THE ULTRASTRUCTURE OF THE EGG AND CENTRAL CELL OF PETUNIA

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SUMMARY

The egg and central cell of *Petunia hybrida* undergo a number of changes and become mature during anthesis. The egg greatly enlarges and becomes highly vacuolated. The nucleus and the major part of the cytoplasm of the mature egg are located at the chalazal pole of the cell. The number of organelles decreases slightly during maturation. The ribosomes of the mature egg are clustered in polysomes. The chalazal part of the mature egg seems to be surrounded by the plasma membrane only, whereas the remainder of the cell has a normal cell wall.

The number of mitochondria and plastids and the amount of endoplasmic reticulum (ER) in the central cell are almost constant during maturation. The number of ribosomes, however, increases greatly. Almost all ribosomes are free and the impression is that they are monosommes. A large amount of starch is formed in the plastids. Two types of dictyosomes are present in the mature central cell. The outer membranes of the polar nuclei are sometimes connected by ER membranes.

1. INTRODUCTION

The egg and central cell are clearly the most important cells of the mature embryo sac. They fuse with the sperm cells and develop into the embryo and the endosperm. The mature embryo sac of *Petunia* has been described by COOPER (1946). His study, however, gives no information about the ultrastructure of the various cell types. The ultrastructural studies of VAN DER PLUIJM (1964), JENSEN (1965a, b), DIBOLL & LARSON (1966), and SCHULZ & JENSEN (1968a, b) show that each cell type of the mature embryo sac is highly differentiated and has a specific ultrastructure.

The present paper describes the ultrastructure of the egg and central cell of *Petunia hybrida*, the synergids of which have been described earlier (VAN WENT 1970). It is part of an electron microscopical study of sexual reproduction.

2. MATERIAL AND METHODS

Plants of *Petunia hybrida*, clone W 166k, were the source of all material. Two different fixation procedures were used. Glutaraldehyde and potassium permanganate were the fixatives in the first one (referred to as GA-$KMnO_4$). In the second one glutaraldehyde and osmium tetroxide were employed (referred to as GA-OsO_4). A complete description of the procedures has been given earlier (VAN WENT 1970).

3. RESULTS

3.1. Egg and central cell just before anthesis

The immature egg is acentrally located below the two synergids with regard to the micropyle. It is pyramid-like in shape and its average diameter is 11 μm. The base of the pyramid forms part of the embryo sac wall. The nucleus is located at the center of the cell and there are only a few small vacuoles.

The immature central cell contains one large vacuole. The two polar nuclei and the major part of the cytoplasm are located at the micropylar pole of the cell.

The ultrastructure of the two immature cells is very similar (*figs. 1* and *2*). The number of mitochondria is moderate. They are oval to spherical in shape and their average diameter is 0.7 μm. They contain a number of short, randomly distributed cristae. The plastids are less numerous than the mitochondria. They are very irregular in shape and their average diameter is approximately 1.5 μm. They contain a small number of thylakoids and sometimes a small starch grain. Dictyosomes are few in number and consist of 3–5 flat cisternae. The ER is poorly developed and most of the sheets are found in the periphery of the cells.

3.2. Egg after anthesis

The mature egg is a long pear-shaped cell, approximately 35 μm long and 15 μm wide at its base (*fig. 4*) (VAN WENT 1970). The top of the egg is 10 μm below the op of the embryo sac. The cell is in contact with the embryo sac wall at the top part only (*figs. 4* and *7*). The rest of it is freely pendulous.

The mature egg contains one large vacuole. The nucleus and the major part of the cytoplasm are located at the chalazal pole of the cell. Only the micropylar part of the cell (two-thirds) seems to be surrounded by a cell wall (*figs. 3* and *8*). The enlargement of the egg during anthesis is apparently not matched by synthesis of sufficient cellwall material.

The matrix of the egg cytoplasm is strongly stained after GA-$KMnO_4$ fixation (*fig. 3*). Randomly distributed polysomes are visible in the matrix after GA-OsO_4 fixation (*figs. 5* and *6*). The mature egg contains only a few mitochondria, plastids, and dictyosomes, and the ER is poorly developed. The ultrastructure of the organelles is the same as in the immature egg. The ER membranes are partly covered with ribosomes. The nucleus is oval in shape and the average diameter is 6 μm (*figs. 3* and *4*). The outer membrane of the nuclear envelope is partly covered with ribosomes. The nucleus contains several chromatin clumps of various size (*figs. 3* and *5*). The spherical nucleolus has a diameter of approximately 2.5 μm. It consists of homogeneously dispersed fine-granular material and is surrounded by a layer of ribosome-like particles (*fig. 5*).

3.3. Central cell after anthesis

The morphology of the mature central cell and its relation to the other cells

of the embryo sac have been described earlier (VAN WENT 1970) and are seen in overall view in *fig. 4*. The fusion of the polar nuclei can occur at different times. They may have fused already before anthesis, but sometimes they remain separate (*fig. 12*). The polar nuclei have a diameter of 10 μm. They lie close to each other, separated by a thin layer of cytoplasm in which organelles may be present. The outer membranes of the two nuclear envelopes are sometimes connected by ER sheets (*fig. 11*). Each polar nucleus contains a large nucleolus, structured like that of the egg nucleus. In contrast with the egg nucleus, the polar nuclei do not contain chromatin clumps. The ultrastructure of the polar nuclei is maintained after fusion.

The ultrastructure of the cytoplasm changes greatly during anthesis. The plasma matrix of the mature central cell has a spotty texture after GA-$KMnO_4$ fixation (*figs. 8* and *11*). After GA-OsO_4 fixation masses of ribosomes are visible (*figs. 6* and *9*). They seem to be monosomes, as no polysomes can be distinguished. The number of mitochondria and their ultrastructure do not change during maturation. The same holds for the number of plastids, but not for their ultrastructure. The plastids of the mature central cell are large (3–5 μm in diameter) and each of them is completely filled with starch grains (*figs. 4* and *12*). The ER is poorly developed. Most of it is found near the plasma membrane and the nuclear envelope. Near the bases of the egg and synergids the ER sheets are parallel and very close to the plasma membrane (*fig. 8*). The ER membranes are partly covered with ribosomes. The mature central cell contains two types of dictyosomes. In the plasma layer which surrounds the central vacuole they are like those of the immature central cell. The dictyosomes in the micropylar part of the cell each consist of one or two short cisternae which are surrounded by vesicles (*fig. 10*). Sometimes there are only vesicles.

4. DISCUSSION

The present data show that both the egg and central cell of *Petunia hybrida*, like the synergids (VAN WENT 1970), differentiate during anthesis. The ultrastructure of the mature egg cytoplasm of *Petunia* differs from that of *Gossypium* (JENSEN 1965b), *Zea* (DIBOLL & LARSON 1966), and *Capsella* (SCHULZ & JENSEN 1968b). The cytoplasm of the *Gossypium* egg is characterized by an extensive ER; that of *Zea* by a large number of mitochondria; whereas the cytoplasm of the *Capsella* egg is literally packed with ribosomes.

The enlarged shape, the polar distribution of the cytoplasm, and the high vacuolization seem to be common properties of plant eggs (MAHESHWARI 1950).

The absence of a wall at the chalazal pole of the egg apparatus has been reported also for *Torenia* (VAN DER PLUIJM 1964), *Gossypium* (JENSEN 1965a, b), and *Zea* (DIBOLL & LARSON 1966). In *Capsella* (SCHULZ & JENSEN 1968a, b) a very thin wall is present at this pole. The absence of a wall could facilitate the passage of the sperm material from one cell to another (DIBOLL & LARSON 1966; JENSEN & FISCHER 1968). Other functions are also possible. In *Petunia* the pollen tube discharges its content into one of the synergids (VAN WENT &

LINSKENS). This synergid increases strongly in volume and imposes a pressure upon the egg. The absence of a wall might be a protection mechanism that keeps the egg very flexible.

The relatively small number of polysomes, the presence of only a few organelles, and the poorly developed ER could indicate a state of low metabolic activity in the mature egg. JENSEN (1965b), DIBOLL & LARSON (1966), and SCHULZ & JENSEN (1968b) also inferred from their results that the mature egg is a rather inactive cell.

The ultrastructural changes of the central cell during anthesis indicate that a large synthesis of RNA and starch has taken place. The rate of respiration might be constant, as the number of mitochondria and their ultrastructure do not change. The ultrastructure of the mature central cell of *Petunia* is very different from those of the species mentioned above.

The presence of ER bridges between the polar nuclei is in agreement with the observations of JENSEN (1964) on the fusion of these nuclei in *Gossypium*. Possibly, the fusion of the polar nuclei occurs in the same way in both species.

From the results presented in this paper and in the previous one (VAN WENT 1970) one can conclude that each cell type of the embryo sac of *Petunia* differentiates in its own way. Each cell type of the mature embryo sac seems to have its own specific metabolic activity.

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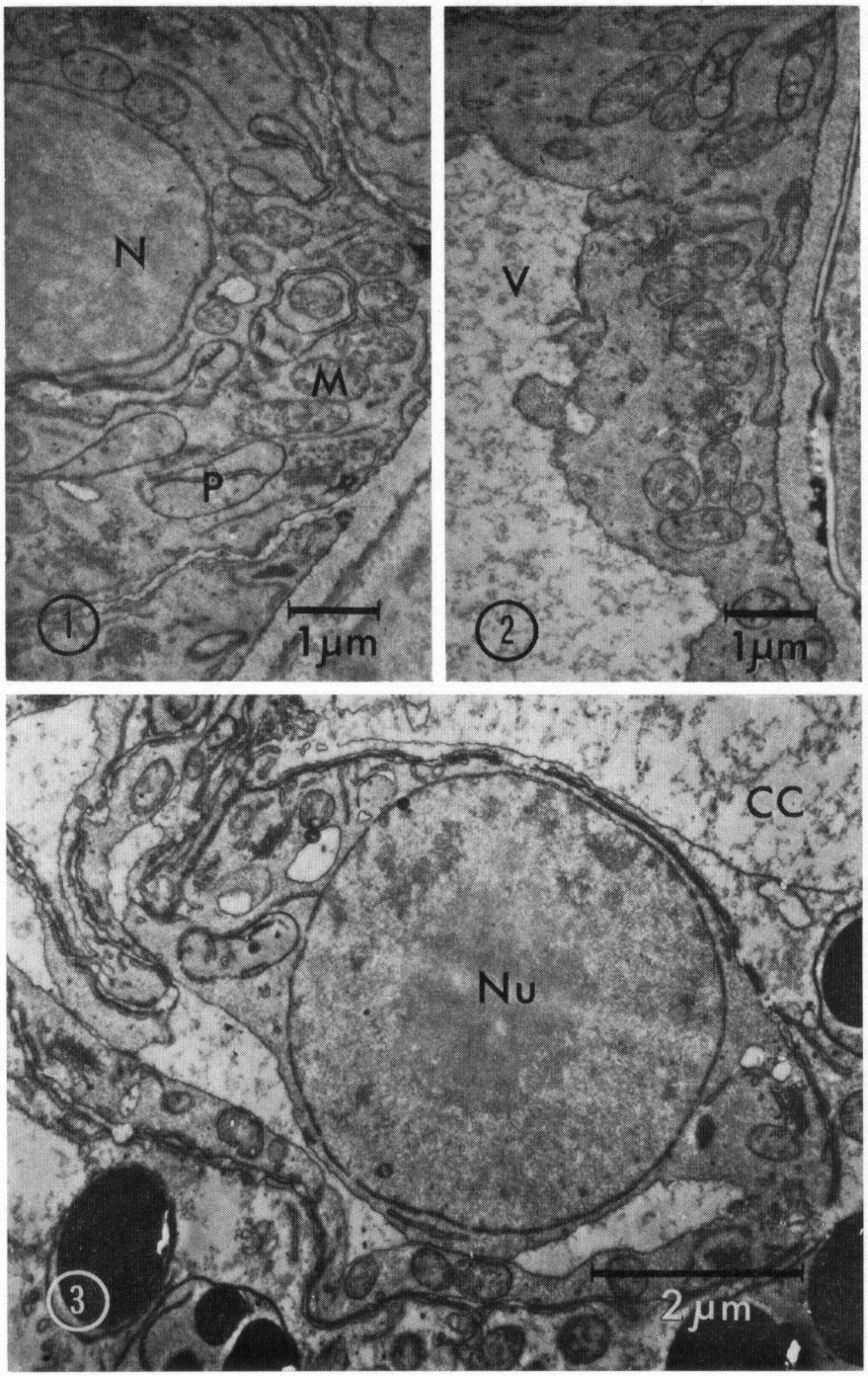
LEGENDS TO THE FIGURES

Fig. 1. Part of the immature egg. GA-$KMnO_4$, × 12,000.
Fig. 2. Part of the immature central cell. GA-$KMnO_4$, × 12,000.
Fig. 3. The chalazal part of the mature egg showing the nucleus and associated cytoplasm. Note the presence of chromatin clumps in the nucleus. GA-$KMnO_4$, × 14,000.
Fig. 4. Longitudinal section through the mature embryo sac showing the pear-like shape of the egg. GA-$KMnO_4$, × 3,000.
Fig. 5. Part of the nucleus and associated cytoplasm of the mature egg. GA-OsO_4, × 25,000.
Fig. 6. Portion of the chalazal part of the egg apparatus and adjacent part of the central cell. Note the intense staining of the central cell cytoplasm. GA-OsO_4, × 25,000.
Fig. 7. The micropylar end of the mature egg. GA-$KMnO_4$, × 10,500.
Fig. 8. Portion of the central cell and chalazal part of the egg. The ER of the central cell parallels and is very close to the plasma membrane (arrow). GA-$KMnO_4$, × 12,000.
Fig. 9. The cytoplasm of the mature central cell. GA-OsO_4, × 30,000.
Fig. 10. Portion of the micropylar part of the mature central cell showing the characteristic dictyosomes. GA-$KMnO_4$, × 12,000.
Fig. 11. Portion of the two polar nuclei. The outer membranes of the nuclear envelopes are connected by a ER sheet (arrow). GA-$KMnO_4$, × 54,000.
Fig. 12. Cross section through the micropylar part of the mature central cell showing the two polar nuclei before fusion. GA-OsO_4, × 6,700.

KEY TO LABELING

CC = central cell; D = dictyosome; E = egg; ER = endoplasmic reticulum; FA = filiform apparatus; L = lipid; M = mitochondrium; N = nucleus; Nu = nucleolus; P = plastid; PN = polar nucleus; S = synergid; St = starch; V = vacuole.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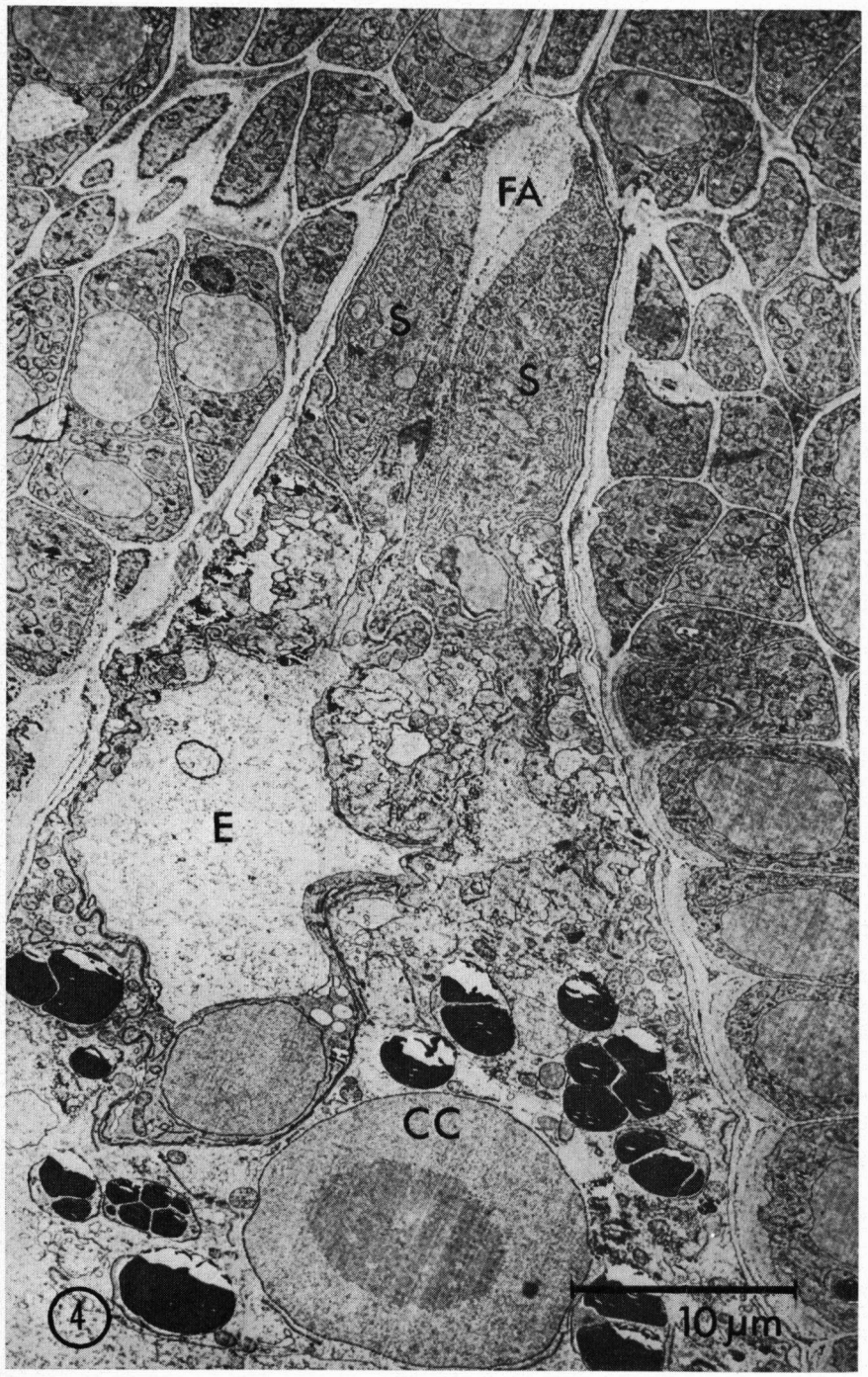
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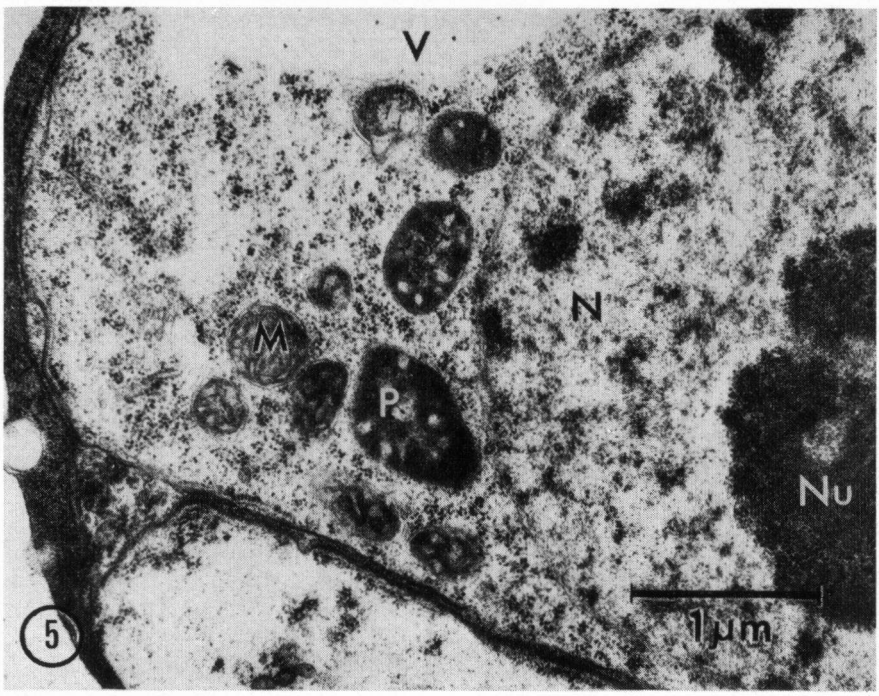
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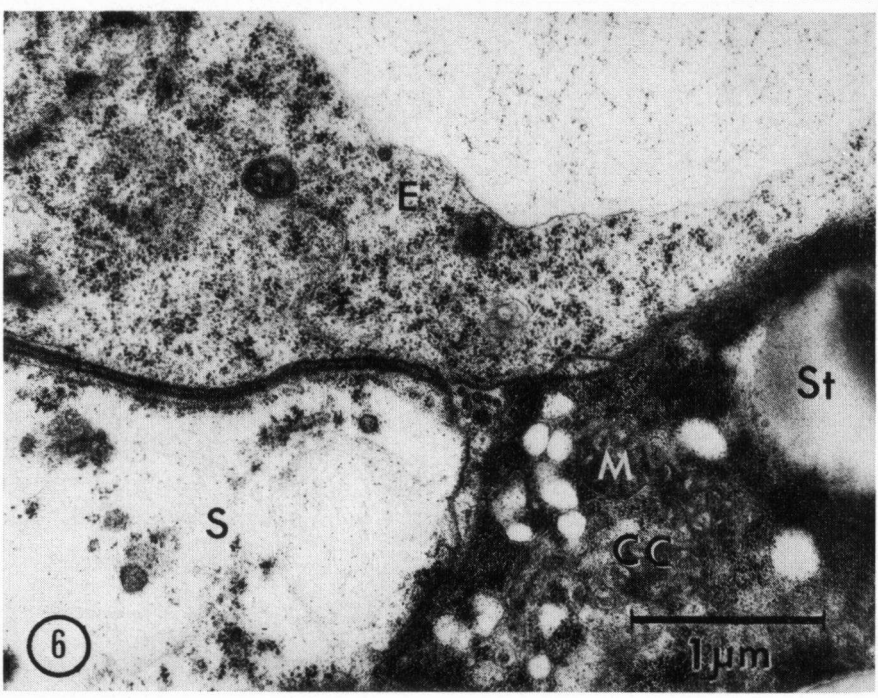
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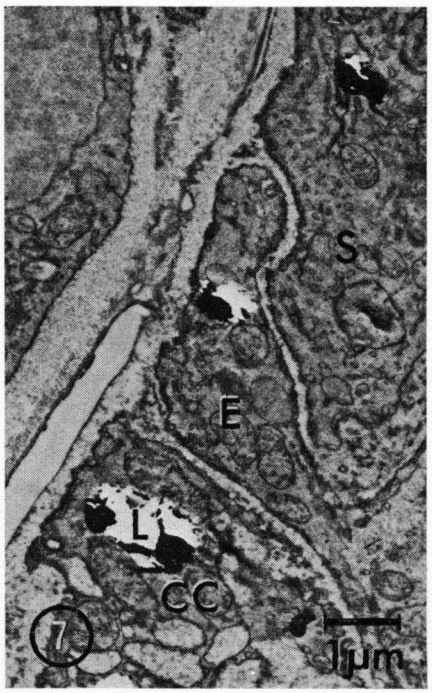
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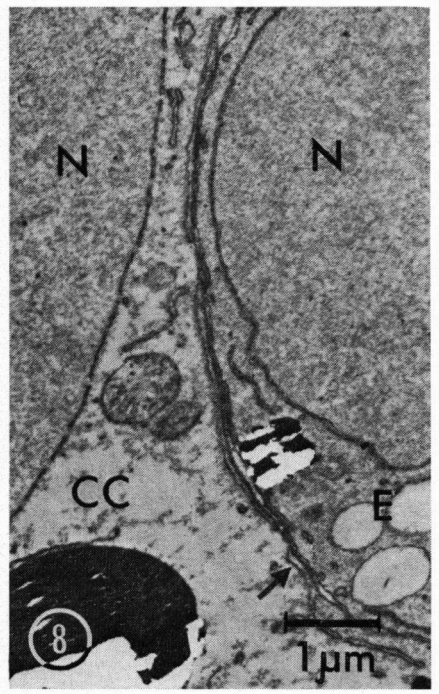
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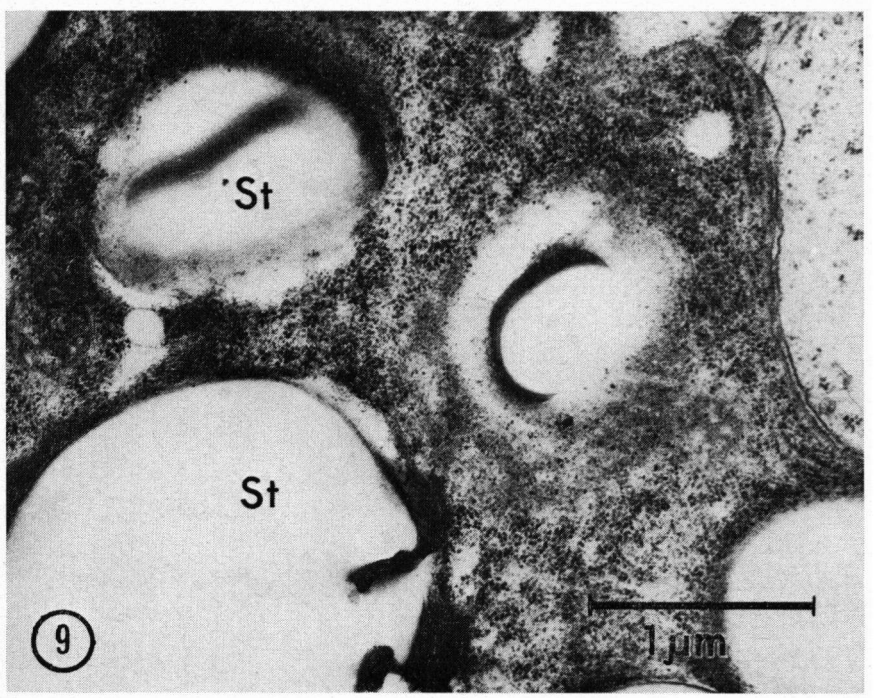
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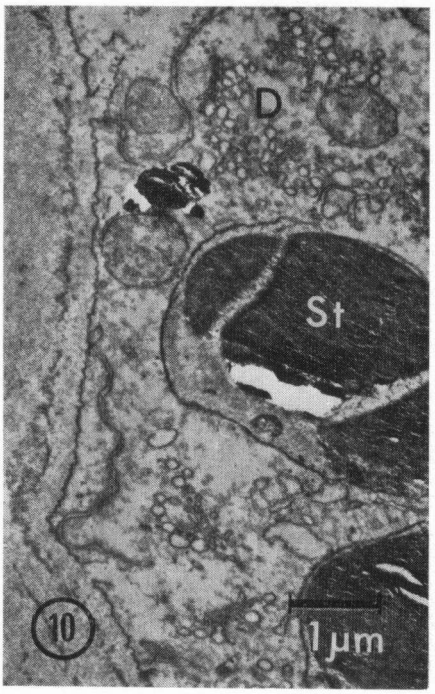
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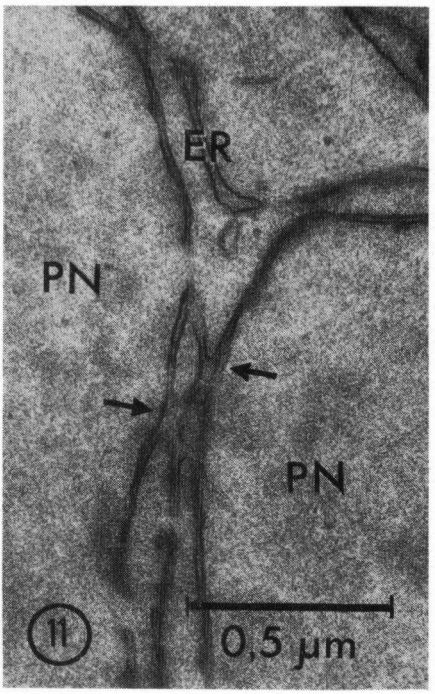
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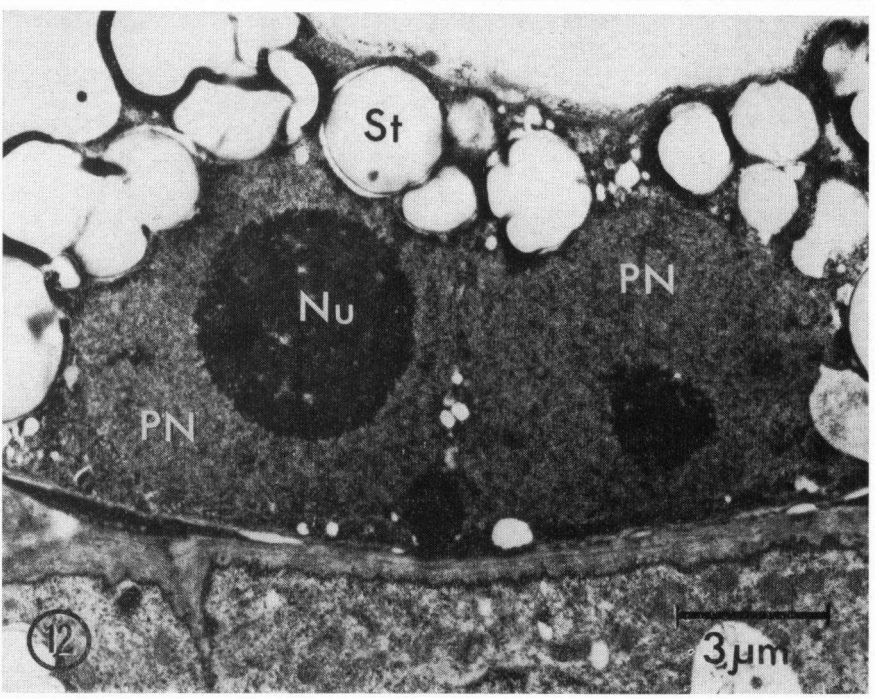
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