MORPHOLOGICAL AND QUANTITATIVE CHANGES IN THE POPULATION OF CELL ORGANELLES DURING MICROSPOROGENESIS OF PINUS SYLVESTRIS L. I. MORPHOLOGICAL CHANGES FROM ZYGOTENE UNTIL PROMETAPHASE I.

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SUMMARY

The membrane-like structures in the nucleus, the nuclear pore, the disappearance of the nuclear membrane, the synaptinemal complex, and changes in the nucleolus and karyoplasm are described and discussed.

Plastids and mitochondria do not change during the microsporogenesis. Until diplotene the lipid granules with electron transparent vesicles are arranged into a "lipid complex". During pachytene rough endoplasmic reticulum changes into smooth endoplasmic reticulum. During diplotene numerous Golgi bodies are present. Microtubules are present from zygotene and scattered in the cytoplasm. At diakinesis many vesicles appear. Ribosomes as well as polysomes are present. From diakinesis only ribosomes are observed.

Callose wall formation starts in diplotene and is connected with the presence of many smooth endoplasmic reticulum cisternae and Golgi vesicles, probably also with polysomes and lipid granules.

1. INTRODUCTION

The most striking process during microsporogenesis is the formation of the callose wall and the pollen wall. Numerous submicroscopical investigations have been made on pollen of various plant species in the successive stages from meiosis to mature pollen.

In Monocotyledons the structure of the synaptinemal complex in the nucleus during the meiotic prophase does not differ much from that in animal organisms (SOTELO 1969), as appeared from investigations with *Lilium*, variety Cinnabar (ROTH & ITO 1967) and *Allium cepa* (STOCKERT *et al.*, 1970). The changes of the nucleolus during cell divisions have been described for different organisms (MILLER & BEATTY 1969). A loss of nucleolar material during cell division has been supposed by BAJER & MOLÈ-BAJER (1969) in *Haemanthus katherinae*, and by DICKINSON & HESLOP-HARRISON (1970) in *Lilium henryi*. In pine, the structure of the nuclear pore is very complex. Morphologically it is different from the pore in amphibian oocytes as described by WISCHNITZER (1958) and FRANKE & SCHEER (1970). As is shown by different descriptions of the nuclear pore, its structure is not fully known (ABELSON & SMITH 1970). During cell division the nuclear membrane is partly broken down and its fragments remain in the cytoplasm (ESAU & GILL 1969).

The morphological changes of the amyloplasts, mitochondria and the Golgi body in the cytoplasm during microsporogenesis have been studied in *Tradescantia paludosa* (MARUYAMA 1968). Changes occur in the starch content of the amyloplasts, mitochondria divide during pachytene.

Before the breakdown of the nuclear membrane the microtubules are oriented in the cytoplasm in a prophase band (NEWCOMB 1968) or in distinct regions, as has been found in *Dactylorchis fuchsii* (BURGESS 1970).

The formation of the callose wall has been described for *Cucurbita ficifolia* by ESCHRICH (1964) and for *Endymion non-scriptus* by ANGOLD (1967). ANGOLD supports the opinion that some relationship should exist between the smooth endoplasmic reticulum (SER) and the callose production.

The present study deals with the morphological changes during microsporogenesis in *Pinus sylvestris* from the zygotene until the prometaphase I. As many events and morphological structures are similar to those in other plant species, comparisons can easily be made.

2. MATERIAL AND METHODS

During the first half of May microsporophylls of the cone of *Pinus sylvestris*, originating from one tree, were collected. Small pieces were fixed for one hour in 1% OsO_4 at 0°C in phosphate buffer pH 7.2. During the time of fixation the specimens were shaken continuously. After 15 minutes washing in water the specimens were stained for 30 minutes in 1% uranyl acetate in water, followed by another 15 minutes washing in water, and were put during 30 minutes into 1% $KMnO_4$ in water. After dehydration in alcohol the specimens were embedded in Epon 812. Sections of 80 nm thickness were cut using a Porter-Blumm ultramicrotome. After 5 minutes staining with lead citrate (REYNOLDS 1963), the material was examined with the Philips EM 300 electron microscope at 60 KV. The different stages were determined by observing 1 μm sections under the phasecontrast microscope.

3. RESULTS

Just before meiosis starts, the pollen mother cells produce a new thin cell wall inside the normal cellulose wall. This new cell wall consists of two electron dense layers of thin fibrillar material with fibrils between these layers (*fig. 19, 26*). The cellulose wall disappears when meiosis begins, whereas the new thin wall remains around the cell.

3.1. Zygotene

The large nucleus has homogeneous nucleoli and the chromatin shows signs of contracting. During late zygotene irregularly shaped structures are present between some of the contracting chromatin masses which structures are visible only for a short time (*fig. 1*). The thickness of the surrounding "membrane" is approximately 11 nm. Sometimes the "membrane" has blebs. Inside the circular

structure there is an electron transparent thin fibrillar material, possibly chromatin fibrils, which makes contact with the membrane-like boundary (*fig. 3*). It seems that the "membranes" become visible only after the formation of the electron transparent space between the contracting chromatine masses (*fig. 2*).

The nuclear pore is very complex and has the same structure in all stages of microsporogenesis. In the electron dense annulus about eight circular regions are present with a diameter of approximately 18 nm. The centre of the pore contains electron dense material (*fig. 4*). In cross section small fragments of the outer nuclear membrane extend to both sides above the pore (*fig. 5*). They do not occupy the whole outline of the annulus, but are situated between the eight regions, thus every pore on both sides has eight extensions. These extensions are tubular with a diameter of 7 nm. The annulus of the pore is composed of eight very small tubules on both sides in a regular arrangement. The tubules have probably closed ends. In the nucleus thin fibrils are connected with the pore (*fig. 7*). *Figure 9* presents a drawing of the supposed structure of the nuclear pore.

During zygotene and pachytene the synaptinemal complex is visible in a number of places (*fig. 8*). Two lateral arms, the intermediate space and the medial ribbon are clearly distinguishable, especially during late zygotene (*fig. 10*). In pachytene the lateral arm is not easily discernible and is probably continuous with the granular chromatin. Bridges between the lateral arm and the medial ribbon become less numerous compared with the situation during late zygotene (*fig. 11*). Finally, during diplotene only an electron transparent oblong band with some electron dense material is observed between the chromatin which disappears rapidly.

The plastids in the cytoplasm have a granular content and rarely contain other elements like little fragments of membranes. Some plastids have a big starch granule. There are also plastids which have a thin long centre piece, which is characteristic for dividing plastids. The mitochondria contain some osmiophilic material and have few cristae. Numerous Golgi bodies are present, but they possess few vesicles only (*fig. 12*).

The lipid granules have osmiophilic dots on their edges, which become visible after poststaining with lead. The lipid granules are connected with large electron transparent vesicles and the whole group is also surrounded by dark dots. This "lipid complex" is visible until diplotene and appears again in the tetrad stage (*fig. 13*).

Rough endoplasmic reticulum (RER) membranes are assembled in packets, and in some places they are not completely covered by ribosomes. These packets of RER are characteristic during zygotene and pachytene (*fig. 16*). Microtubules are rare and are situated mainly against the nuclear membrane. Large numbers of ribosomes and some polysomes give a grey tint to the cytoplasm (*fig. 7, 13, 16*).

3.2. Pachytene

The karyoplasm is highly electron transparent. The nucleolus is still homogeneous (*fig. 8*). In the cytoplasm the dark dots around the lipid granules disappear

and the "lipid complex" begins to disperse. In this stage the Golgi bodies possess more vesicles, but they are still small in size (*fig. 14*). Packets of RER are observed repeatedly, the lumen of the membranes shows dilatations and the ribosomes on the membranes are lacking in some places. More SER is present (*fig. 15, 17*). In all directions and everywhere in the cytoplasm microtubules become visible. Ribosomes as well as polysomes are present (*fig. 15*).

3.3. Diplotene

During early diplotene the karyoplasm contains very thin fibrillar material and electron dense granules. Locally it shows light zones (*fig. 18, 23*). After the initial phase the granules between the contracting chromosomes increase in number (*fig. 19, 28*). The nucleolus, which was initially homogeneous (*fig. 18*), becomes more heterogeneous and is connected with less electron dense granular material (*fig. 20, 21*). The more electron dense part of the nucleolus has the same structure as the electron dense bodies, which become perceptible in the cytoplasm from diplotene until interphase I (*fig. 20, 22, 26*). This part has often granules on its margin (*fig. 21*). Finally the nucleolus shifts towards a reticulate form (nucleolemma) and disappears (*fig. 19*).

In comparison with the preceding phases the plastids and mitochondria do not change. The cytoplasm has electron dense lipid granules with dark dots and less electron dense lipid granules without dark dots. The Golgi bodies are present in large numbers and produce many small and some large vesicles (*fig. 24, 29*). SER is dispersed in the cytoplasm, RER strands are scanty. The microtubules are stretched in all directions. Polysomes as well as ribosomes are present (*fig. 25, 26*).

During diplotene the callose wall formation starts. The small space between plasma membrane and cell wall contains only some small granules and fibrils (*fig. 27*). The small space grows when the callose wall formation begins. Outside the plasma membrane showing sometimes undulations, a fine electron dense fibrillar material has accumulated against the cell wall (*fig. 26*). Thereafter a fine fibrillar network against the cell wall becomes visible, which changes into a highly electron transparent line between the cell wall and the flat plasma membrane (*fig. 28, 29*). The callose wall envelops the whole cell and grows in thickness until the tetrad stage (*fig. 30*).

At the start and during the callose wall formation many SER cisternae and Golgi vesicles are present in the cytoplasm (*fig. 24*). Between the plasma membrane and cell wall the material of the growing callose wall has a similar structure as the content of the cisternae of the SER and Golgi vesicles (*fig. 24, 26, 29*).

3.4. Diakinesis

In early diakinesis the nuclear membrane shows great undulations. The chromosomes are visible as blocks of electron dense granular material. The karyoplasm possesses granules with a size of about 30 nm and less numerous of 15 nm (*fig. 32*). The breakdown of the nuclear membrane starts with a local widening

of the perinuclear space. Thereafter, the membrane structure fades; here very small pieces of membrane remain in an osmiophilic zone (*fig. 31*).

No morphological change occurs in the plastids and mitochondria. The Golgi bodies produce small vesicles. Lipid granules are less electron dense and lack their surrounding dark dots. Vesicles with an electon transparent content are numerous. Round and large vesicles with a clear membrane are present during all phases. During diakinesis and the following metaphase, however, there are also very dilated cisternae of SER and many small vesicles (*fig. 34*). Some microtubuli are situated perpendicular on both sides of the disappearing nuclear membrane (*fig. 31*). In the cytoplasm and karyoplasm more microtubules are oriented parallel; in cross section some microtubuli are surrounded by an electron transparent core (*fig. 32, 33*). Ribosomes are distributed in the cytoplasm, polysomes are not observed in this stage.

4. DISCUSSION AND CONCLUSION

4.1. The nucleus

In the karyoplasm membrane-like structures between the contracting chromatin may be caused by a change of molecular charge due to the contraction of the chromatin. Thereafter a demixture and separation between karyoplasm and chromatin takes place. In the region of this demixture there could be a reorientation of molecules according to their charge. For a moment thin films could be formed locally which are visible as membrane-like structures. The whole process may be analogous to the formation of coacervate droplets (BUNGENBERG DE JONG 1949).

During pachytene the karyoplasm is highly electron transparent, granules of approximately 30 nm appear in diplotene. Such granules, but also granules of 15 nm, increase in number during diakinesis. Mixing of these granules with the cytoplasm takes place after telophase I. When the granules in the karyoplasm appear, the nucleolus shows some changes. The heterogeneous nucleolus has granules on its margin. MILLER & BEATTY (1969) found granules around the nucleolus in oocytes of *Rana calamitans*, which were apparently related to the nucleolar RNA metabolism. These granules may be considered as the 32S RNA particles which are produced in the nucleolus and become visible later as larger granules in the karyoplasm. It may be possible that in pine the granules in the karyoplasm have originated from the nucleolus. The more electron dense part of the nucleolus in diakinesis has the same structure as the electron dense bodies in the cytoplasm during this phase. Dense bodies derived from the nucleolus are also found in the cytoplasm of the dividing endosperm cell of *Haemanthus* (BAJER & MOLÈ-BAJER 1969) and during meiosis in *Lilium* (DICKINSON & HESLOP-HARRISON 1970). The changes in the karyoplasm during meiotic prophase are also related to the contraction of the chromatin and nucleolar activities during diplotene.

In pine the ultrastructure of the synaptinemal complex is in agreement with numerous descriptions in animals and plants (ROTH & ITO 1967; SOTELO 1969;

STOCKERT, GIMENEZ-MARTIN & SOGO 1970). Remarkable is the high number of synaptinemal complexes during zygotene and pachytene. The medial ribbon is composed of two layers as is found in *Periplaneta americana* (SOTELO 1969).

The annulus of the nuclear pore is an arrangement of eight small circular regions which are situated between eight very small tubular extensions on the outer nuclear membrane. These tubules are bowed and extend on both sides of the pore. An octagonal pattern of the nuclear pore has been reported by WISCHNITZER (1958) in amphibian oocytes. The author supposes that the annulus possesses eight microcylinders. An octagonal pore in amphibian oocytes has been described by GALL (1967) and in *Haemanthus* by BAJER & MOLÈ-BAJER (1969). FRANKE & SCHEER (1970) suppose that the eight subunits in the annulus are granules; on the contrary, ABELSON & SMITH (1970) are of the opinion that the subunits are minitubules.

In pine the eight small circular structures are the regions between the eight thin tubules. It cannot be excluded that in pine another type of a nuclear pore is present, the dimensions of the pore are smaller than in amphibian oocytes (GALL 1967). The diameter of the thin tubules, about 7 nm, fits the thickness of the unit membrane.

Before breakdown the nuclear membrane shows undulations. Dilatations between the two membranes become visible and locally the two membranes become diffuse. Parts of the nuclear membrane remain intact. At places where the breakdown, probably by enzymes, takes place, sometimes small membrane fragments are visible. Thus a local breakdown exists (ESAU & GILL 1969).

4.2. The cytoplasm

Until prometaphase I the plastids and mitochondria in pine do not change in their morphology. The plastids have an electron dense content or they may contain a large starch granule. On the contrary, in *Tradescantia* the size of the starch granule in the plastids increases after the leptotene up to the young microspore (MARUYAMA 1968). In the mitochondria few cristae are present. Dividing plastids as well as mitochondria are found in all stages.

The "lipid complex" decomposes after zygotene. The black dots around the lipid granules are visible after poststaining with lead and sometimes are spread out over other organelles lying in the vicinity of the lipid granule. Therefore these dots are probably artifacts, closely connected with the lipid granules.

Vesicles in the cytoplasm have a clear electron dense membrane and a rather large size. The high number of vesicles just before the division is due to the presence of SER cisternae and small Golgi vesicles, which ar more dilated now.

The Golgi bodies produce vesicles. They increase in number during pachytene and diplotene, and this augmentation may possibly be related to the formation of the callose wall.

The RER is mainly perceptible in groups of membranes. After zygotene the RER has lost its ribosomes and in diplotene only SER is visible.

During zygotene until diakinesis the microtubules are distributed at random in the cytoplasm. Contrary to what has been found in mitotic cells (NEWCOMB

1969), in pine no prophase band exists, and the microtubules are not oriented in distinct regions (BURGESS 1970). The number of microtubules increases until prometaphase. During the breakdown of the nuclear membrane the microtubules penetrate the karyoplasm at right angles to the disappearing nuclear membrane. Possibly this penetration of microtubules into the nucleus occurs as a result of a destruction and rebuilding of the microtubuli (ESAU & GILL 1969).

In all stages ribosomes are visible, contrary to the situation in *Lilium* (MACKENZIE & HESLOP-HARRISON 1967) where the ribosomes disappear during pachytene. In *Petunia* (LINSKENS 1969) and *Trillium* (HOTTA & STERN 1963) a decrease of the RNA content in pachytene is demonstrated as well. It appears from histochemical studies in *Cosmos* by KNOX, DICKINSON & HESLOP-HARRISON (1970), that RNA disappears between zygotene and pachytene; this does not necessarily imply that also the ribosomes should be absent in the cytoplasm. Up to diakinesis in pine polysomes are present.

4.3. The callose wall formation

A small zone of thin electron dense fibrils, which converts into an electron transparent line, is the first sign of callose wall formation during diplotene. The plasma membrane sometimes has local undulations and vesicles are found near this membrane. But the plasma membrane is principally flat and lies near to or in direct contact with the coming callose wall. The plasma membrane in pine is always intact, contrary to that in *Cucurbita* (ESCHRICH 1964).

Many cisternae of SER are present in the cytoplasm and possibly a relation exists between these cisternae and the formation of the callose wall, as ANGOLD (1967) has reported for *Endymion*. In pine the SER is possibly derived from the RER. The presence of Golgi vesicles lying near the plasma membrane may be connected with the callose wall formation and their content may be excreted. A high number of polysomes in the cytoplasm is observed and a decomposition of the lipid complex occurs during callose wall formation. It is remarkable that all these phenomena are repeated during the callose wall formation in the tetrad. For this reason the callose wall formation will be fully discussed with the description of that phase.

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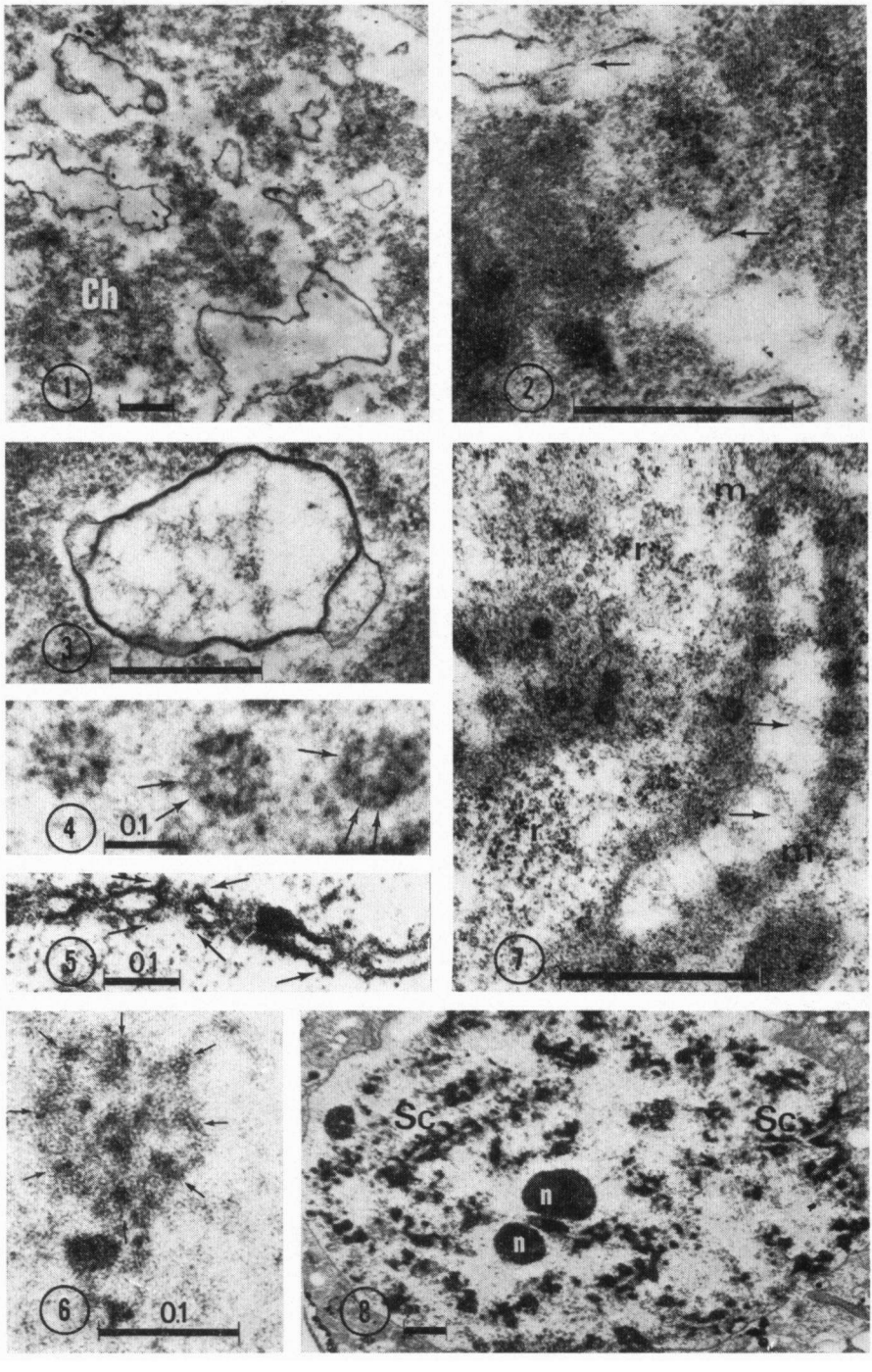
Unless mentioned otherwise, the line on the figures represents a length of 1 μm.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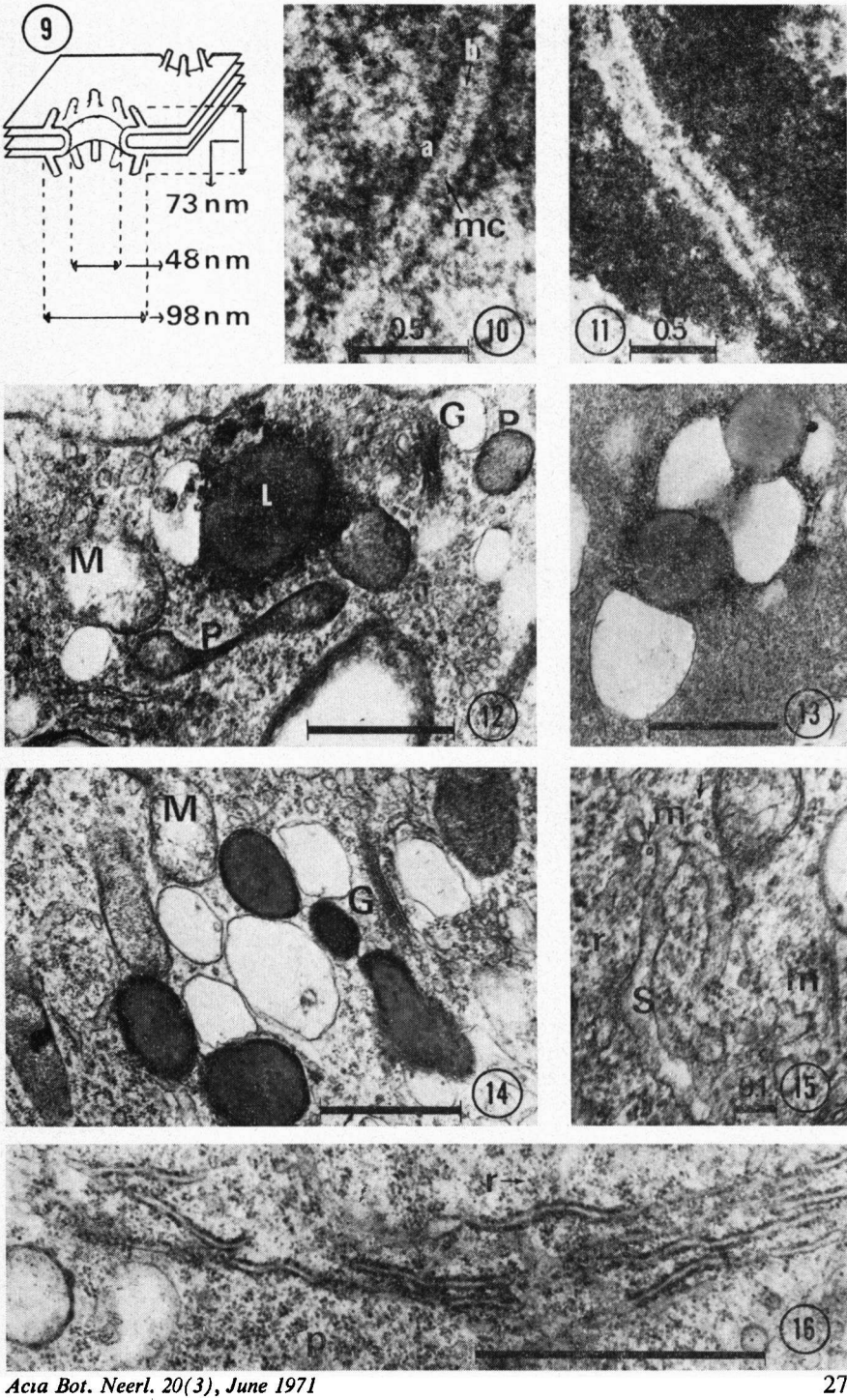
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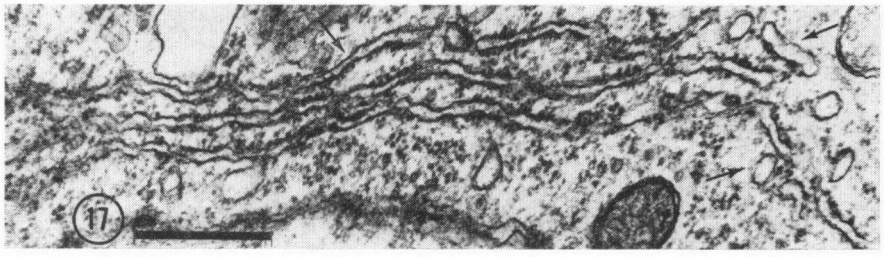
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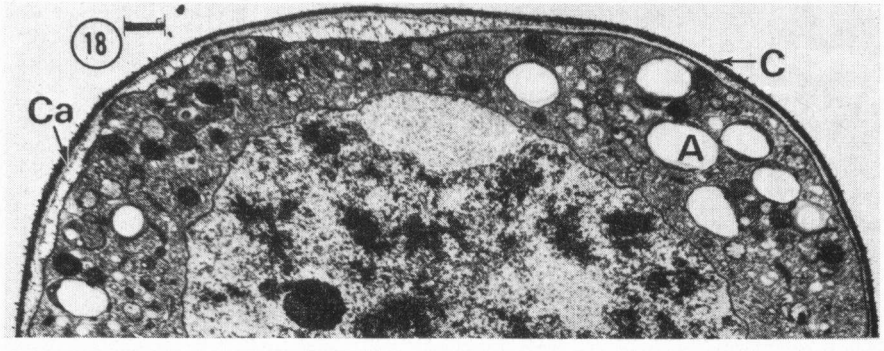
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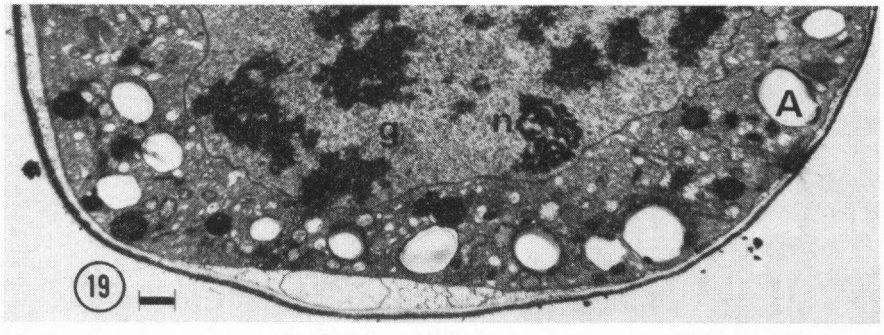
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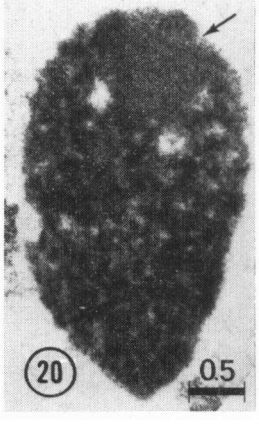
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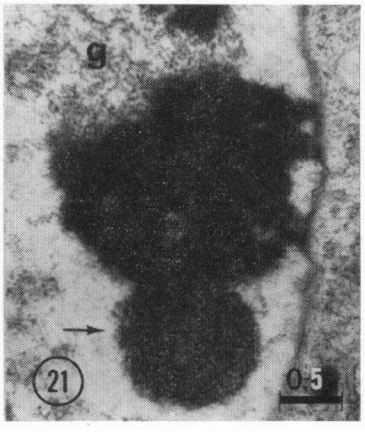
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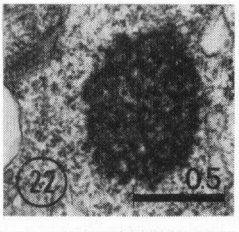
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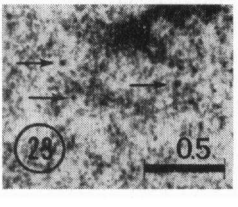
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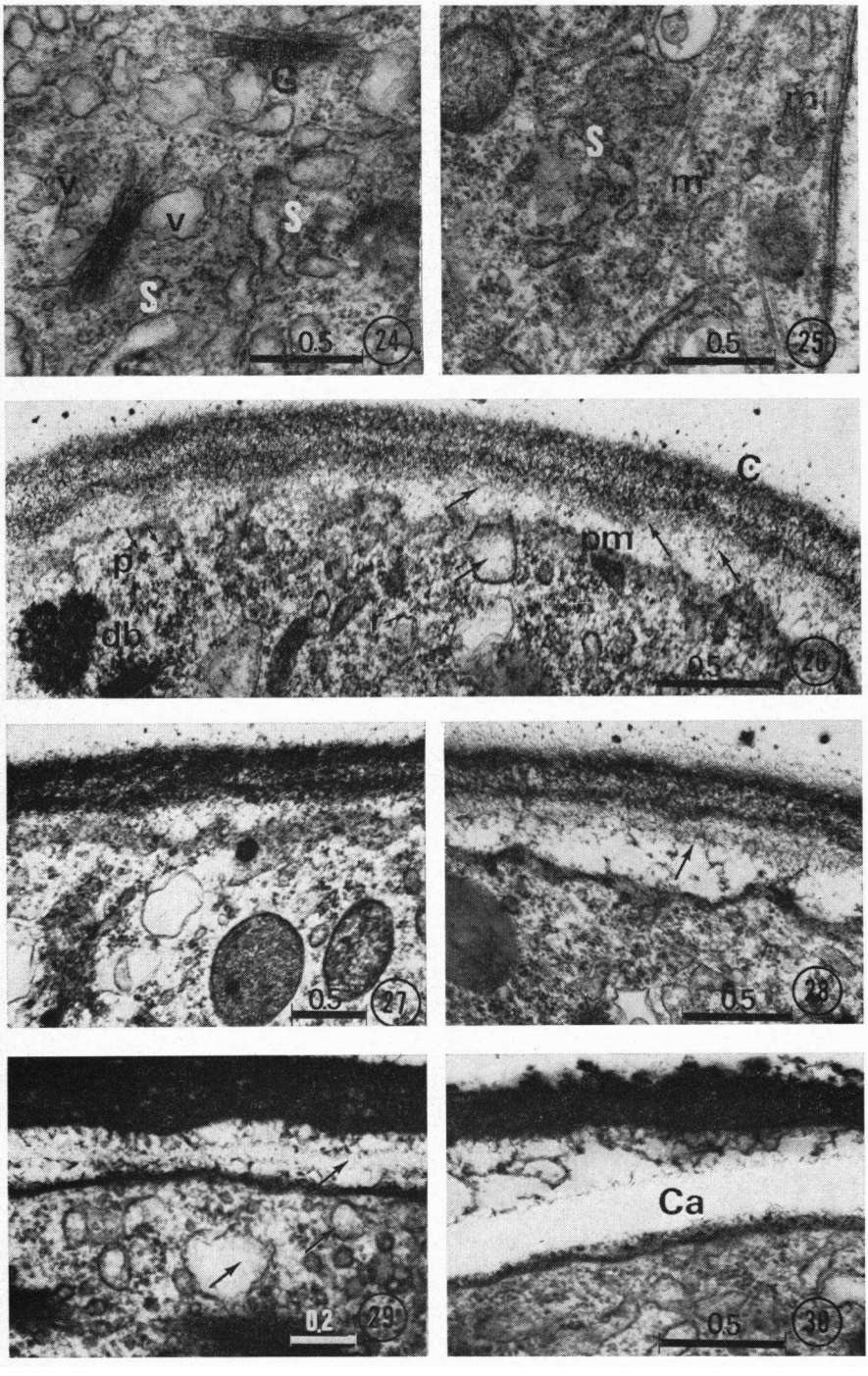
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V
V
S
S
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24
S
m
m
0.5
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C
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pm
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26
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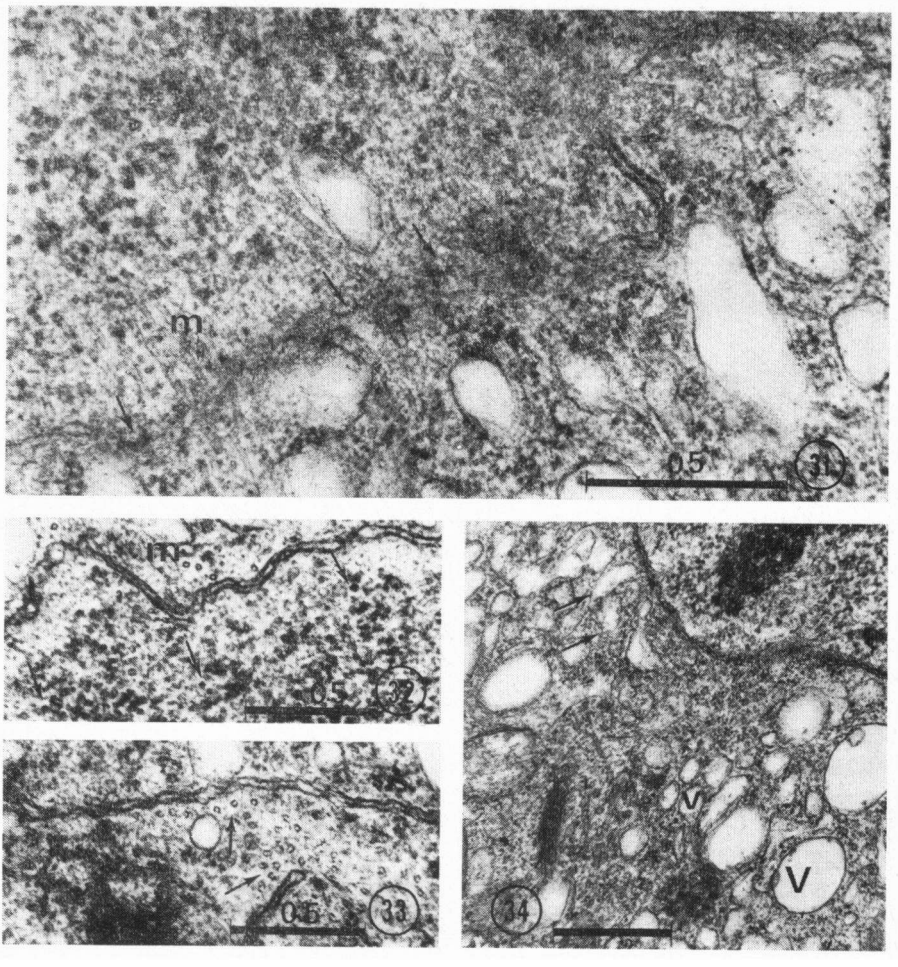
27
0.5
28
0.2
29
Ca
0.5
30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m
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32
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0.5
33
V
V
34