). Most authors assume that the synergids are directing the growth of the pollen tube. VAN DER PLUIJM (1964) and DIBOLL & LARSON (1966) inferred from their results that the synergids probably produce and secrete chemotropical active substances. JENSEN (1965) and SCHULTZ & JENSEN (1968), however, inferred from their results that the synergids probably not secrete, but absorb substances from the micropyle. They suggest that the synergids have merely a nourishing function.

The electron microscopical study of the synergids of *Petunia*, of which the results are presented in this paper, is an attempt to throw new light on the various hypotheses on the ultrastructure and function of the synergids.

2. MATERIAL AND METHODS

Plants of *Petunia hybrida*, clone W 166k, were grown in the greenhouse at approximately 25°C. The ovaries were cut into sections, 0.5 mm thick and fixed in 5% glutaratdehyde (GA) in 0.1 M phosphate buffer at pH 7.2 for 3 hrs at 4°C. After fixation, the tissue was washed overnight in 0.1 M phosphate buffer of pH 7.2 and subsequently post-fixed in either 5% $KMnO_4$ for 7 hrs or in 2% OsO_4 for 24 hrs at room temperature. The fixed material was then washed in water and the ovules were prepared free from the ovary sections. The isolated ovules were dehydrated in ethanol and embedded in Epon via propylene oxide. The gelatine capsules into which the ovules were transferred, were provided with a bottom of polymerized Epon. The straight surface obtained in the capsules in this way, enabled manipulation of the ovules before sectioning. Sections of material fixed in GA and OsO_4 were post-stained with 2% uranyl acetate and lead citrate (REYNOLDS 1963).

For the examination of the cell walls we used the following methods. The fresh ovules were treated either with a mixture of one part acetic acid and one part 30% hydrogen peroxide for 15 min at 100°C (MOOR 1959), or with the same mixture for 15 min at room temperature, or with 0.01 M acetate buffer of pH 4.4 for 15 min at room temperature. After these treatments, the ovules were washed in water and stained for 20 min in 1% aniline blue. They were then dehydrated in ethanol and subsequently embedded in butyl methacrylate. After sectioning, the butyl methacrylate was dissolved from the sections by amyl acetate. Finally, the sections were shadowed with platinum.

3. RESULTS

The synergids of *Petunia* show a number of changes in size, shape and ultrastructure during anthesis. Before anthesis the synergids are approximately 14 μm long and 11 μm wide and surrounded by a wall with an average diameter of 0.1 μm. Their ultrastructure is relatively simple and is similar to that of the egg, central cell and surrounding vegetative tissue (VAN WENT & LINSKENS 1967). The nucleus is located in the center of the cell and there are only a few organelles, which are randomly distributed (*fig. 2*). Only a few small vacuoles are present at this stage of development.

After anthesis, the picture is completely different. The general topography of the micropylar part of the mature embryo sac is shown in *fig. 1*. The synergids and the egg are long pear-shaped cells, approximately 35 μm long and 15 μm wide. The two synergids partially surround the egg. Together, these three cells form the egg apparatus. Two-third of the egg apparatus at the chalazal pole is surrounded by the central cell. A filiform apparatus (FA) lies between the tops of the synergids. It is approximately 15 μm long and separates the tops of the synergids like a wedge (*fig. 3*). Where the FA borders on the micropyle, it has a cap-like shape. This cap-shaped part of the FA is not completely symmetrical. One of the two halves, covering the synergids, is always thinner

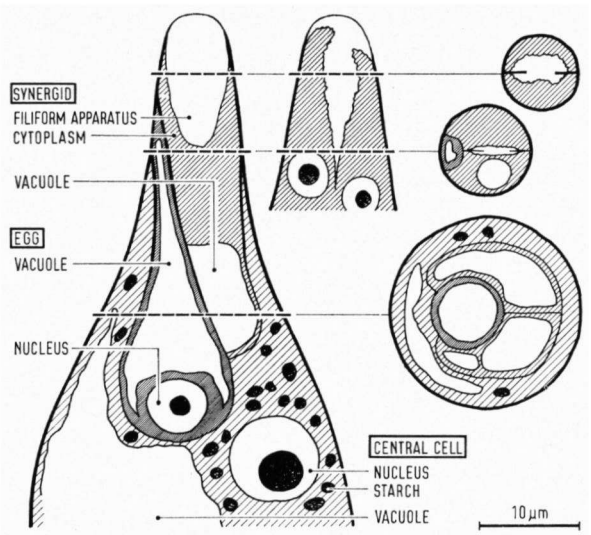


Fig. 1. Diagrammatic drawings of longitudinal and cross sections of the micropylar part of the mature embryo sac of *Petunia*.

than the other half. The major part of the cytoplasm is located in two-third of the synergids at the micropylar pole. The chalazal parts of the synergids are filled with a number of vacuoles. The nucleus is located near the center of the cell just above the vacuoles.

The cell wall surrounding the mature synergid varies in thickness. Near the micropyle the cell wall is thickest. This thickness diminishes with increasing distance from the micropyle. The wall is extremely thin at the chalazal pole of the synergid. Plasmodesmata are rare in the synergid cell wall. The FA is continuous with the cell wall and completely covered by the plasma membrane (*fig. 3, 8*). The surface of the FA, directed toward the cytoplasm, is irregular and consists of a great number of small invaginations and lobes.

The FA consists of two structural components. The major component is electron-translucent after GA-$KMnO_4$ fixation, as are the other cell walls of the ovule (*figs. 3, 8*). Only the middle lamellae are weakly stained. More electron-dense material is observed after GA-OsO_4 fixation. The amount of osmiophilic material in the cell walls of the surrounding vegetative tissue is larger than in the major component of the FA (*fig. 5*). The osmiophilic material shows a net-like arrangement in the part of the FA near the micropyle (*fig. 4, 5*). This net-like arrangement passes into a more fibrillar one in the chalazal part (*fig. 7*).

The minor component, found in the lateral parts of the FA, is continuous with the thin lateral walls of the synergids (*fig. 8*). In cross sections it looks like a tape, starting in the thin lateral wall and penetrating into the FA. The component is very electron-dense after both GA-$KMnO_4$ and GA-OsO_4 fixation (*fig. 5, 8*).

After treatment with either the mixture of hydrogen peroxide and acetic acid, or acetate buffer at 25°C, a skeleton of cellulose fibers is visible in the major component of the FA (*fig. 6*). In the cell walls of the surrounding vegetative tissue and in the minor component of the FA, the cellulose fibers remain invisible after either treatment. The cellulose fibers in these parts become visible only by treatment with hydrogen peroxide and acetic acid at 100°C for at least 15 min. The extremely thin parts of the wall at the chalazal pole of the synergids have disappeared completely after these treatments.

The synergid nucleus is spherical; there are no lobes or invaginations. The diameter is approximately 6.5 μm. The nucleus is surrounded by an envelope in which many pores are present.

Contacts between the nuclear envelope and the endoplasmic reticulum are rare. The outer nuclear membrane is partly covered with ribosomes. The structure of the nucleoplasm is homogeneous. Numerous small concentrations of chromatine are regularly spread over the whole nucleus. The nucleus contains one nucleolus, approximately 2 μm in diameter. After GA-OsO_4 fixation, the nucleolus consists of a network of fine granular material. Within the meshes of this network, especially at the periphery, large numbers of ribosome-like particles are present (*fig. 9*).

The synergids contain large numbers of mitochondria, which are uniformly distributed in the cytoplasm. All mitochondria have approximately the same size and structure. Their shape is oval and their average diameter is 0.7 μm. They contain a moderate number of short cristae, which partly appear to be tubular. There is no special arrangement of the cristae. After GA-OsO_4 fixation, the mitochondria show an electron-dense matrix and the cristae are somewhat swollen.

The number of plastids in the synergids is low. There is about one plastid to every ten mitochondria. The plastids, like the mitochondria, are randomly distributed in the cytoplasm. The shape of the plastids varies from dumb-bell like to spherical. The average diameter is approximately 1.5 μm. Their ultrastructure is very simple. They contain only a few thylakoids. In most cases there is one long thylakoid, lying parallel to the outer membranes, and a few short ones, intruding the interior of the plastid. The plastids of the synergids never contain starch, even when in all other cell types of the ovule they do contain it.

The synergids contain large numbers of dictyosomes. They too are randomly distributed in the cytoplasm. Each of the dictyosomes consists of 3–5 flat cisternae, of which the middle ones are the longest. The average length of the cisternae is 0.7 μm. The cisternae appear always to be swollen at their ends. Associated with the dictyosomes are numerous vesicles, located at the ends of the cisternae. The content of the vesicles is always electron-translucent.

An extensive system of endoplasmic reticulum (ER) is present in the synergids (*fig. 7, 8*). Most ER membranes lie parallel to the long axis of the cell. In the neighbourhood of the nucleus they are parallel to the nuclear envelope. Near the wall and the FA they parallel the plasma membrane. The ER sheets

are concentrated in groups of 2–6. The membranes of a group are always parallel. The measurements of the sheets vary.

All membranes of the ER are covered with ribosomes (*fig. 7, 9*). The synergids contain, besides the membrane-bound ribosomes, also free ribosomes.

The vacuoles of the synergids are almost exclusively located in the chalazal part of the cell, as was mentioned already. In the micropylar part there are sometimes a few small vacuoles. The vacuoles contain little or no electron-dense material after both GA-$KMnO_4$ and GA-OsO_4 fixation. The thin plasma sheath surrounding the vacuoles contains only a few organelles.

After anthesis, the micropyle contains some electron-dense material which always lies in the vicinity of the FA (*fig. 4*).

4. DISCUSSION

The data presented in this paper, show that the synergids of the mature embryo sac of *Petunia* are highly differentiated cells. The changes in size, shape and ultrastructure arise during anthesis and differentiation is over in about 24 hrs.

The cavity, described by COOPER (1946) between the tops of the synergids, is in fact a filiform apparatus. This FA is part of the synergid cell walls, since it is continuous with the thin lateral walls and completely separated from the cytoplasm by the plasma membrane. The same conclusion was drawn for the FA's of *Torenia* (VAN DER PLUIJM 1964), *Gossypium* (JENSEN 1965), *Zea* (DIBOLL & LARSON 1966), and *Capsella* (SCHULZ & JENSEN 1968). This confirms the theory of STRASBURGER (1884), who stated that the FA is an elaboration of the synergid cell wall.

The FA of *Petunia* has an interesting ultrastructure. The generally accepted theory is that the angiosperm cell wall consists of a skeleton of cellulose fibers embedded in a matrix of pectin and hemi-cellulose (FREY-WYSSLING & MÜHLETHALER 1965). A cellulose skeleton is present in both the major and minor component of the FA. The matrices of the two components are clearly different and both are different from the matrix of the other cell walls of the ovule. The differences in the amounts of electron-dense material after GA-OsO_4 fixation could mean that the amount of matrix in the major component of the FA is small and in the minor component large, in comparison with the amount in the other cell walls. The fact that only the minor component is strongly stained after GA-$KMnO_4$ fixation indicates that its matrix is chemically different from that of the major component and the other cell walls. The electron microscopic appearance suggests the presence of a lipid-like compound in this matrix. Unfortunately, attempts to locate a lipid-like compound histochemically failed. The following concept is proposed for the ultrastructure of the FA of *Petunia*. The major part consists of a loose network of cellulose fibers embedded in a small amount of matrix. The minor part consists of a cellulose skeleton embedded in a larger amount of matrix, part of which is probably of lipid nature. If this idea is correct, it could mean that the major

component is highly permeable to water and dissolved substances, whereas the minor is not.

Two differently structured parts were also found in the FA's of *Gossypium* (JENSEN 1965), *Zea* (DIBOLL & LARSON 1966), and *Capsella* (SCHULZ & JENSEN 1968), whereas the FA of *Torenia* (VAN DER PLUIJM 1964) has a homogeneous structure. However, a part structured like the minor component of the FA of *Petunia* was not observed in the FA's of these species. All authors mentioned above concluded from their results that the FA probably serves as a diffusion pathway.

The changes in ultrastructure of the cytoplasm during differentiation of the synergids indicate that the metabolic activity increases. Part of this increasing metabolic activity can be attributed to the formation of the FA. The presence of a large number of dictyosomes is in agreement with the conclusions of WHALEY & MOLLENHAUER (1963) and others on cell wall formation.

According to JENSEN (1965) the synergids of *Gossypium* have a nourishing function. They should absorb, store, and transport compounds from the nucellus. In *Gossypium* a column of cells disintegrate in the region of the micropyle. The FA is thought to function as a diffusion pathway for compounds released by these disintegrating cells. Consistent with this idea is the fact that the plastids near the FA contain large amounts of starch. The same function was proposed for the synergids of *Capsella* by SCHULZ & JENSEN (1968).

A function as proposed by Jensen seems unlikely for the synergids of *Petunia*, as there are no disintegrating cells in the micropylar region of the ovule and the plastids of the synergids never contain starch. The nourishing of the embryo sac probably takes place at the chalazal pole, where a small xylem strand ends.

It has been proposed by many authors that the synergids produce and secrete substances which direct the growth of the pollen tube (VAZART 1958; VAN DER PLUIJM 1964). Chemotropic activity has been reported for the ovules of a few species (WELK *et al.* 1965).

In *Petunia*, the pollen tube grows through the micropyle and enters the embryo sac by growing through the FA into one of the synergids (VAN WENT & LINSKENS 1967). The micropyle is very narrow, much smaller than the diameter of the pollen tube. It seems likely that a stimulus is needed to make the pollen tube grow into it. The same holds for the pollen tube growing into the FA. That stimulus might be provided by chemotropic substances produced in the synergids. The accumulation of mitochondria and the presence of an extensive rough ER in the micropylar part of the synergid after anthesis could be interpreted in terms of a large metabolic activity in that region. This activity might be the production of these chemotropic substances, which are secreted through the large plasma membrane area into the FA. From the latter they could diffuse into the micropyle.

According to FAWCETT (1966) in animal tissue the rough ER is most prominent in glandular cells with secretorial functions. The presence of an extensive rough ER in the synergids is consistent with this conclusion. The only direct evidence that there is any secretion, is the presence of electron-dense material

in the micropyle after anthesis. The position of this material suggests that it is secreted by the synergids through the FA.

ACKNOWLEDGEMENTS

The author thanks Mr. A. W. Dicke for skillful technical assistance and Dr. Ir. G. W. M. Barendse, Dr. H. J. Wilson, and Dr. A. F. Croes for correction of the english text.

REFERENCES

COOPER, D. C. (1946): Double fertilization in Petunia. *Amer. J. Bot.* 33: 54–57.

DIBOLL, A. G. & D. A. LARSON (1966): An electron microscopic study of the mature megagametophyte in Zea mays. *Amer. J. Bot.* 53: 391–402.

FAWCETT, D. W. (1966): *The cell, its organelles and inclusions.* W. B. Saunders Co., Philadelphia-London.

FREY-WYSSLING, A. & K. MÜHLETHALER (1965): *Ultrastructural plant cytology.* Elsevier Publ. Co., Amsterdam-London-New York.

JENSEN, W. A. (1965): The ultrastructure and histochemistry of the synergids of cotton. *Amer. J. Bot.* 52: 238–256.

MAHESHWARI, P. (1950): *An introduction to the embryology of angiosperms.* McGraw-Hill, New York.

MOOR, H. (1959): *Platin-Kohle-Abdruck-Technik angewandt auf den Feinbau der Milchröhren.* Thesis, Zürich.

PLUIJM, J. E. VAN DER (1964): An electron microscopic investigation of the filiform apparatus in the embryo sac of Torenia fournieri. In: H. F. LINSKENS (ed.) *Pollen physiology and fertilization*, p. 8–16. North Holl. Publ. Co., Amsterdam.

REYNOLDS, E. S. (1963): The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17: 208–212.

SCHULZ, R. & W. A. JENSEN (1968): Capsella embryogenesis: the synergids before and after fertilization. *Amer. J. Bot.* 55: 541–552.

STRASBURGER, E. (1884): *Neuere Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen als Grundlage für eine Theorie der Zeugung.* G. Fischer, Jena.

VAZART, B. (1958): Différenciation des cellules sexuelles et fécondations chez les phanérogames. *Protoplasmatologia* 8: 1–158.

WELK, M., W. F. MILLINGTON & W. G. ROSEN (1965): Chemotropic activity and the pathway of the pollen tube in Lily. *Amer. J. Bot.* 52: 774–781.

WENT, J. L. VAN & H. F. LINSKENS (1967): Die Entwicklung des sogenannten Fadenapparates im Embryosack von Petunia hybrida. *Genet. Breed. Res.* 37: 51–56.

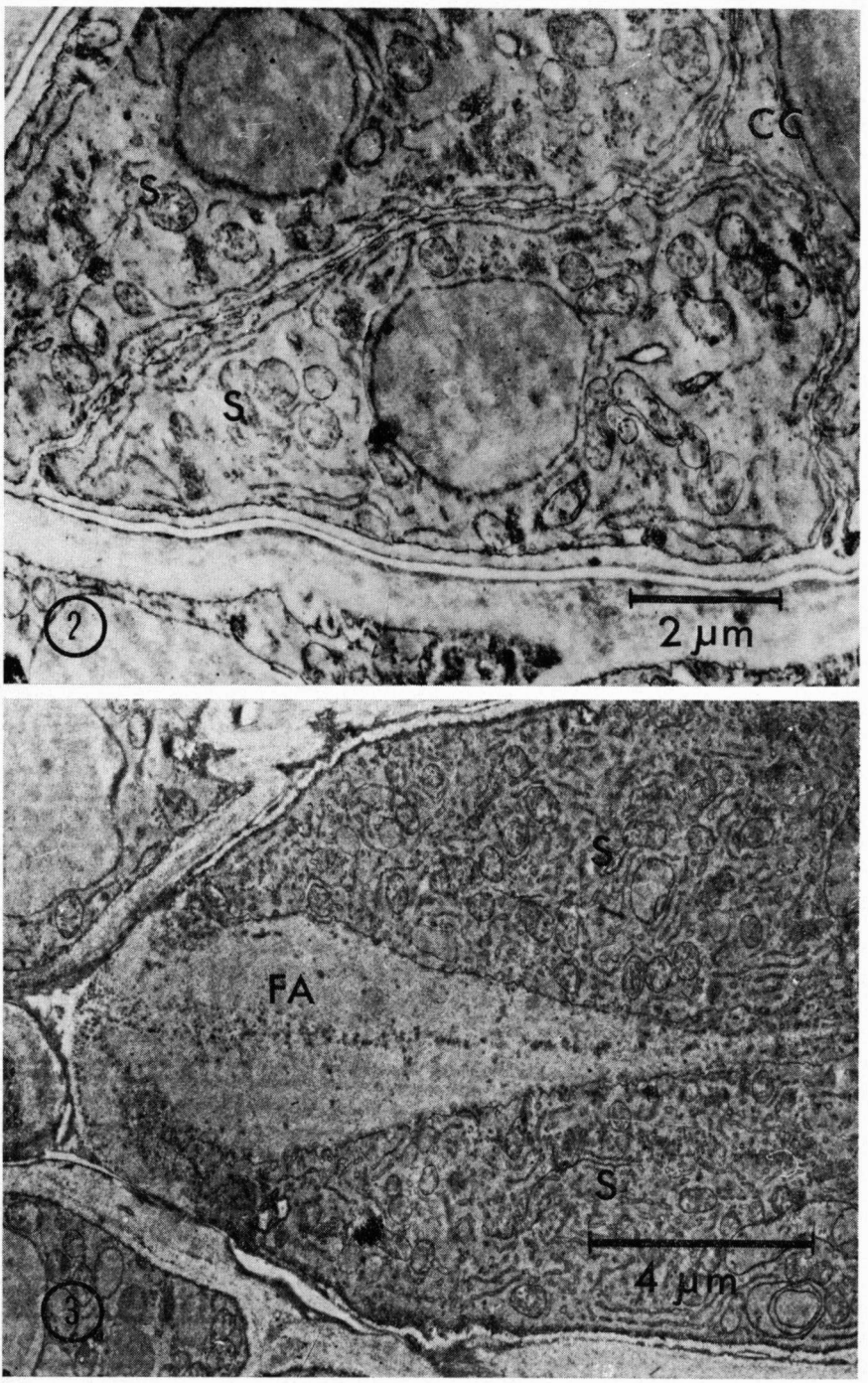
WHALEY, W. G. & H. H. MOLLENHAUER (1963): The golgi apparatus and cell plate formation. *J. Cell. Biol.* 17: 216–221.

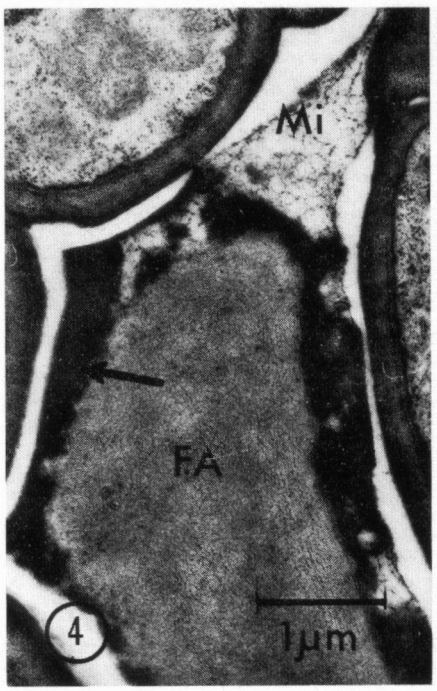
KEY TO LABELING:

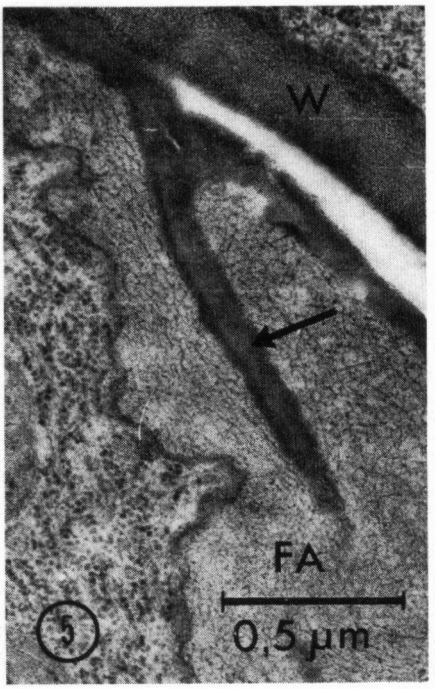
CC = central cell; Cy = cytoplasm; D = dictyosome; ER = endoplasmic reticulum; FA = filiform apparatus; M = mitochondrium; Mi = micropyle; N = nucleus; Nu = nucleolus; P = plastid; S = synergid; V = vacuole; VT = vegetative tissue of the ovule; W = cell wall.

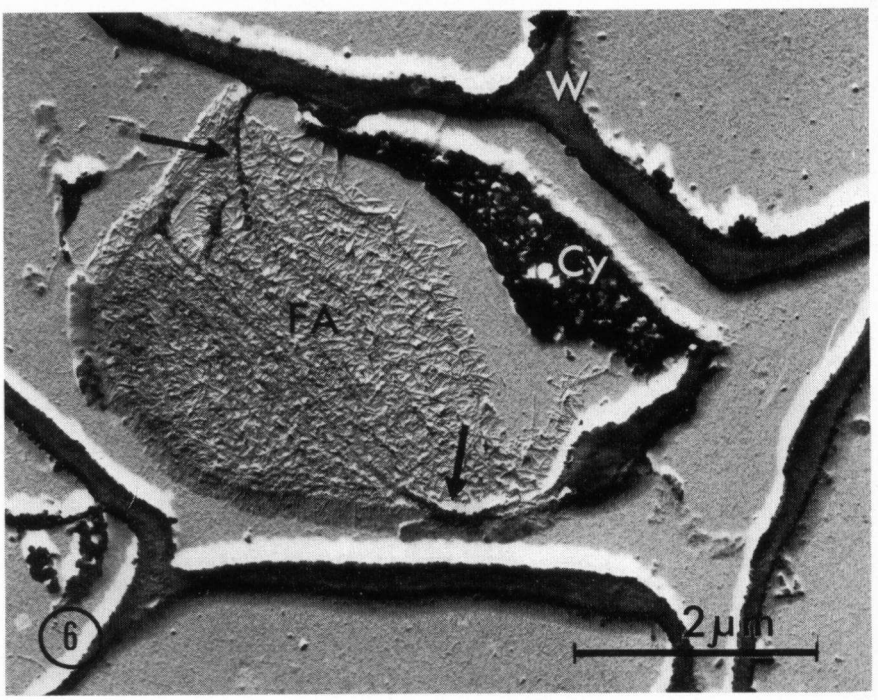
LEGENS

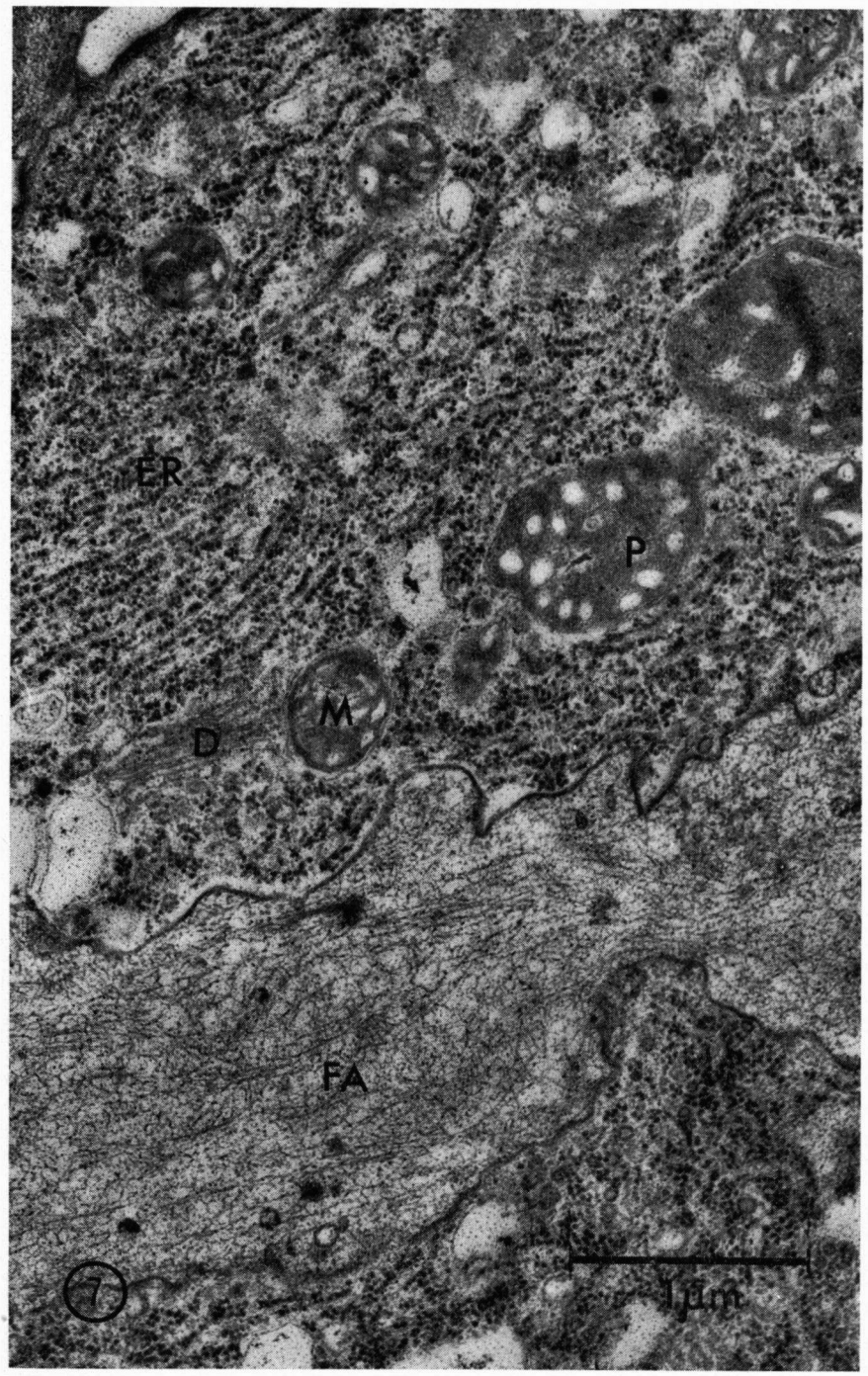
Fig. 2. Longitudinal section of the immature synergid. GA–$KMnO_4$ fixation, × 10,000.
Fig. 3. Longitudinal section of the micropylar parts of the mature synergids. GA–$KMnO_4$ fixation, × 7,500.
Fig. 4. Cross section of the FA near the micropyle. Note the presence of very electron-dense material in the micropyle (arrow). GA–OsO_4 fixation, × 17,700.
Fig. 5. Detail of the FA near the tops of the synergids. Note the differences in appearance between the major and minor component of the FA (arrow) and the cell walls of the vegetative tissue. GA–OsO_4 fixation, × 48,000.
Fig. 6. The FA and cell walls of the vegetative tissue after treatment with a mixture of acetic acid and hydrogen peroxide for 15 min at 25 °C. Arrows indicate the location of the minor component of the FA. × 16,000.
Fig. 7. The FA and associated cytoplasm just above the nucleus. GA–OsO_4 fixation, × 32,000.
Fig. 8. Cross section of the synergids just above the nucleus. Arrow indicates the location of the minor component of the FA. GA–$KMnO_4$ fixation, × 9,000.
Fig. 9. The synergid nucleus and associated cytoplasm. GA–OsO_4 fixation, × 25,000.

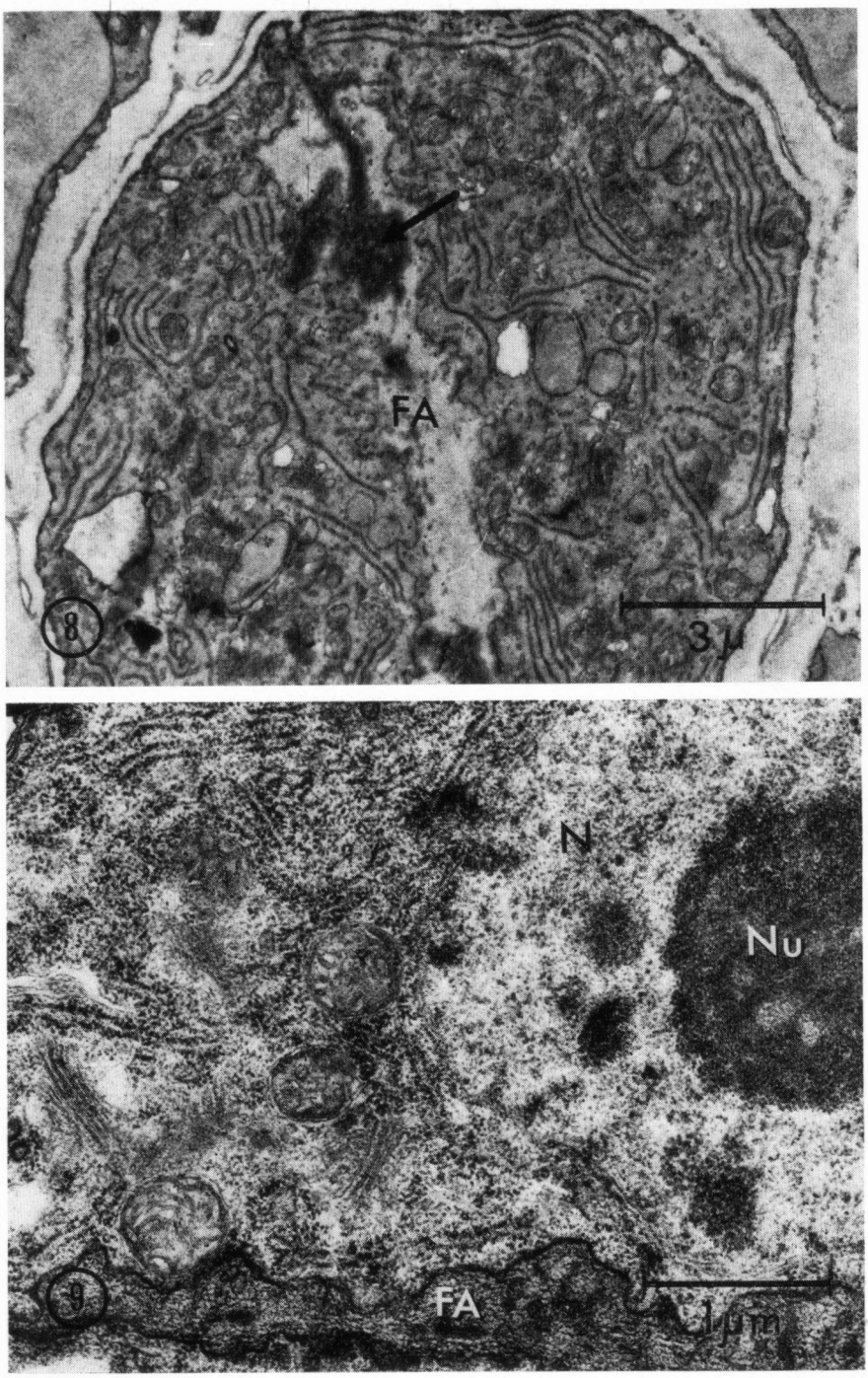

CC
S
S
2
2 µm
S
FA
S
3
4 µm


Mi
FA
4
1µm


W
FA
5
0,5 µm


W
Cy
FA
6
2µm


ER
P
M
D
FA
7
1µm


FA
8
3 µ
N
Nu
9
FA
1 µm