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ULTRASTRUCTURE OF THE DEVELOPING EMBRYO SAC OF SPINACH

H. J. WILMS

Vakgroep Plantencytologie en -morfologie, Landbouwhogeschool, Arboretumlaan 4, 6703 BD Wageningen.

SUMMARY

The ultrastructure of the embryo sac of spinach (*Spinacia oleracea* L. cv. Prévital) has been studied from the inception of the constituent cells to the time of fertilization. The development shows two phases. One is from cell formation until cell maturity and the second from maturity to the fertilizable stage. During the first phase the cell dimensions, areas and volumes of the various cells and cell parts have been measured and compared. The protoplasm of the antipodals hardly increases, whereas that of the egg and central cells multiplies 10 times. But the enlargement of all cells is mainly due to vacuolation. During the second phase the cells develop their final ultrastructure. Initially they are similar – have irregular nuclei, abundant ER, common mitochondria and dictyosomes, scanty plastids and no lipid. The antipodals attain their final structure sooner, are ephemeral and start degenerating. The egg cell differentiates fast, subsequently grows slowly and at maturity a renewed increase, in particular of mitochondria, occurs. The development of synergids and central cell is gradual. Before fertilization one of the synergids degenerates and in the central cell the polar nuclei form numerous long protrusions which fuse partly. The ultrastructural changes of each cell type have been related to their possible functions. Accumulation and degradation of reserve materials in various cells is discussed in relation with the nutritional supply to the differentiating female gametophyte.

1. INTRODUCTION

The embryo sac of spinach develops according to the *Polygonum* type. Earlier studies on other species have shown that each constituent cell of this embryo sac has its precise ultrastructure indicating specific functions. The antipodals are thought to be secretory and possibly synthesize enzymes, which digest the adjacent nucellus (DIBOLL & LARSON 1966; VAZART 1968; SCHULZ & JENSEN 1971; RIFOT 1973; ZHUKOVA & SOKOLOVSKAYA 1977). Many hypotheses have been postulated on the function of the synergids (VAZART 1958). It is obvious that their main function is related to the transfer of male gametes to the egg and central cells. Furthermore, most authors assume that the synergids direct the growth of the pollen tube (VAN DER PLUYM 1964; DIBOLL & LARSON 1966; VAN WENT 1970a). JENSEN (1965a) and SCHULZ & JENSEN (1968a), on the other hand, suggest that they have a nourishing function. The egg cell and also the central cell are the most important cells with respect to reproduction. They fuse with the sperm cells and develop into the embryo and endosperm, respectively.

The present paper is a part of the investigation on the early embryogenesis in spinach, starting with the formation of the ovule up to completion of fertilization. It describes and relates the ultrastructure of different cell types with their possible functions, from their inception to the time of fertilization.

2. MATERIAL AND METHODS

Spinacia oleracea L. cv. Prévital was grown in the greenhouse at approximately 25 °C. The dissected ovaries were fixed and embedded according the GA-OsO_4 method, which has been described previously (WILMS 1980).

For detection of polysaccharides on electron microscopic level the method described by THIÉRY (1967) was used.

Measurement of length, width and area were taken with a Kontron MOP AM-2 on at least three ovules of each developing stage.

3. RESULTS

The cellular development of *Spinacia oleracea* embryo sac shows two phases. The first phase is from the formation of cells (young stage) until the attainment of their largest dimensions (mature stage). The latter stage is also determined by the presence of intact antipodals and identically structured synergids. The second phase starts at the mature stage and ends at the fertilizable stage during which cells develop their final ultrastructure. At the time of fertilization, however, the antipodals as well as one of the synergids degenerate.

The dimensions of various cell types and of the embryo sac at the young and mature stages are given in *table 1*. Since in transverse sections the cellular areas are nearly circular only length and width are included. The areas of protoplasm, vacuoles and total cell are measured from median longitudinal sections. The dimensions of constituent cells are also presented as percentage of the total area. The increase in volume is calculated in the following way: the volume of the mature embryo sac is nearly $\pi r_m \times \text{median area}_m$, in which r_m is the ray length in cross sections and for πr_m the width of the mature embryo sac is used. This volume is $55 \times 11{,}400\ \mu m^3$. The volume of the young embryo sac, calculated in the same way, is $17 \times 950\ \mu m^3$. The factor for the increase in volume of the embryo sac is $55 \times 11{,}400/17 \times 950 = 39$.

The development of the embryo sac from young to mature stage takes about 10 days, and from mature to fertilizable stage about 4 days. When young, the embryo sac is 69 μm long and 17 μm wide, but at maturity it measures 350 × 55 μm (*table 1*).

At the fertilizable stage there are considerable developmental differences between the persistent and the degenerated synergids. At the same time, the antipodals are also in various stages of degeneration.

3.1. Embryo sac at young stage

The ultrastructure of various cells of the young embryo sac is initially the same. The first structural differences become visible when vacuolation starts and the random distribution of the organelles in the cytoplasm becomes lost, first in the egg and central cells, next in the antipodals and finally in the synergids (see *diagrams 1–4*).

Table 1. Mean size and areas from median longitudinal sections at young and mature stages of embryo sac. Each number represents mean of measurements on three different ovules (prot = protoplasm, vac = vacuoles, tot = total).

	Young stage					Mature stage					Volume increasing factor		
	size	area in μm²				size	area in μm²						
	(μm)	prot	vac	tot	%	(μm)	prot	vac	tot	%	prot	vac	tot
embryo sac	69×17	720	230	950	100	350×55	2000	9400	11400	100	9	130	39
3 antipodals	24×17	210	10	220	23.2	65×18	200	200	400	3.5	1	21	2
synergid	16×10	70	–	70	7.4	64×22	200	500	700	6.1	6	>200	22
egg cell	18×14	80	50	130	13.7	70×28	400	400	800	7.0	10	16	12
central cell	42×17	360	170	530	55.7	300×55	1200	8300	9500	83.4	10	160	57

The nuclei are irregular in shape and approximately 5–6 μm in diameter with homogeneous nucleoli of about 4 μm in diameter. The size of the polar nuclei is, however, larger.

ER (endoplasmic reticulum) is abundant but its distinction as SER or RER is not clear. In the central cell most of the ER cisternae are interconnected or arranged in circular patterns which encircle small vacuoles.

Mitochondria are spherical and oval, are regularly spread in the cytoplasm, and have few tubular cristae, except for those in the synergids which have more cristae.

The number of plastids is low and they are oval to pear-shaped with a diameter of approximately 0.3 μm. Their ultrastructure is rather simple. A small starch grain is observed in the plastids of the egg cell and of the synergids, whereas plastoglobuli sometimes occur in the plastids of the synergids.

Dictyosomes are frequent. Each consists of 4–6 mostly flat cisternae with an average length of 0.7 μm. The dictyosomes of the egg cell have flat as well as circular cisternae. All cisternae form many small vesicles with an electron-transparent content.

Lipids are absent in the young stage but a very small amount is seen in the synergids.

3.2. Antipodals

The partitioning of the chalazal cytoplasm at the coenocytic stage results in the formation of three antipodals (*fig. 1*). Separating walls are formed from the periphery of the embryo sac to the centre. Dictyosomes are frequently at the places of wall formation. Wall synthesis also occurs in relation to the side walls of the antipodals. All walls of the antipodals possess wall projections (*fig. 2*) and have plasmodesmata (*figs. 3–5*). The longitudinal walls inbetween the antipodal cells and nucellus do not have plasmodesmata connections. In the degenerating phase no plasmodesmata are observed anymore.

The morphology of the antipodals, marked with A1, A2 and A3, at the mature stage is shown in *fig. 2*. A1 is the antipodal bordering the central cell and A3 is the

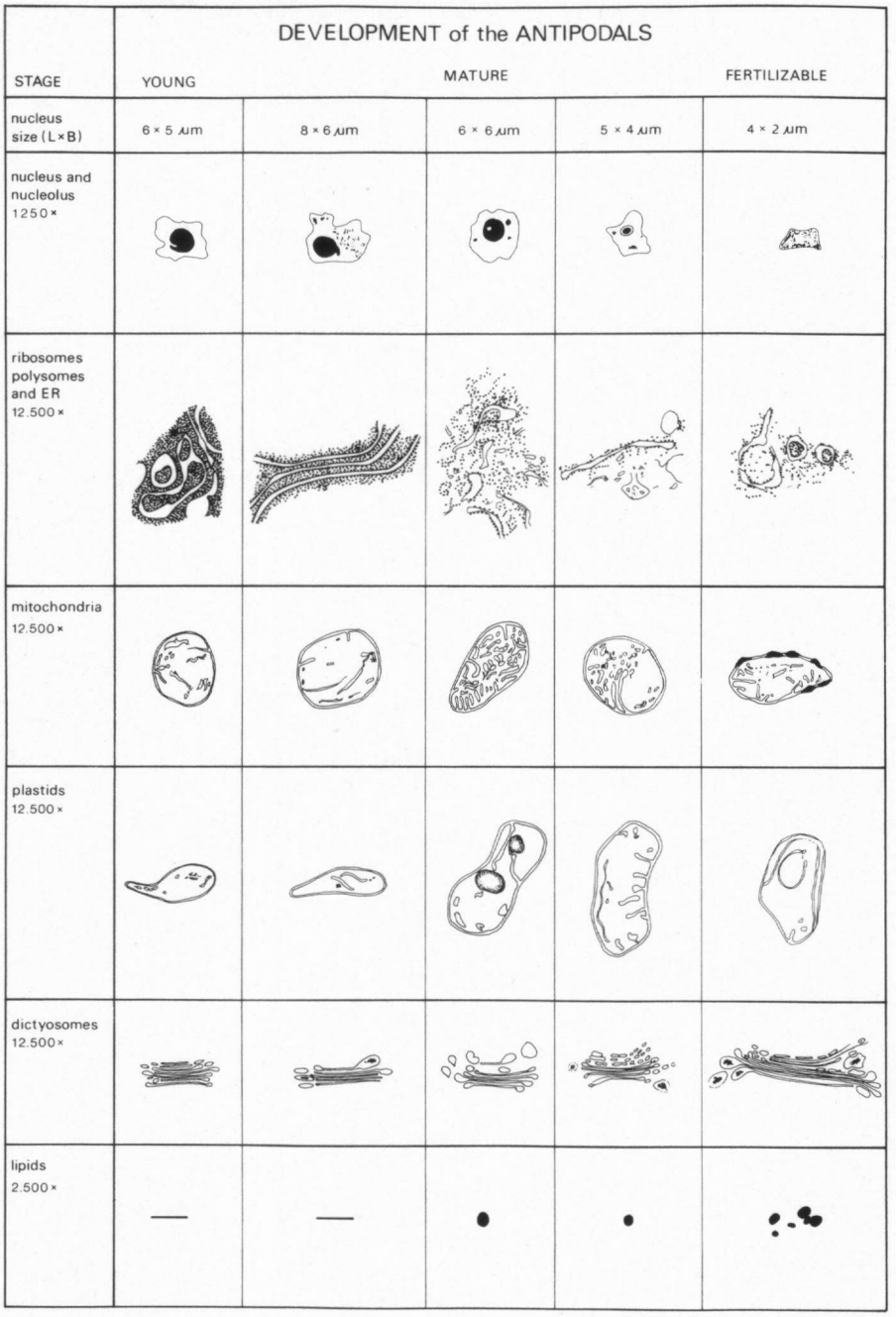

Diagram 1. Morphological changes of antipodal cell organelles during development of embryo sac. Sign – is used when no lipid granules are observed.

one bordering the chalazal nucellus. At maturity their average sizes (L × B in μm) in median sections are A1: 24 × 16, A2: 36 × 10 and A3: 40 × 12. Soon after the mature stage has been reached degeneration starts successively in A1, A2 and finally A3.

At maturity the nucleus becomes almost spherical and has a diameter of approximately 6 μm. A small amount of chromatin is spread over the entire nucleus. In a later stage it becomes irregular again and decreases in size. The nucleolus disintegrates and eventually disappears. The nuclear envelope often shows separated membranes with large electron-transparent spaces in between. The karyoplasm develops very condensed chromatin clumps.

During the development of the antipodals there is an increase in vacuolation whereas the organelles undergo a number of changes (*diagram 1*). In the first phase the amount of ribosomes per cytoplasm area diminishes. Long ER cisternae with attached ribosomes develop and become arranged parallel to each other. At the mature stage irregularly distributed cisternae, often with sacculate endings, are observed. The amount of attached ribosomes decreases as compared to the other polysomes as well as monosomes. When degeneration sets in RER is often observed as circular strands (*fig. 6*), sometimes encircling small vacuoles or cytoplasmic islands with accumulated ribosomes.

The mitochondria show an increase in diameter up to 0.9 μm and many tubular cristae also develop. They begin to degenerate simultaneously with the antipodals. The number of cristae decreases and this is accompanied by the accumulation of strongly stained material in the surrounding membranes and perimitochondrial space.

Plastids are not common in the antipodals. A few long thylakoids and some short sacculate protrusions of the inner membrane develop. Sometimes single starch grains occur, which subsequently disappear.

The number of dictyosomes remains the same during cell maturation, whereas the number of cisternae becomes less. During degeneration the dictyosomes produce many vesicles, often large ones, with an electron-dense content.

A few droplets of lipid with a maximal diameter of 0.6 μm appear when the antipodals become mature. During degeneration a slight increase in the amount of lipid occurs.

3.3. Synergids

The general topography of the micropylar part of the mature embryo sac is shown in *figs. 7* and *10*. The cells of the egg apparatus are only partly attached to the micropylar embryo sac wall. Because of this and because of the triangular arrangement of the cells, a number of cell walls are common or shared, including the common synergid wall, the egg cell-synergid wall, the synergid-central cell wall and the egg cell-central cell wall. The boundaries of the cells of the egg apparatus at the fertilizable stage vary considerably, from two membranes only at the chalazal side of the cells to a specialized thickened structure, the filiform apparatus (FA), at the micropylar pole of the synergids. In between these extremes the thickness of the remaining cell walls is irregular. The base of the egg

cell is attached at the side of the embryo sac, about 10 μm from the most micropylar part (*fig. 7*).

The major part of the synergid cytoplasm and the nucleus are located in the micropylar region of the cell. The chalazal portion is filled with a number of vacuoles. One of the synergids disorganizes before fertilization (*fig. 10*) whereas the other (psy) does so soon after double fertilization. In a few older but unfertilized ovules the synergids start degenerating simultaneously.

At the young stage each synergid is completely surrounded by a cell wall of equal thickness (about 0.1 μm) over the entire length (*fig. 1*). With the formation of the FA, the remaining parts of walls at the micropylar side become thicker (*fig. 8*). Both the FA and other parts of the cell wall show an increase in density during development (*fig. 9*). At the chalazal side there is less increase in wall thickness and this is accompanied by decrease in density.

The differentiation of FA starts simultaneously with the formation of wall protrusions at different places of the merged synergid-embryo sac wall. Rod-, club-, finger- and plate-like extensions of the wall grow into the cytoplasm. As a result of their fusion and formation of new wall protrusions finally a complex striated filiform apparatus develops. A plasmalemma separates the wall material from the cytoplasm but the interface is so convoluted that the FA appears as a sponge-like mass of wall material interpenetrated by cytoplasm.

In one and sometimes both synergids a discontinuity in FA wall formation is observed close to the common synergid wall. On these places the original wall is still present in close connection with the cytoplasm and the FA shows a slit (*fig 12*). The other small cytoplasmic inclusions in the FA are round and show all organelles. Initially the FA has the same homogeneous ultrastructure (*fig. 9*). After the mature stage however, different wall material is deposited. At the time of fertilization, a number of layers are already deposited (*fig. 11*). The electron density of the successively formed layers gradually decreases (*fig 11*). In GA-OsO_4 fixed material the density of the later deposits corresponds with the density of the content of the dictyosome vesicles present at that time (*fig. 11*).

The FA gives a strong PAS positive reaction for insoluble carbohydrates and also a positive reaction on the Thiéry-test for polysaccharides.

Plasmodesmata connect the two synergids and also the synergids with the egg cell and the central cell. They are observed only in the micropylar half of the synergid.

The various organelles show ultrastructural changes during the course of differentiation and maturation (*diagram 2*) accompanied by an ultimate polar distribution. The nucleus elongates towards the FA and then the shape changes to oval. It is approximately 12 μm long and about 4 μm in thickness. The total length of the nuclear envelope in median sections increases about twice. The nuclear envelope has many pores. Contact between the nuclear envelope and the ER is rare. The outer nuclear membrane is partly covered with ribosomes. At maturity numerous small concentrations of chromatin are regularly spread over the entire nucleus. During the development to the fertilizable stage the nucleolus of the degenerated synergid disintegrates almost totally, whereas in the persistent

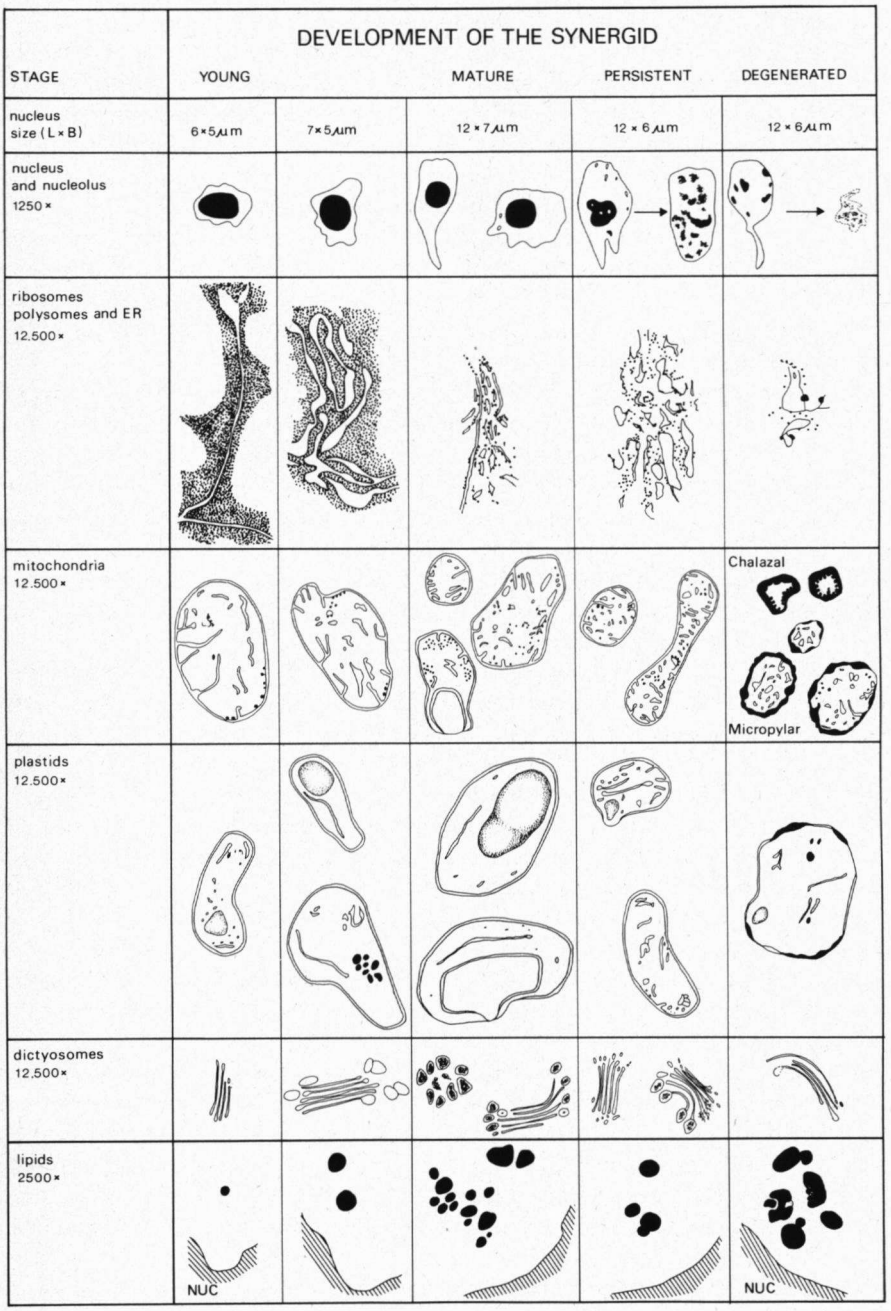

Diagram 2. Synergid cell organelles as seen during embryo sac development. Lines in lipid columns represent micropylar parts of synergid walls (NUC – nucellus).

synergid its diameter decreases to about 1 μm.

The number of mitochondria increases tremendously up to maturity. They are elongated and their average diameter is 0.5 μm. The tubular cristae become well developed. When degeneration of the synergid starts, electron-dense material accumulates at the membranes. The cristae collapse and then disappear whereas a thick electron-dense layer accumulates at the perimitochondrial space. The inner and outer membranes cannot be distinguished any more.

The plastids are distributed mostly in the cytoplasm around the nucleus and partly in between the nucleus and the FA. They become cup-shaped and subdense. In the persistent synergid they appear translucent, and have some electron-dense material. In the degenerated synergid very few vesicles, all without electron-dense material, are visible.

The distribution of dictyosomes in the cytoplasm increases at random, except near the nucleus where they are absent. The cisternae of each dictyosome increase in number to 4 or 5 and in length to 1.0 μm. Associated with the cisternae are vesicles of different sizes and of various degrees of density according to the developmental stage of the synergid. Shortly after the coenocytic stage electron-transparent vesicles are observed and at maturity most of these become electron-dense. In the persistent synergid they appear translucent, and have some electron-dense material. In the degenerated synergid very few vesicles, all without electron-dense material, are visible.

The RER cisternae become dilated and rearranged towards maturity, each resulting in single ER cisternae and having short and swollen lamellae, both covered with ribosomes. The number of ribosomes greatly decreases at maturity. Lipid bodies increase during the development of the synergids, both in number and diameter. Intensification of this process occurs in the degenerating synergid.

The thin plasma strands surrounding the vacuoles contain only a few organelles. The plasma membrane of the degenerated synergid is no longer distinguishable at the chalazal side.

3.4. Egg cell

The egg cell is a polarized cell (*fig. 1*). with its cytoplasm and nucleus located at the chalazal end, and vacuoles at the micropylar side. The mature egg cell is approximately 70 μm long, 30 μm wide at the chalazal and 18 μm wide at the micropylar part (*table 1*). The micropylar half is surrounded by a wall with intermediate density whereas at the chalazal half the membrane is in close contact with that of the central cell. During the development from mature to fertilizable stage the wall between the egg and central cells in the region of the degenerated synergid gets an irregular and unusual morphology. In this area the plasma membranes of the egg and the central cells become intermittently separated by gaps which contain randomly distributed granular dense material (*fig. 15*). In between the gaps the membranes of the egg and the central cells remain close together.

The partial egg cell wall only contains plasmodesmata where it borders the synergids and the central cell.

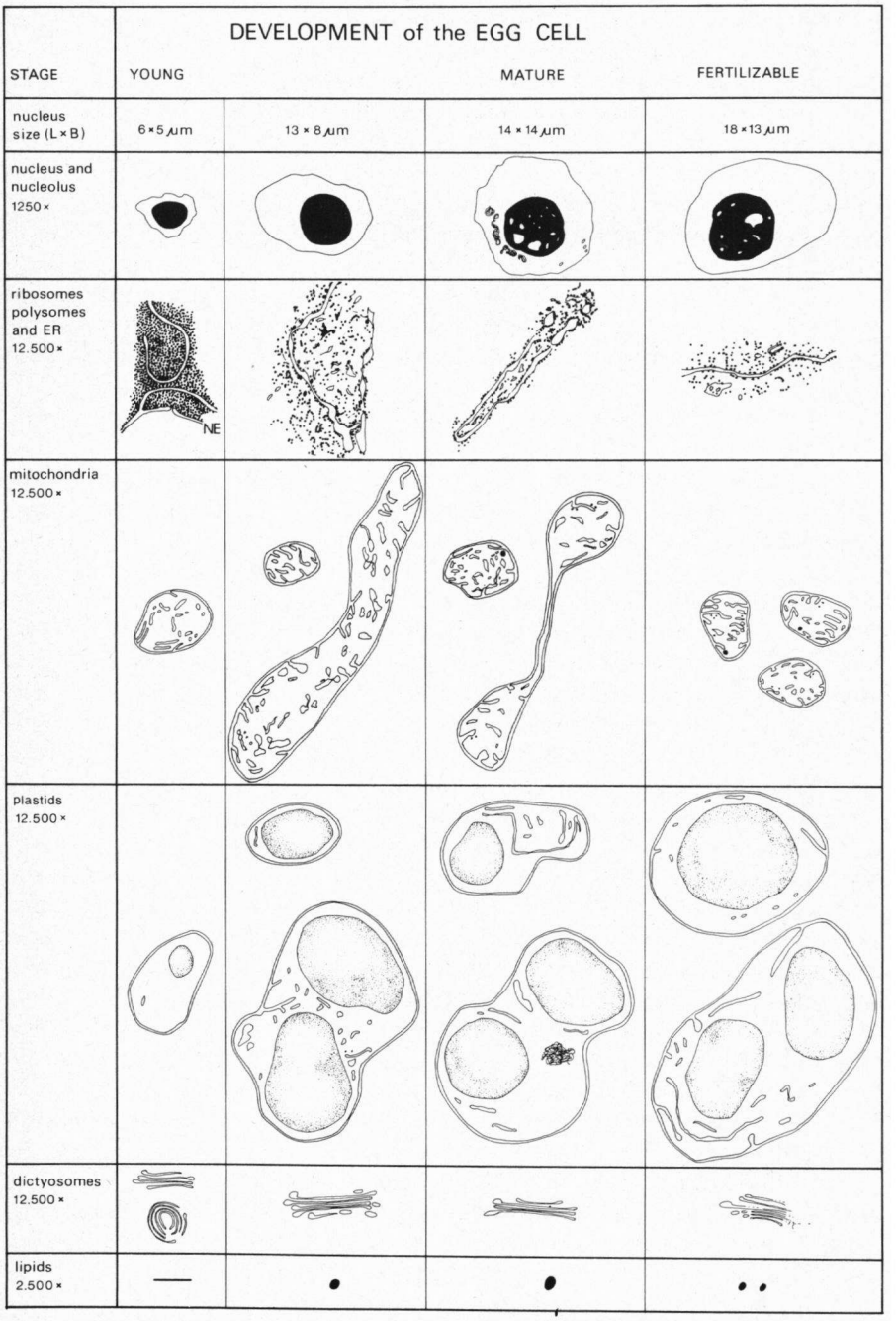

Diagram 3. Egg cell organelles during development of embryo sac. Sign – indicates absence of lipid granules (NE – nuclear envelope).

The nucleus is located at the chalazal tip of the egg cell. At maturity the nucleolus has an electron-dense granular structure with some electron-opaque vacuoles. Then the karyoplasm often contains some multi membrane-like structures resembling myeline (*fig. 13*) and some electron-translucent vacuolar material (*fig. 14*).

The changes in the organelles during the course of differentiation and maturation of the egg cell are depicted in *diagram 3*.

Mitochondria elongate and their number increases. At the fertilizable stage many mitochondria become clustered in the micropylar part of the egg cell.

The plastids are located near the nucleus and possess single starch grains. These amyloplasts grow to an average diameter of 2.5 μm at maturity. Also the amount of starch increases, whereas usually more than one large starch grain develops.

The number of dictyosomes remains low during the maturation of the egg cell. Their ultrastructure stays simple.

The number of ribosomes gradually decreases during development. Single strands of RER and free mono- and polyribosomes are present at mature stage. Some small lipid droplets are also present.

3.5. Central cell

The central cell comprises all the original megaspore cytoplasm that is not included in the antipodals, synergids and egg cell. A large central vacuole restricts the cytoplasm to peripheral areas whereas in the vicinity of the egg apparatus an accumulation of cytoplasm, including the two polar nuclei, is observed. A few strands run through the vacuole, connecting the cytoplasm associated with polar nuclei to the peripheral cytoplasm of the cell. The mature central cell is approximately 300 μm long and 55 μm wide (*table 1*).

The central cell is surrounded by a rather irregular wall. At the place of contact with the antipodals the wall shows projections alternated with plasmodesmata. The wall bordering the nucellus is equally thin, except for the micropylar region near the egg apparatus, where projections are present (*fig. 7*). Both parts are without plasmodesmata. The central cell shares a wall with plasmodesmata with the synergids and egg cell at the micropylar part of their contact.

The two polar nuclei approach each other and the chalazal tip of the egg apparatus during the development of the embryo sac. In the nuclei electron-opaque vacuoles appear and the nucleolar diameter increases to 8 μm. The size of the polar nuclei also increases to about 20 × 14 μm. After maturity the overall shape still remains spherical but numerous long protrusions are formed into the surrounding cytoplasm (*fig. 16*). The protrusions are enveloped by both nuclear membranes. Meanwhile the nuclear size diminishes to about 14 × 12 μm. The polar nuclei fuse partly before fertilization, but only after pollination has taken place. The partial fusion of the nuclei takes several hours and usually begins by fusion of the membrane of the nuclear protrusions, occasionally by the joining of the nuclear membranes directly.

While the number and shape of mitochondria hardly changes, their length

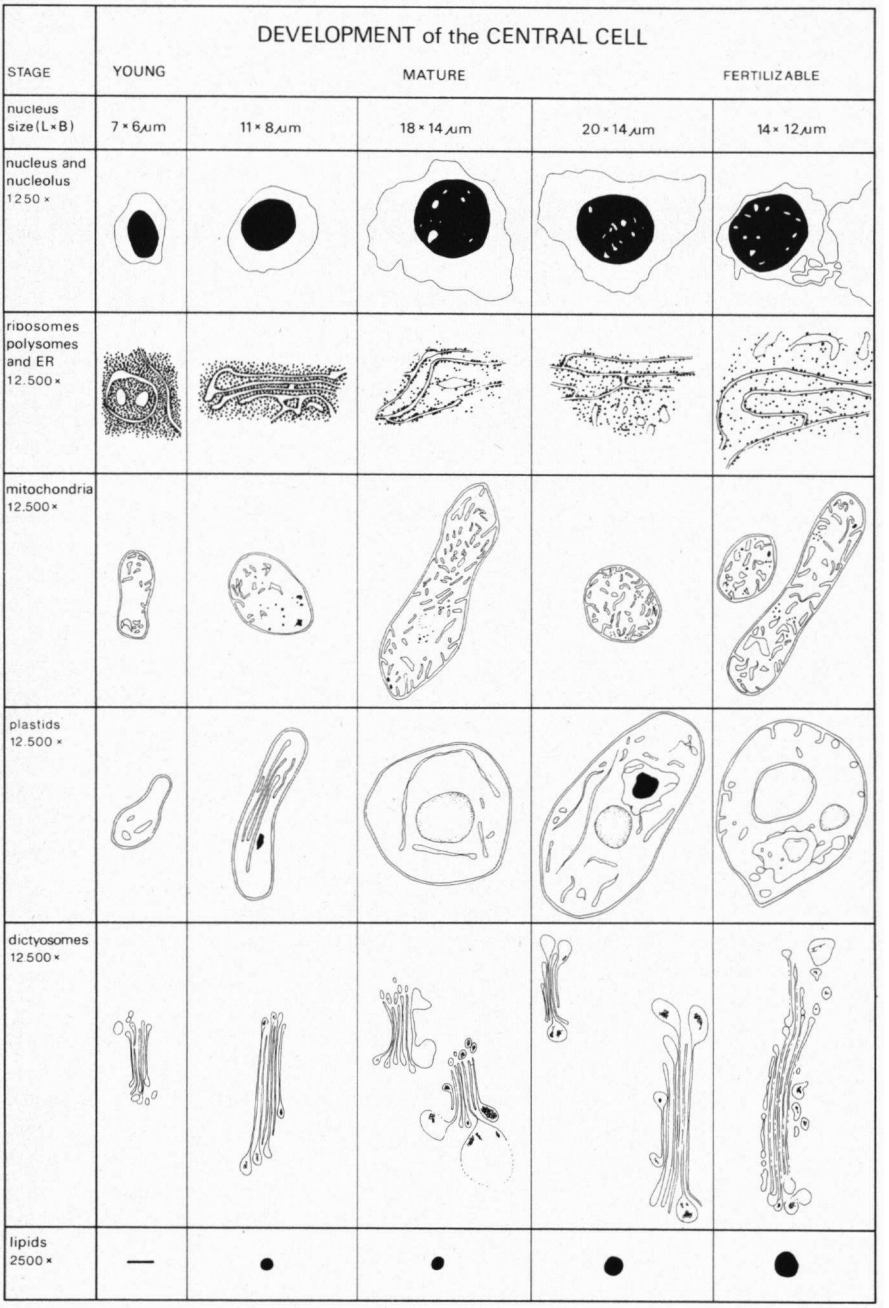

Diagram 4. Cell organelles of central cell during development of embryo sac. Sign – shows absence of lipid granules.

increases to 2.5 μm at maturity (*diagram 4*) and a large number of short tubular cristae develops. Ribosome-like bodies also are present at maturity.

The plastids, restricted in number and position, grow and develop during central cell maturation. They become almost spherical (2–3 μm in diameter) and usually possess one small starch grain, some long thylakoids, and plastoglobuli. In a later stage a sacculate reticulum develops.

The decrease of ribosomes in a cytoplasmic area results in a distinction between ribosomes associated with the ER and those lying free. Whorls of RER are commonly found when the mature stage is reached. Near the egg apparatus usually 2 or 3 parallelly arranged RER cisternae are visible along the plasma membrane.

The dictyosomes develop differently depending on their location. In the peripheral cytoplasm surrounding the central vacuole they are similar to those at the young stage. Sometimes larger vesicles with and without electron-dense material are formed. The dictyosomes in the micropylar part of the developing central cell first decrease in number and then become more elongated, to about 2.5 μm, showing an increasing number of accompanying vesicles containing some electron-dense material.

4. DISCUSSION

The development of the spinach embryo sac from the formation of the individual cells to the fertilizable stage can be seen in two phases: 1) enlargement and differentiation of the cells; 2) realisation of the final functional situation.

Cell enlargement ends at the mature stage. The area in median longitudinal sections, which is occupied by the antipodals in the young stage is about the same as that of the egg apparatus, whereas that of the central cell is more than twice. During maturity the central cell increases maximal, the egg cell and the synergids enlarge relatively much less, whereas the antipodals increase very little. The enlargement is mainly the result of an increase of vacuoles and partly of protoplasm. The egg and central cells produce relatively more new protoplasm as compared to the other cells of the embryo sac. (see *table 1*). In fact the protoplasm of the antipodals hardly increases. This may be considered as a first indication of differences in the developmental stages of the various cells of the embryo sac.

Realisation of the final functional situation, starting with the degeneration of the antipodals and one of the synergids, seems to be related to pollination. Degeneration of one synergid in general starts after pollination, but further study is needed to confirm this. The functional differentiation of the cells is also indicated by the changes in wall structure and their protoplasmic ultrastructure.

4.1. Antipodals

Wall protrusions occur mainly in the chalazal antipodal cell(s) (MASAND & KAPIL 1966; FISHER & JENSEN 1969; D'ALASCIO-DESCHAMPS 1973; GODINEAU 1973; NEWCOMB 1973a RIFOT 1973; KAPIL & BHATNAGAR 1978). Initially the

wall between the micropylar antipodal and the central cell is relatively thin, at maturity this wall thickens (D'ALASCIO-DESCHAMPS 1973; NEWCOMB 1973a; RIFOT 1973). Plasmodesmata are present in the walls of the chalazal antipodal, in between the antipodals and in the walls between the micropylar antipodal and the central cell (SCHULZ & JENSEN 1971; GODINEAU 1973; NEWCOMB 1973a). In spinach all antipodals have wall protrusions. The location of plasmodesmata is similar to that described for other species. That means that plasmodesmatal contact between sporophytic and gametophytic tissue is restricted to the extreme chalazal position. During megasporogenesis and megagametogenesis the entire development is turned towards the elimination of plasmodesmatal contact between the sporophyte and gametophyte. On the other hand, the absence of plasmodesmata means an obstruction in the supply of metabolites. The maintenance of a limited area (the extreme portion of the chalazal antipodal) with plasmodesmata can be considered as an intermediate situation between the isolation mechanism and the maintenance of required nutrition at this time of development. In a later stage these plasmodesmata are not observed any more.

The final degeneration of the antipodals starting with the micropylar one can be an effect of the continuing contact between genetically different sporophytic and gametophytic protoplasm. But it can also be due to a definite break of the protoplasmic contact in view of the future fertilization, to prevent direct contact between the two, genetically different, sporophytes. From this stage on contact and nutrition have to go via cell walls and plasma membranes. This results in a shift of transport routes and confirms earlier statements (WILMS 1980). Transport no longer takes place through the original chalazal nucellus tissue, but through the chalazal proliferating tissue and subsequently through the entire embryo sac wall.

The ultrastructure of the cytoplasm of antipodals of spinach differs in various stages of development. During the process of development they have many mitochondria, ribosomes and RER, whereas the number of dictyosomes is variable GODINEAU; 1973; RIFOT 1971, 1973; D'ALASCIO-DESCHAMPS 1973; ZHUKOVA & SOKOLOVSKAYA 1977; DUMAS 1978; NEWCOMB 1973a), although poorly developed antipodals are often reported during embryogenesis (BANNIKOVA 1971; SCHULZ & JENSEN 1971). In *Spinacia* initially the dictyosomes generate vesicles which are likely to be related to the formation of irregular wall thickenings. At maturity the mitochondria develop many tubular cristae, which suggests a high activity of the cells. The antipodals function till the embryo sac is mature but in the following period, to the fertilizable stage, degeneration sets in, beginning with the micropylar antipodal. The ephemeral antipodals do not secrete enzymes which can digest the enclosing nucellus as is suggested in other taxa (SCHULZ & JENSEN 1971; RIFOT 1973), because no degeneration of the surrounding nucellus is observed.

4.2. Synergids

In spinach the FA is an elaboration of the wall as in other species. Its material is

synthesized within the synergids. The FA consists of a homogeneous osmiophilic structure, much more osmiophilic than the cell wall parts of the surrounding nucellus (WILMS 1980), indicating that this consists of a major non-cellulosic compound. The FA of *Petunia* (VAN WENT 1970a) or *Helianthus* (NEWCOMB 1973a) is only a thickening of the cell wall. In other species such as *Capsella* (SCHULZ & JENSEN 1968a), cotton (JENSEN 1965a), *Zea mays* (DIBOLL & LARSON 1966), barley (CASS & JENSEN 1970), *Linum* (VAZART 1971), *Aquilegia* (FOUGERE-RIFOT 1975), *Plantago* (VANNEREU 1978) and in *Spinacia* too the formation of the FA implies an increase of the plasma membrane area. These synergids might be considered as 'transfer cells' (GUNNING & PATE 1969; PATE & GUNNING 1972).

It is of interest that the time of synergid degeneration varies from plant to plant: from before pollen tube entry, as in cotton (JENSEN 1965a), *Hordeum vulgare* (CASS & JENSEN 1970), *Stipa elmeri*, *Epidendrum scutella* (COCUCCI & JENSEN 1969), *Linum* (VAZART 1971), *Acer* (HASKELL & POSTLETHWAIT 1971), *Helianthus* (NEWCOMB 1973b) and perhaps *Zea mays* (DIBOLL 1968), to after pollen tube entry, as in *Capsella* (SCHULZ & JENSEN 1968a) and *Petunia* (VAN WENT 1970a). The degeneration of one synergid in spinach seems a direct response to pollination as reported for *Gossypium* (JENSEN & FISHER 1968). An indication for this is that in some older unfertilized ovules both synergids are equally degenerated.

According to MAZE & LIN (1975) the FA in each of the two synergids has specific functions. In the penetrated synergid it controls the pollen tube growth or the transfer of pollen-tube-growth-directing substances out of the synergid. The FA of the persistent synergid transfers material into the megagametophyte (GUNNING & PATE 1969; JENSEN 1965a; SCHULZ & JENSEN 1968a; NEWCOMB 1973b). Neither VAN WENT (1970a) nor MOGENSEN (1972) interpret the persistent synergid as having a transference function. In spinach no supply route goes this way till fertilization (WILMS 1980) and after fertilization the persistent synergid starts degenerating quickly. This indicates that the assumed transference function of the persistent synergid may be of minor importance. The FA of the degenerated synergid seems to control and regulate its molarity. After degeneration this synergid probably has a lower molarity than the persistent synergid and also lower than the intercellular spaces of the bordering conductive nucellar tissue.

The synergids show in the young stages many well developed mitochondria and dictyosomes and long strands of RER, which are indicatives of a high metabolic activity. The increase in the number of dictyosomes and vesicles with an osmiophilic content indicates production of secretory compounds.

When one synergid degenerates, its organelles lose their internal structure and an accumulation of lipid in between the membranes occurs. Lipid granules accumulate and aggregate in the cytoplasm. This can possibly be related to growth stimulating or growth inhibiting effects of fatty acids in pollen tubes (IWANAMI 1980), since in *in vitro* experiments the monocarboxylic acids inhibit pollen germination and pollen tube growth, whereas dicarboxylic acids stimulate pollen tube growth, both related to IAA activity.

The very thin cell walls at the chalazal part of the young synergid disappear, and during the following stages only a plasma membrane can be seen. Finally, when pollen tube penetration is about to occur this membrane disappears and the chalazal part of the cell collapses, possibly due to a decrease in molarity of this cytoplasm. Absence of starch, or presence of only small amounts, and presence of much lipid suggest a small amount of water-soluble and molarity-increasing compounds.

4.3. Egg cell

The changes in size, shape and ultrastructure of the differentiating egg cell occur mainly in the first days after formation. At the end of the differentiation the number of mitochondria increases much in the micropylar part. Such a large number of well-formed mitochondria is also recorded in *Zea mays* (DIBOLL & LARSON 1966). According to RUNNSTRÖM, HAGSTRÖM and PERLMAN (1959) this does not necessarily mean that a high rate of respiration is taking place; rather it might indicate a potential for a high metabolic rate, generally associated with postfertilization activity. The relatively small number of other organelles and their internal morphology indicate a low rate of activity in the mature egg cell. The occurrence of starch in *Spinacia* egg cells seems to be common as it is also in other angiosperms (JENSEN 1965b); DIBOLL & LARSON 1966; SCHULZ & JENSEN 1968b; VAN WENT 1970b; NEWCOMB 1973a). This is probably needed shortly after fertilization for the development of the proembryo, since it disappears then.

The occurrence of myeline- and vacuole-like structures in the karyoplasm during a short period of the mature stage is exceptional for interphase nuclei. Similar membranous bodies are reported in megaspore mother cells at the meiotic prophase of *Allium* (DE BOER-DE JEU 1978) and for microspore mother cells at the zygotene stage in *Pinus sylvestris* (WILLEMSE 1971). In *Pinus banksiana* DICKINSON & BELL (1976) report myeline-like figures in the meiotic nuclei of the microspore mother cells, whereas they suppose that their initiation is by invagination of the inner membrane of the envelope. In the development of the egg nucleus there seems a moment which corresponds with the meiotic stage of the spore mother cell. In the egg cell the nucleus is preparing for fusion while the nucleus of the spore mother cell is still in the stage of division.

4.4. Central cell

The structural changes during development of the central cell of *Spinacia* are rather slow and spread over the total developing period. In the cytoplasm around the polar nuclei an increasing ER system develops. At the same time nuclear extensions, visible as long thread-like runners, develop and get connected with each other. The tremendous increase in surface area of the nuclear envelopes may be of importance for the fusion of the nuclei with the sperm nucleus, but is not yet understood. The fusion of the polar nuclei occurs in a similar way as reported in cotton (JENSEN 1964, 1965b) *Zea* (DIBOLL 1968) and *Petunia* (VAN WENT 1970b). They partially fuse before fertilization, whereas in other species total nuclear fusion is completed by this time (GODINEAU 1973).

At the fertilizable stage partly fused nuclei, newly formed ER-cisternae, free ribosomes and many mitochondria with tubular cristae in the neightbourhood of the egg apparatus suggest that the central cell is awaiting fertilization.

The structural development of the cell types of the embryo sac of spinach can be summarized and correlated with the developing time of the embryo sac (*diagram 5*). The ultrastructure at the formation is the reference. The degree of changes is considered as degree of development, either positive as it reflects increase of complexity, or negative as it is interpreted as degeneration. When the organelles are optimal in development and maximal in number the cell type gets a development degree of 100, and each cell type seems to have its own specific metabolic activity.

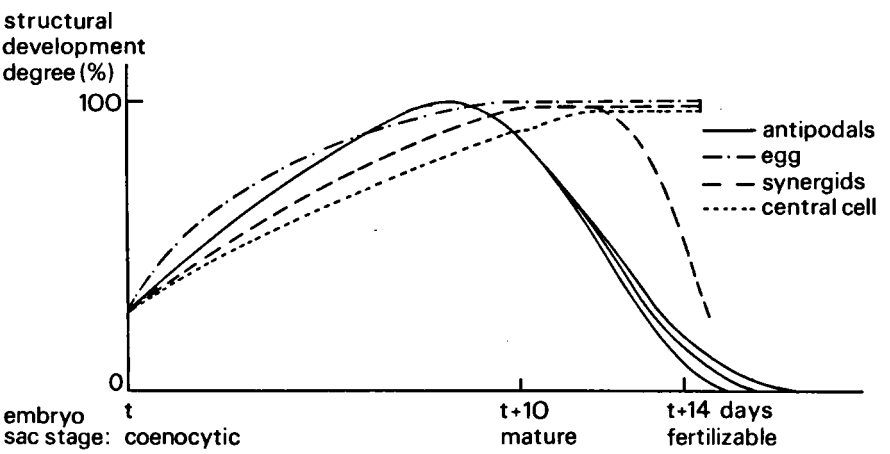

Diagram 5. Correlation between development of various cell types of embryo sac and development, differentiation and degeneration of their organelles.

In the young stage of development of the embryo sac the main supply of metabolites is via the antipodals to the central cell to the egg and synergids (WILMS 1980). Storage of reserve material starts in the egg as starch, and increases during its entire development until the fertilizable stage. At the same time a little amount of starch and some lipid are stored in the synergids. During the first phase of cell enlargement and differentiation lipid granules are formed in the central cell, egg cell and synergids, whereas the storage of starch stops in the synergids.

In the latter some plastoglobuli appear for a short while in their plastids. Near maturity starch is formed also in the central cell and the antipodals, suggesting more metabolic supply needed for further cell development. At maturity this metabolic supply stops and in the antipodals the starch is broken down gradually whereas lipid formation continues. In the central cell and also in the synergids a similar but slower process occurs.

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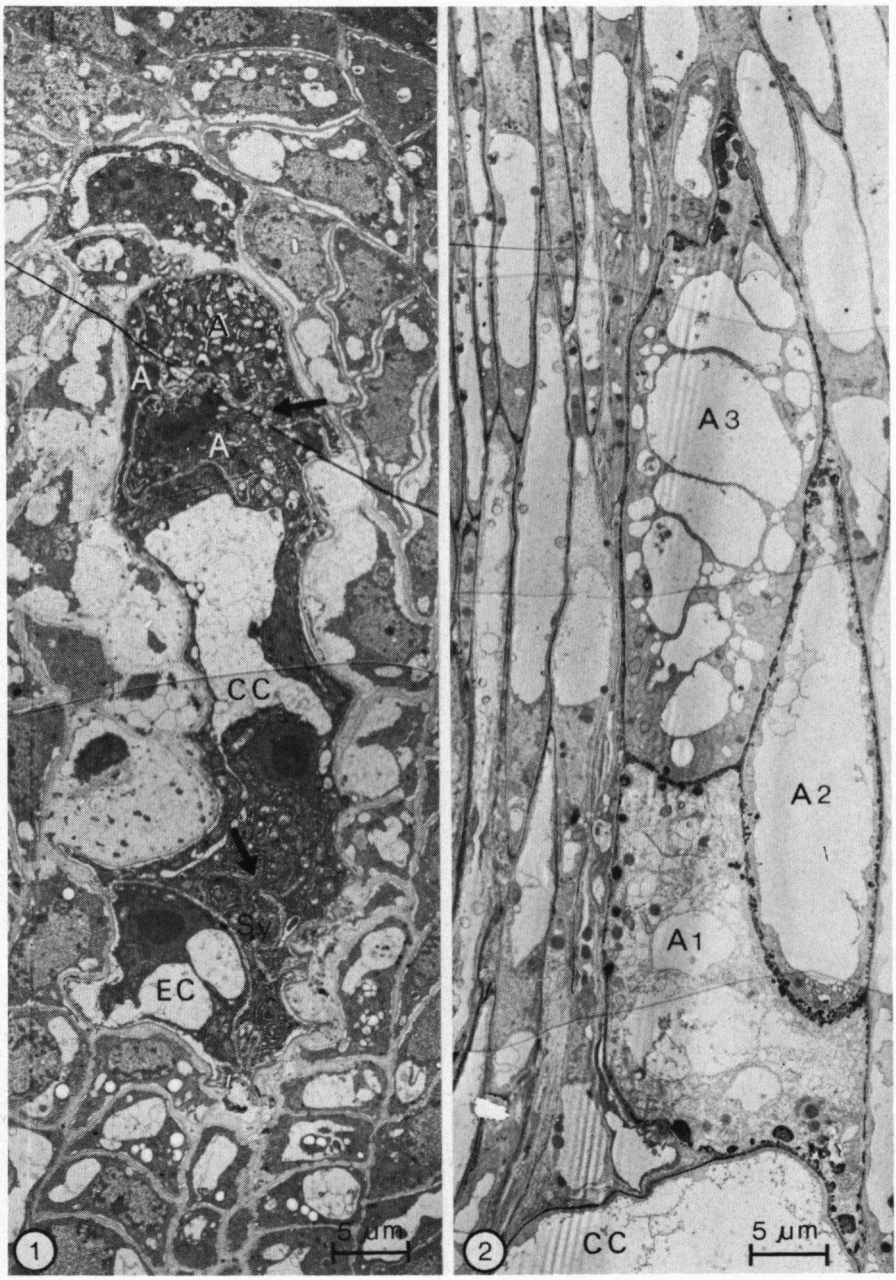

Fig. 1. Young embryo sac just after coenocytic period. Cell wall of antipodals (A) and synergids (Sy) is not yet formed (arrows). 2,000 ×. Fig. 2. Antipodals (A1, A2, A3) at fertilizable stage. 2,000 ×. (CC – central cell, EC – egg cell).

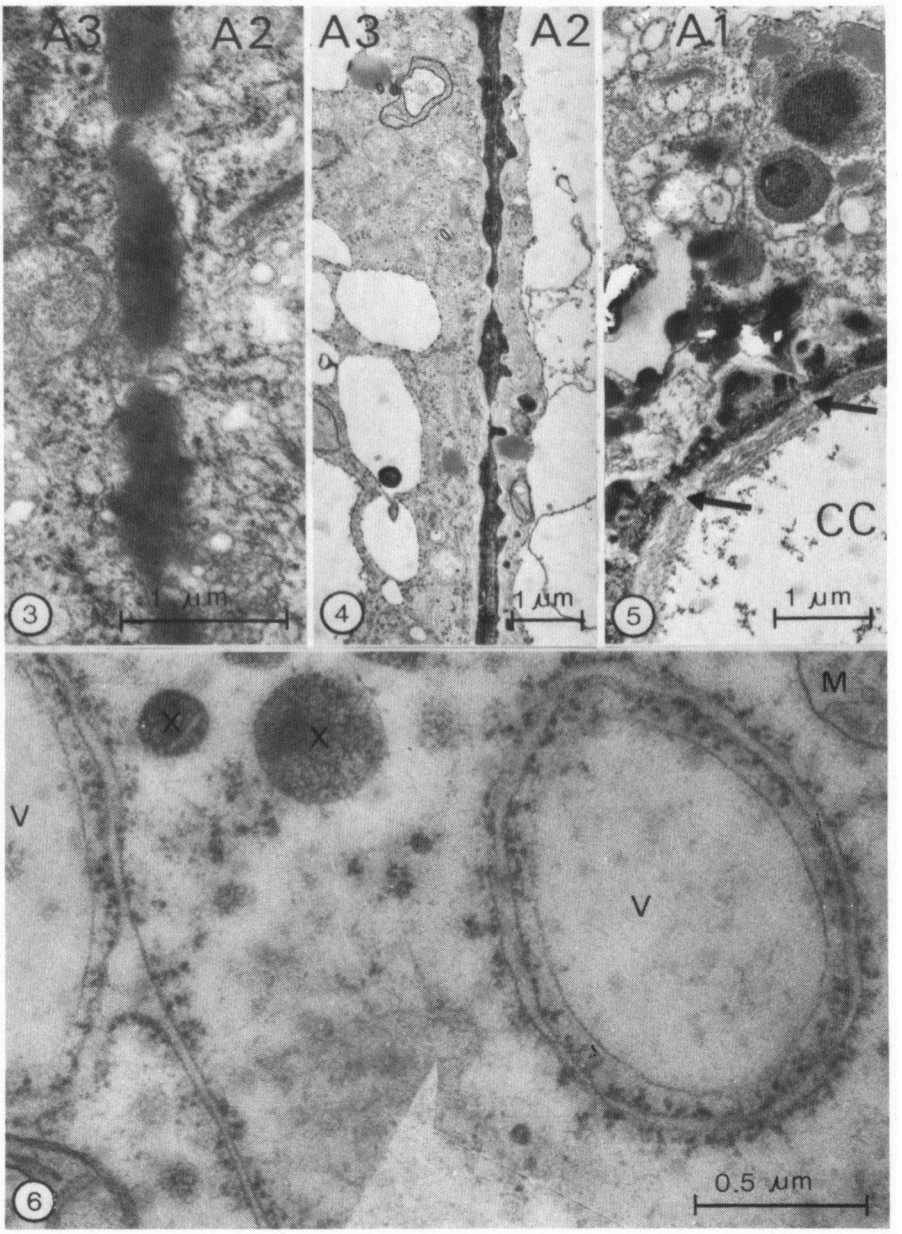

Fig. 3. Part of antipodals (A) when mature showing wide plasmodesmatal connections between common cell wall. 22,000 ×. Fig. 4. Portion of A3 – A2 enlarged from fertilizable stage depicted in fig. 2. 9,000 ×. Fig. 5. Portion of the A1 – central cell (CC) magnified from fig. 2. 13,000 ×. Fig. 6. Cytoplasm of A3 during fertilizable stage exhibiting circular strands of RER around vacuoles. 45,000 ×. (M – mitochondrium, X – cluster of degenerating ribosomes, V – vacuoles).

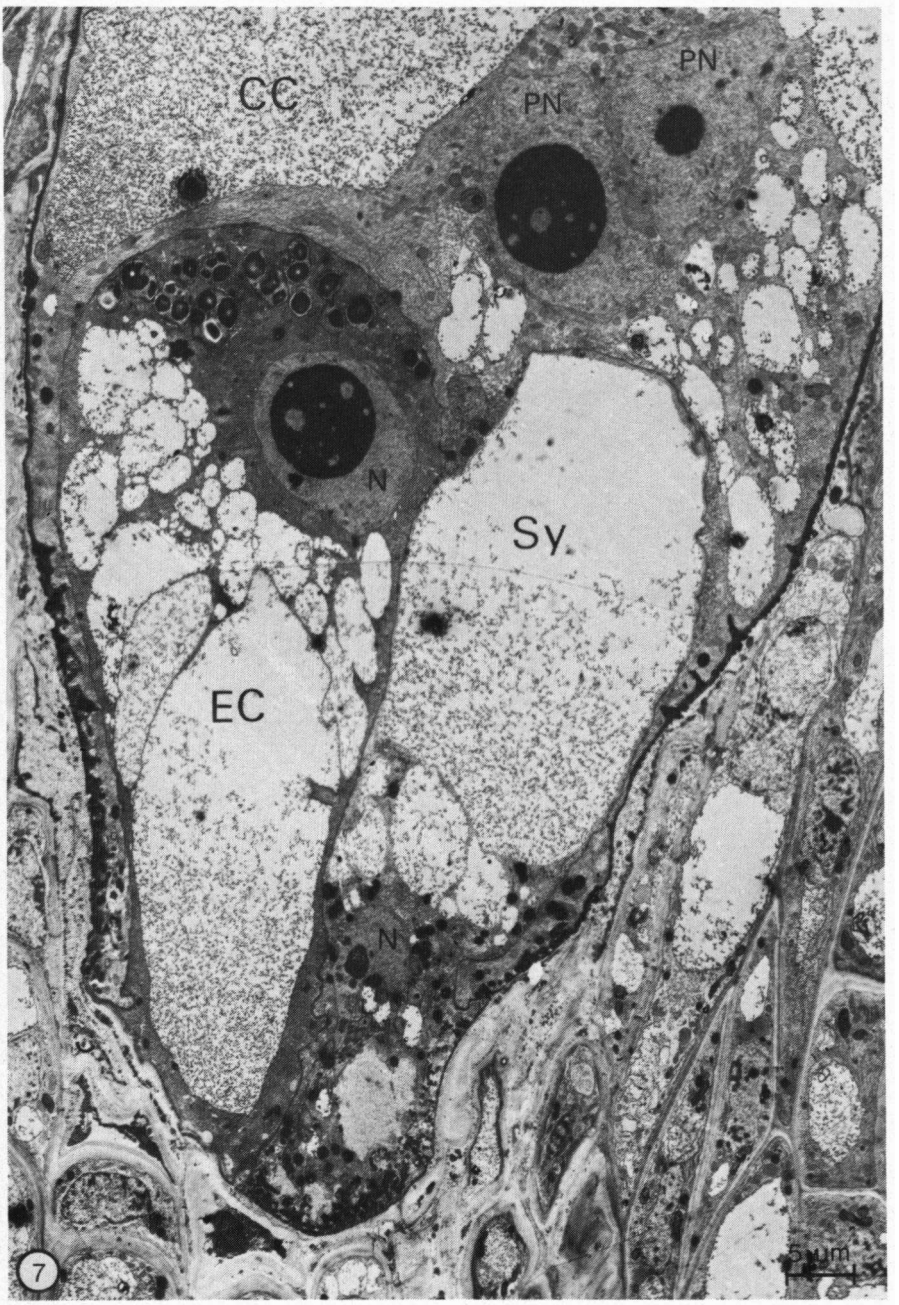

Fig. 7. Longitudinal section through egg apparatus and polar nuclei (PN) at fertilizable stage. 2,200 ×. (CC – central cell, EC – egg cell, N – nucleus, Sy – synergid).

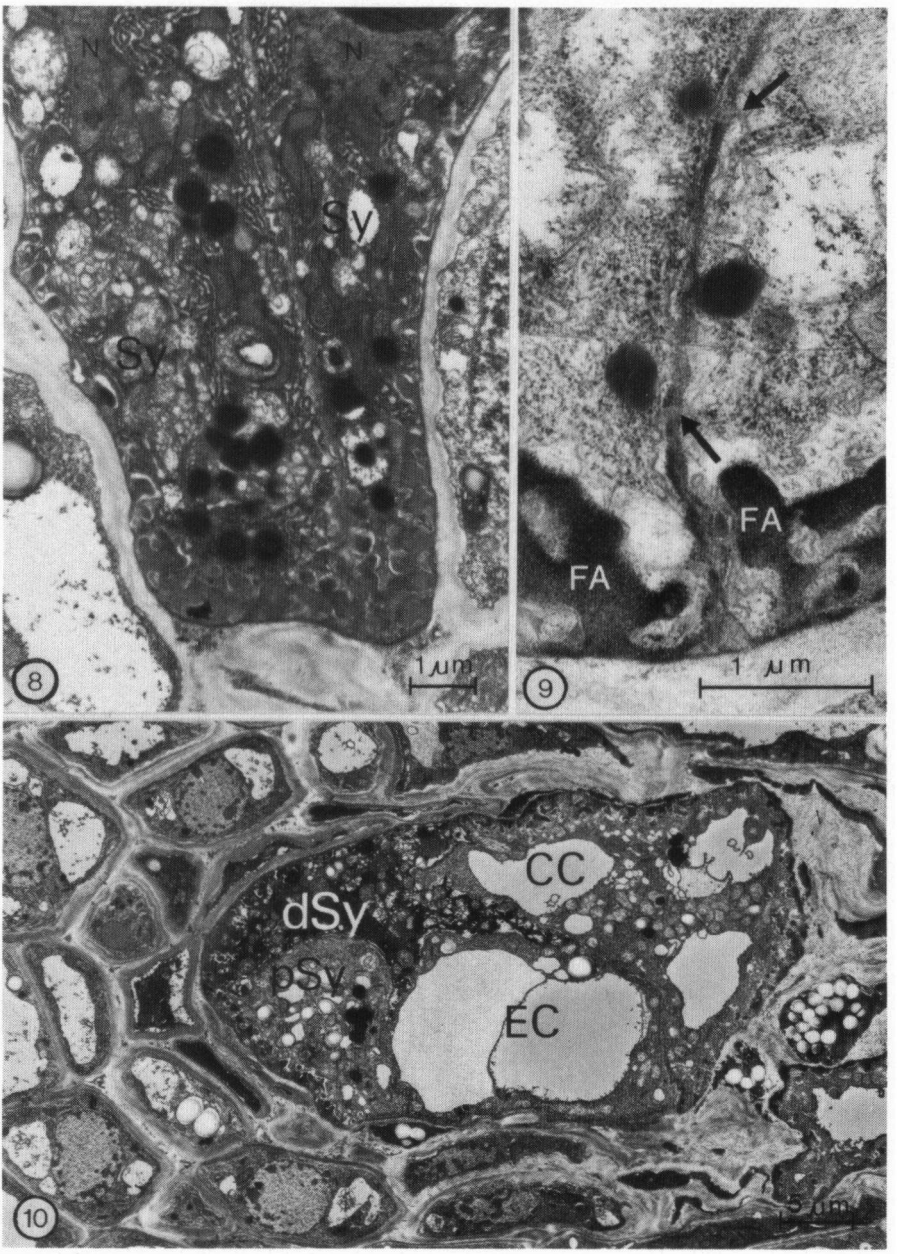

Fig. 8. Formation of the cell wall protrusions (FA) in basal part of synergids (Sy). 9,000 ×. Fig. 9. Enlarged view of a part of FA and common synergid cell wall with plasmodesmata (arrows). 22,000 ×. Fig. 10. Cross section through basal part of embryo sac at fertilizable stage. 1,800 ×. (CC – central cell, dSy – degenerated synergid, EC – egg cell, N – nucleus, pSy – persistent synergid).

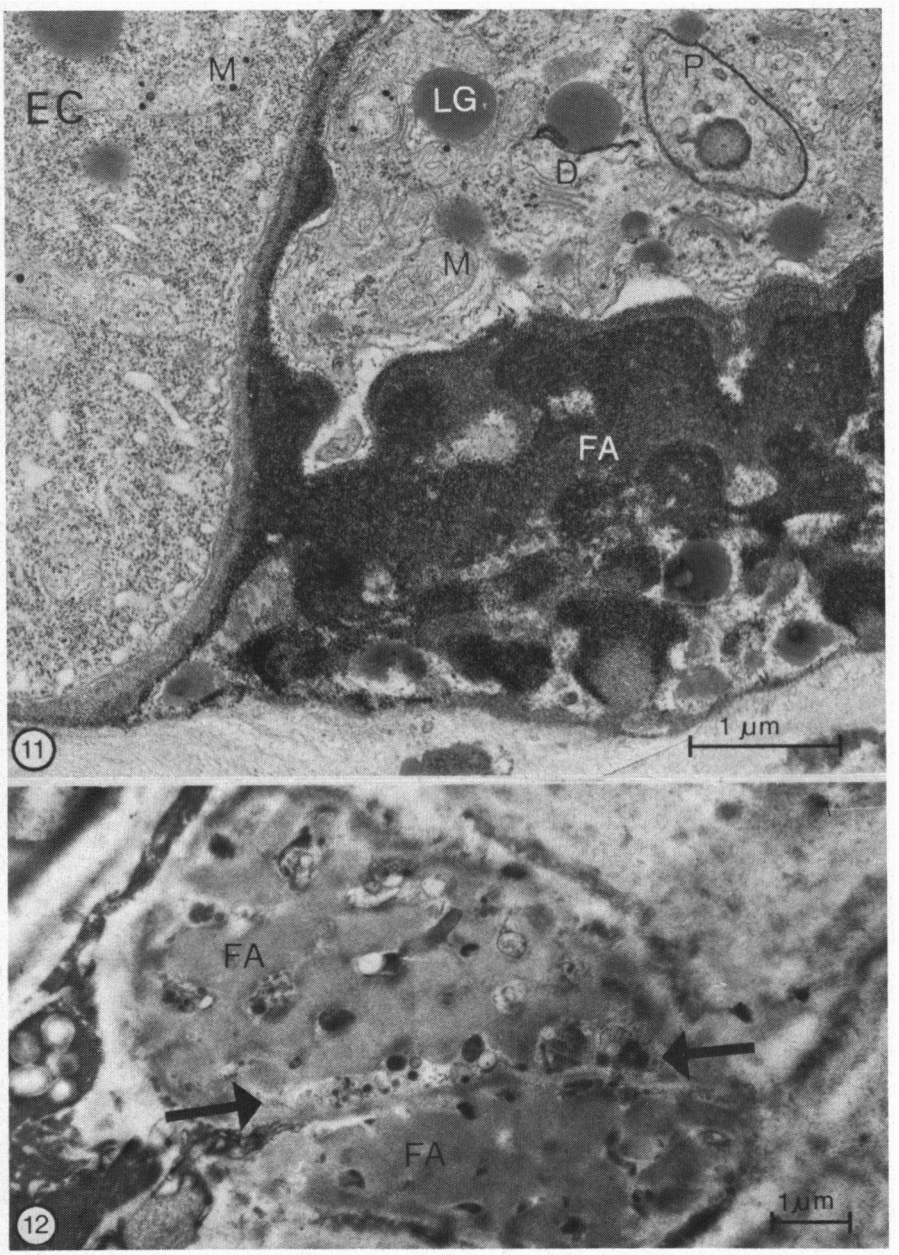

Fig. 11. Enlarged part of a synergid with FA and egg cell (EC). Note cell wall thickening at synergid side of common synergid-egg cell wall. 20,000 ×. Fig. 12. Cross section through FA. Long slit with cytoplasm is seen between arrows. 10,000 ×. (D – dictyosome, LG – lipid granule, M – mitochondrium, P – plastid).

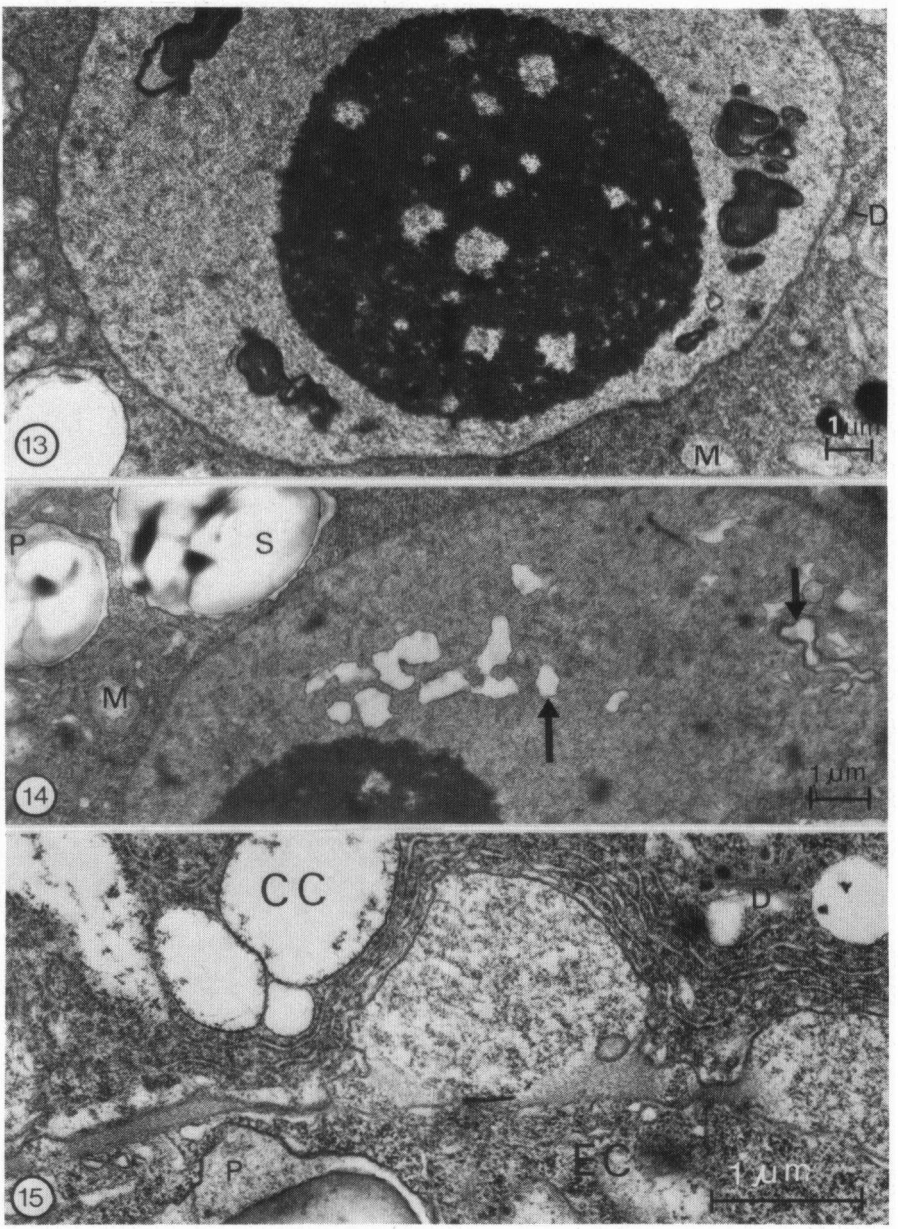

Fig 13. Egg cell nucleus with multi membrane-like structures. 6,000 × . Fig. 14. Egg cell nucleus with electron-transparant vacuole-like structures (arrow). 8.000 × . Fig. 15. Contact between chalazal part of egg cell (EC) and bordering central cell (CC). Dissolution of thin cell wall causes disintegration, while 'puffs' develop. 20,000 × . (D – dictyosome, M – mitochondrium, P – plastid, S – starch).

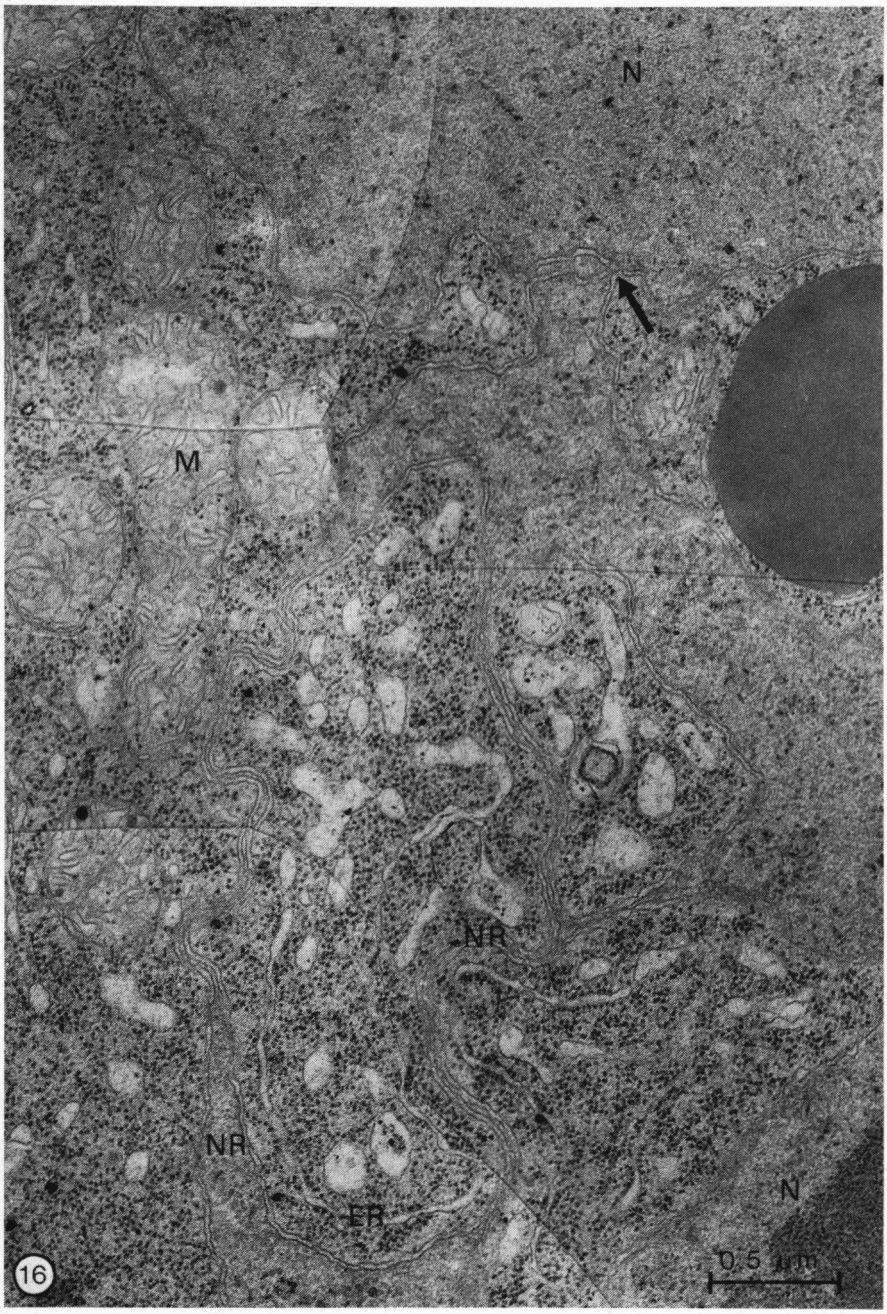

Fig. 16. Enlarged view of polar nuclei (N) with long nucleoplasm runners (NR) which fuse at several places. Nuclear membrane fusion is normal (arrow). 35,000 ×. (ER – endoplasmic reticulum, M – mitochondrium).