Acta Bot. Neerl. 33(1), February 1984, p. 111–122.

HISTOCHEMICAL, STRUCTURAL AND ULTRASTRUCTURAL FEATURES OF ENDOSPERM IN ALYSSUM MARITIMUM LAM.

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SUMMARY

The primary endosperm nucleus in *Alyssum maritimum* is surrounded by a large number of polysaccharide grains. The grains are utilized during the nuclear development of the endosperm. The nuclear endosperm stains intensely for proteins, DNA and RNA but the concentration of these metabolites declines during cellularization, whereas the polysaccharide content increases again. The cellular endosperm in mature seeds stores polysaccharides mainly in the form of cell walls, and proteins as proteinaceous masses.

The endosperm is rich in ribosomes, mitochondria and plastids. The nuclei show different morphology. Nucleoli are either vacuolated or compact. In the endosperm extrusion of spherical bodies from the nuclei occurs.

1. INTRODUCTION

The endosperm usually is regarded as a nutritive tissue. Recently this function of providing nutrition to the embryo by the young and developing endosperm is, however, debated (NEWCOMB 1973, YEUNG & CLUTTER 1978, PRABHAKAR 1979). Accumulation of endosperm at the micropylar and chalazal ends of the embryo sac is believed to play a haustorial role in absorption of nutrients. Whether the endosperm haustorium is active during the entire embryogenesis or only during a particular phase is not clear. The pattern of utilization of endosperm by the growing embryo is not known. The present study on ontogenetical, histochemical and ultrastructural aspects of endosperm in *Alyssum maritimum* was, therefore,undertaken to fill these gaps in the literature.

2. MATERIALS AND METHODS

The plants of *Alyssum maritimum* Lam. were grown in the garden of the Botany Department, Delhi. For light microscopy seeds were fixed in FAA or in AA. Dehydration, infiltration and embedding were done as usual. Sections were cut at 12 μm. Insoluble polysaccharides were located with the PAS reaction; proteins with mercuric bromophenol blue (MAZIA et al. 1953); DNA with Feulgen's reaction and RNA with the pyronin Y method (TEPPER & GIFFORD 1962).

For electron microscopy seeds were fixed in 3% glutaraldehyde for 3 hrs (pH 7.2) and post-fixed in 1% OsO_4 for 1 hr, both at room temperature, and kept ovenight in a refrigerator. The specimens were dehydrated in an acetone series and embedded in Epon-Araldite. Sections were stained with uranyl acetate and lead citrate.

3. RESULTS

3.1. Histology

The primary endosperm nucleus divides earlier than the zygote. The initial endosperm development is nuclear. Endosperm cytoplasm and nuclei aggregate at both the micropylar (*fig. 1*) and the chalazal end of the embryo sac. The central portion of the endosperm consists of a peripheral layer of cytoplasm with a row of nuclei. The endosperm, thus, can be divided into three regions: micropylar (*fig. 2*), central and chalazal (*fig. 6*).

At about the young dicotyledonous embryo stage nuclear divisions in the endosperm become asynchronous. Vacuolation and cell formation, initiated in the micropylar region, gradually proceed towards the central portion of the endosperm (*fig. 3*). The endosperm cells formed are highly vacuolated and possess small nuclei and nucleoli. The endosperm layer adjacent to the endothelium, however, shows dense cytoplasm with prominent nuclei (*fig. 4*). The large central vacuole is reduced and the embryo sac cavity is filled with endosperm cells (*figs. 5, 12*). The chalazal portion of the endosperm remains coenocytic up to the early dicotyledonous embryo stage (*fig. 6*) and continues to show karyokinesis (*fig. 12*). This nucleated portion eventually becomes cellular during seed maturation (*figs. 7, 8*). All endosperm, except a single layer along the periphery of the embryo sac, is crushed in the mature seed.

3.2. Histochemistry

The primary endosperm nucleus gives an intense reaction for proteins, has a strongly pyroninophilic nucleolus, stains faintly for DNA and is surrounded by many polysaccharide grains. With the onset of free nuclear divisions the concentration of cytoplasmic proteins and RNA increases concomitantly. The endosperm nuclei at the micropylar and chalazal ends stain feebly for DNA.

The endosperm cytoplasm at the micropylar and the chalazal end of the embryo sac show abundant polysaccharide grains during the pre-globular embryo stage.

At the globular proembryo stage the endosperm tissue is PAS-negative (*fig. 2*). The micropylar, central and chalazal portions of the endosperm stain with equal intensity for cytoplasmic and nuclear proteins. The chalazal portion of the endosperm initially reveals a uniform staining for proteins but later the upper half stains more intensely than the lower half. The distribution of RNA follows a similar pattern.

At the late hart-shaped embryo stage a large number of polysaccharide grains

appear in the micropylar portion of the endosperm (*fig. 3, 5*). In this region concomitant with the appearance of PAS-positive grains there is a decline in the level of proteins (*figs. 10, 12*).

Subsequently polysaccharide grains are also formed in the central endosperm portion. The peripheral endosperm cells show a higher concentration of polysaccharide grains than those in the centre of the embryo sac (*fig. 15*). The concentration of proteins and RNA concomitantly decreases with the accumulation of polysaccharides in the central portion, in contrast to the chalazal portion which stains intensely (*figs. 11, 12*). The endosperm layer subjacent to the endothelium shows dense proteins and RNA (*fig. 14*).

The coenocytic chalazal portion of the endosperm remains PAS-negative up to the early dicotyledonous embryo stage (*fig. 7*). After the laying of cell walls the cells thus derived from this portion show an accumulation of polysaccharide grains (*figs. 7, 8*). During subsequent seed maturation the number of these grains decreases; in the mature seed they are absent (*fig. 9*).

The asynchronous nuclear divisions in the coenocytic chalazal portion are followed by a decline in the level of cytoplasmic proteins (*fig. 12*). The endosperm cells derived from the chalazal portion initially reveal intense staining for cytoplasmic proteins (*fig. 13*) and RNA, but later the staining intensity declines. The distribution of RNA in the coenocytic chalazal portion follows the same pattern as that of the proteins. The nuclei in this portion are variously sized but faintly stained. The single persistent layer of endosperm subjacent to the endothelium shows well-stained nuclei, intensely pyroninophilic nucleoli, negligible staining for cytoplasmic RNA, and deeply stained granular protein masses.

3.3. Ultrastructure

Micropylar portion of the endosperm: At the heart-shaped embryo stage the endosperm adheres closely to the suspensor cells and surrounds the embryo proper. The endosperm cytoplasm has many vacuoles of various sizes and dilated cisternae of RER (*figs. 15, 17*). The electron-translucent cytoplasm is rich in ribosomes and mitochondria and has a few plastids with starch grains (*fig. 15*).

Nuclei in this portion of the endosperm show variation in size, shape, extent of lobing, types of nucleoli and extrusion of nucleolar material. The differences in the endosperm nuclear morphology observed are: (a) the nucleus shows electron-translucent nucleoplasm whereas the nucleolus has a dense granular zone interspersed with few fibrillar zones (*fig. 19*); (b) a few nuclei show only one lobe and the nucleolus reveals distinct zones of pars granulosa and pars amorpha (*fig. 10*); (c) the nucleus acquires a lenticular shape due to flattening of the central region and stretching of lobes on either side (*fig. 17*); (d) a few exhibit an amoeboid appearance with two lobes that grow like pseudopodia and fold inward.

A number of nuclei show nucleolar extrusion and blebbing of the nuclear envelope. Nucleolar blebs are seen in the nucleoplasm or the cytoplasm (*figs. 18, 19*). There is no uniformity in the size of the vesicles pinched off from the nuclear membrane (*figs. 18, 19*). Incidently, nuclei show paracrystalline inclusions in the nucleoplasm (*fig. 17*).

Central portion of the endosperm: The cytoplasm is electron-translucent but rich in ribosomes, mitochondria, plastids, and vacuoles. The plastids rarely contain starch grains. The nuclei in this region are similar in size and shape but lack lobing. The nuclear membrane does not show blebbing and the nucleoli have distinct zones of pars granulosa and pars amorpha.

Chalazal portion of the endosperm: The cytoplasm is electron-translucent. Ribosomes either occur as individual units or aggregate to form polysomes. Numerous dilated cisternae of RER (*fig. 20*) and many pleiomorphic mitochondria are observed. The nuclei are large, lobed and generally show a single nucleolus (*fig. 20*). The chromatin is dispersed and clumped near the periphery of the nucleus. At places the outer membrane of the nuclear envelope is continuous with the endoplasmic reticulum. Nucleolar material apparently is extruded by blebbing of the nuclear envelope.

The endosperm sheath enclosing the embryo proper: At the heart-shaped embryo stage the cotyledonary primordia occupy the space initially filled with still nuclear endosperm. The endosperm adjacent to the embryo boundary shows various organelles like endoplasmic reticulum, mitochondria and plastids at different stages of lysis (*fig. 16*). The dilated cisternae of RER rupture, followed by loss of ribosomes from the ER surface. Many dilated ER cisternae enclosing cytoplasm are seen.

4. DISCUSSION

The micropylar, chalazal, and central portions of the endosperm of *Alyssum maritimum* are not separated by cell walls, but reveal characteristic histochemical features. During the initial free nuclear divisions in the endosperm the concentration of polysaccharides decreases. This decline may be due to the utilization of this metabolite for early growth and development of the endosperm. Further, a high concentration of protein, DNA and RNA is observed in the endosperm, as also seen in *Stellaria media* (PRITCHARD 1964), *Linum usitatissimum* and *Ranunculus sceleratus* (DHAR 1976). These observations indicate clearly that the endosperm is metabolically very active and lacks any storage metabolites during early ontogeny. With the onset of cellularization a strong decline is, however, observed in the level of macromolecules like proteins, DNA and RNA. Concomitantly polysaccharide grains appear and show gradual accumulation in the endosperm. On the basis of the present histochemical studies it is postulated that actively dividing endosperm during early ontogeny needs nutrients for its own growth. At this stage the endosperm may not contribute significantly to the nutrition of the proembryo. After cellularization the endosperm acts as a sink for metabolites since the content of polysaccharides then gradually increases. The depletion of polysaccharides from endosperm tissue during seed maturation indicates utilization of this metabolite for growth and differentiation of the embryo. The possibility of other, indirect roles however, cannot be ruled out. The intense metabolic activity of the young endosperm may in part reflect

the production of hormones and other growth substances influencing the morphogenesis of embryos. In leguminous plants the liquid endosperm surrounding the embryo has been reported to contain different hormones (EEUWENS & SCHWABE 1975). Another function of the endosperm during early embryogeny may be the maintenance of an osmotic gradient. RIJVEN (1952), NORSTOG (1967) and NORSTOG & KLEIN (1972) postulated that a high osmotic value of the environment is essential for the normal development of the embryo and also prevents the embryo from precocious germination. The endosperm therefore, may play an important morphogenetic role in the regulation of the growth of the embryo (YEUNG & CLUTTER 1978).

Ultrastructure: Electron microscopy shows that the cytoplasm of the endosperm is rich in mitochondria, plastids, and ribosomes. This shows that the endosperm is metabolically an active tissue (cf. BUTTROSE 1963; MARINOS 1970; NEWCOMB 1973, 1978; SCHULZ & JENSEN 1974, 1977; WEINBAUM & SIMONS 1974; TURALA-SZYBOWSKA 1975; SINGH & MOGENSEN 1976). In *A. maritimum* the endosperm nuclei are polyploid, variously sized and shaped. Further nucleolar extrusions including a portion of the nuclear membrane are also observed. Extrusion of nucleolar material is also reported in *Phaseolus coccineus* (CIONINI & CREMONINI 1970), *Capsella bursa-pastoris* (SCHULZ & JENSEN 1973) and *Gossypium hirsutum* (SCHULZ & JENSEN 1977). SZOLLOSI (1965) concluded that in the rat the extrusion bodies are similar in composition to the nucleolus. SZOLLOSI (1965), HAY (1968) and SCHULZ & JENSEN (1973) believe that nucleolar extrusions represent the mass transport of ribosomal precursors or possibly other kinds of RNA into the cytoplasm.

A clear unstained gap is observed around the globular proembryo and its later stages in *A. maritimum*. Many organelles near the embryo boundary are at various stages of lysis. ER cisterns engulfing cytoplasm and interpreted as autophagic vacuoles are also observed in the endosperm near the embryo boundary. The gap, thus, is not due to mechanical pushing of the embryo but is formed by gradual lysis and utilisation of the endosperm by the embryo. NEWCOMB (1973) reported a clear area around the embryo devoid of any endosperm cells and also suggested that the endosperm is digested by the developing embryo.

The present histochemical and ultrastructural studies show clearly that the endosperm is metabolically very active during early embryogenesis. The accumulation of reserve metabolites and the utilization of endosperm by the embryo from the heart-shaped stage onwards support the view that the endosperm performs an important role of providing nutrition to the embryo only during the later stages of embryogenesis.

ACKNOWLEDGEMENTS

Dr. (Mrs.) Kumkum Prabhakar thanks the Council of Scientific and Industrial Research, New Delhi, for the award of a Research Associateship. We thank Professor Van Went for critically going through the manuscript and offering valuable suggestions for improvement. The publication was made possible by a grant from the DPC-fund of the Royal Netherlands Botanical Society.

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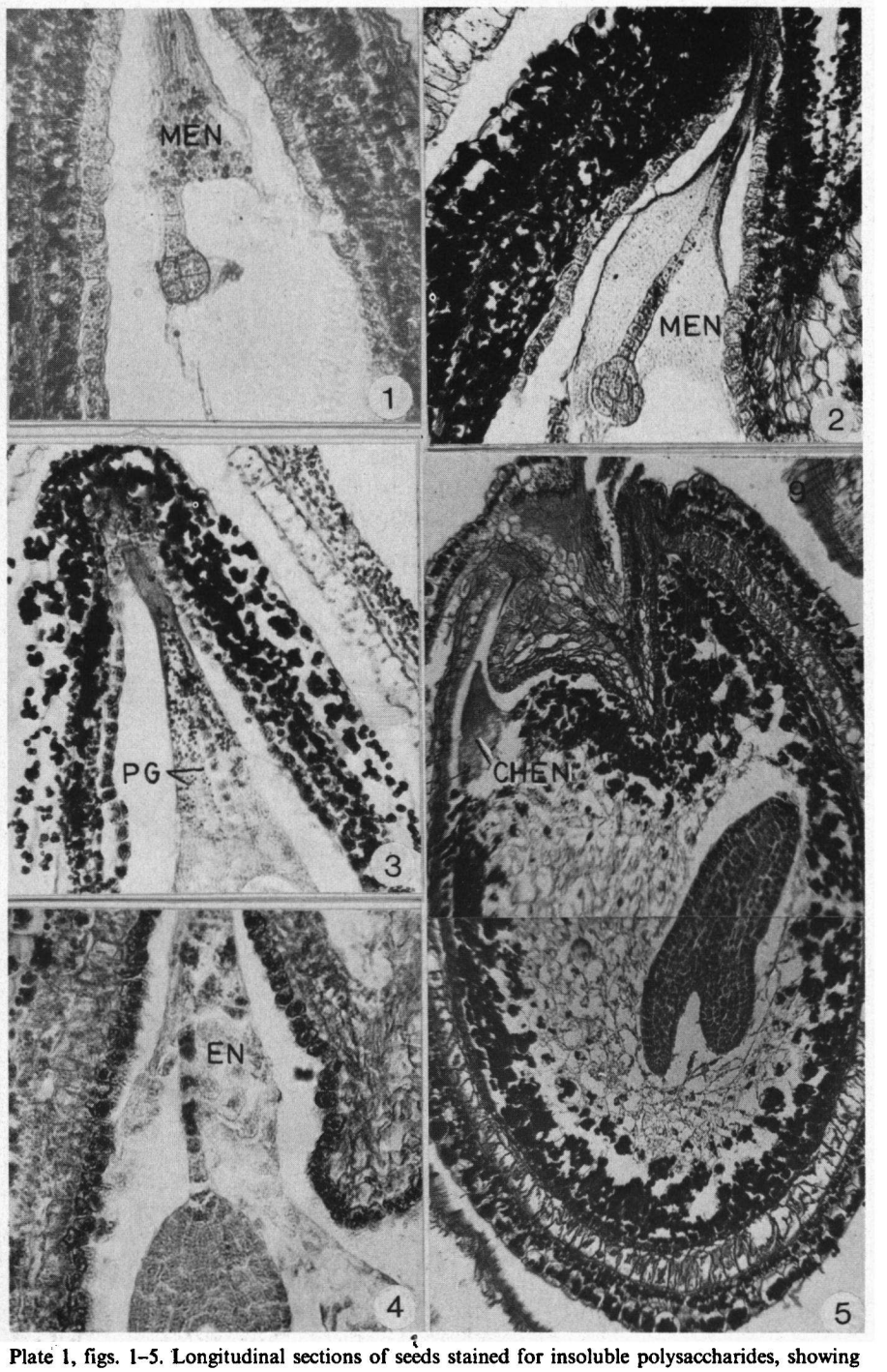

Plate 1, figs. 1–5. Longitudinal sections of seeds stained for insoluble polysaccharides, showing initial decline (1–2) and subsequent accumulation (3–5) of polysaccharides in the micropylar portion of the endosperm (MEN). 1. Quadrant proembryo stage, × 340; 2. early globular proembryo stage, × 180; 3. heart-shaped embryo stage with polysaccharide grain (PG), × 340; 4. young dicotyledonous embryo stage (EN = endosperm), × 340; 5. older dicotyledonous embryo stage (CHEN = chalazal portion of endosperm), × 340.

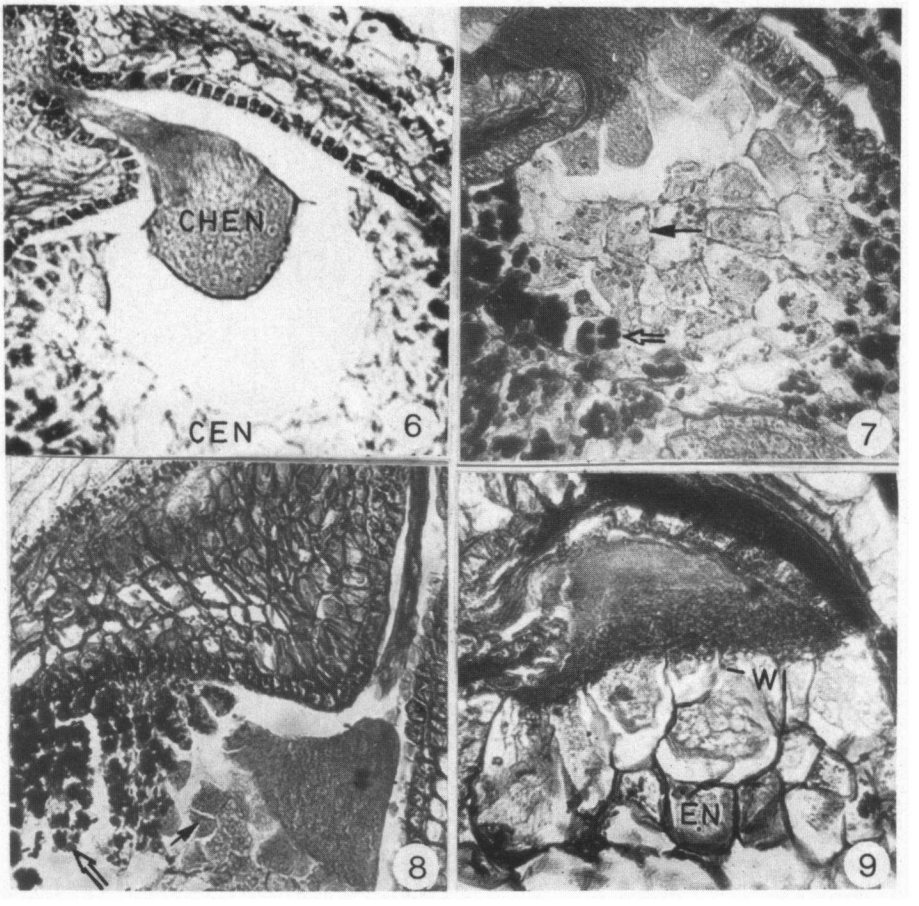

Plate 2, figs. 6–9. Longitudinal sections of seeds stained for insoluble polysaccharides, showing the chalazal portion of the endosperm. 6. Early dicotyledonous embryo stage. PAS-positive grains are present in the central portion of the endosperm (CEN) but are absent in the chalazal portion (CHEN), × 340; 7, 8. Maturation stages to show compartmentation of the coenocyytic chalazal endosperm; difference in the concentration of polysaccharide grains in the central part (thick arrows) and the cells derived from the chalazal portion (thin arrows), × 340; 9. portion of mature seed showing intensely stained wall ingrowth (WT) at the extreme chalazal end; endosperm cells show well-stained walls, × 340.

Plate 3, figs. 10–14. Longitudinal sections of seeds stained for proteins. 10. Young dicotyledonous embryo stage; the micropylar part of the endosperm (MEN) stains intensely for cytoplasmic and nuclear proteins, × 340; 11, young dicotyledonous embryo stage to show the difference in staining intensity of the coenocytic chalazal portion of the endosperm (CHEN) and endosperm cells in the central region, × 340; 12. early dicotyledonous embryo stage to show chalazal endosperm (CHEN) with protein-rich cytoplam, × 98; 13. late dicotyledonous embryo stage showing protein-rich cells derived from the chalazal portion of the endosperm; newly formed cells show more cytoplasmic proteins than older cells, × 340; 14. Part of a seed during maturation; the endosperm layer (EN) adjacent to the endothelium shows more cytoplasmic proteins than subjacent layers, × 340.

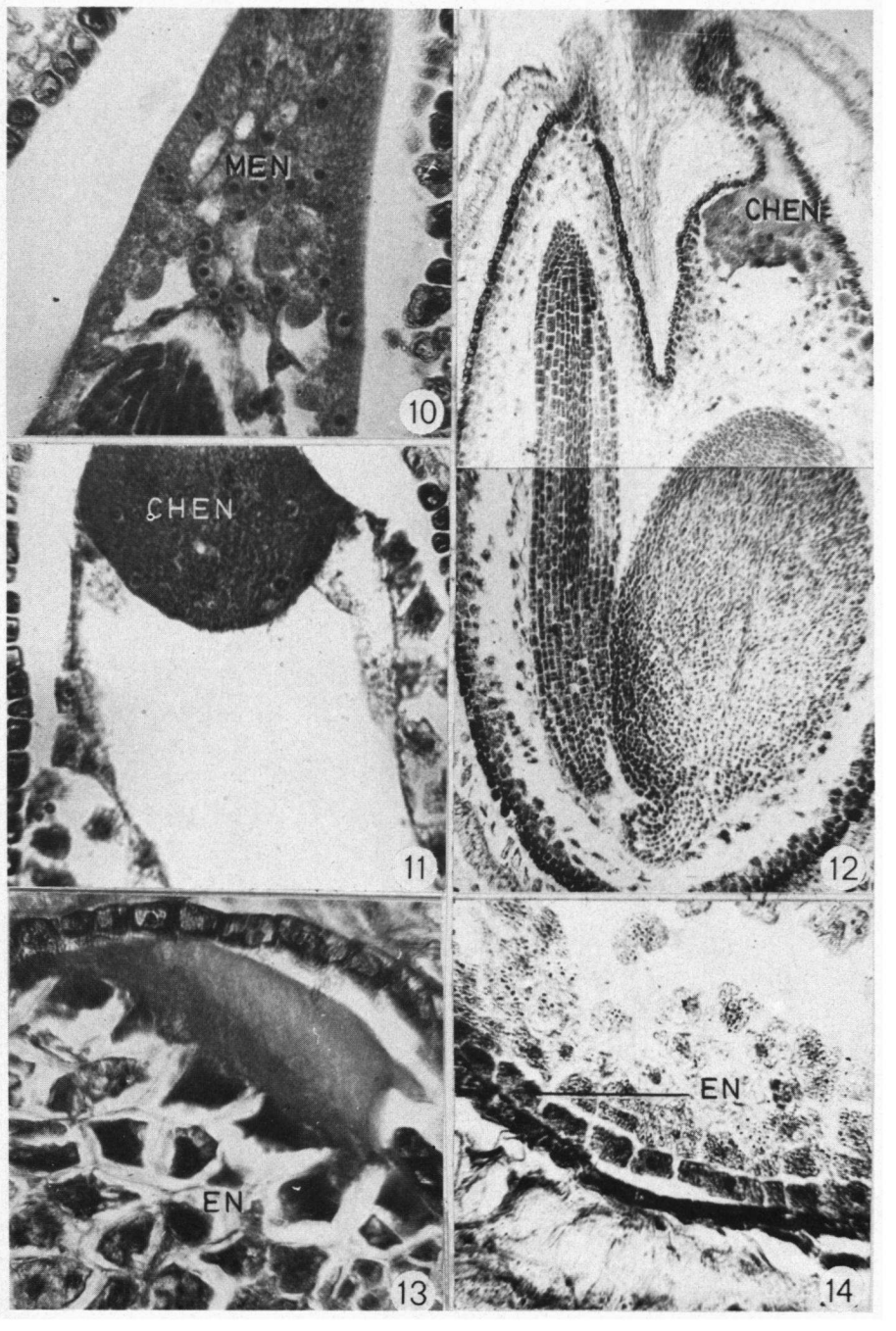

Plate 3.

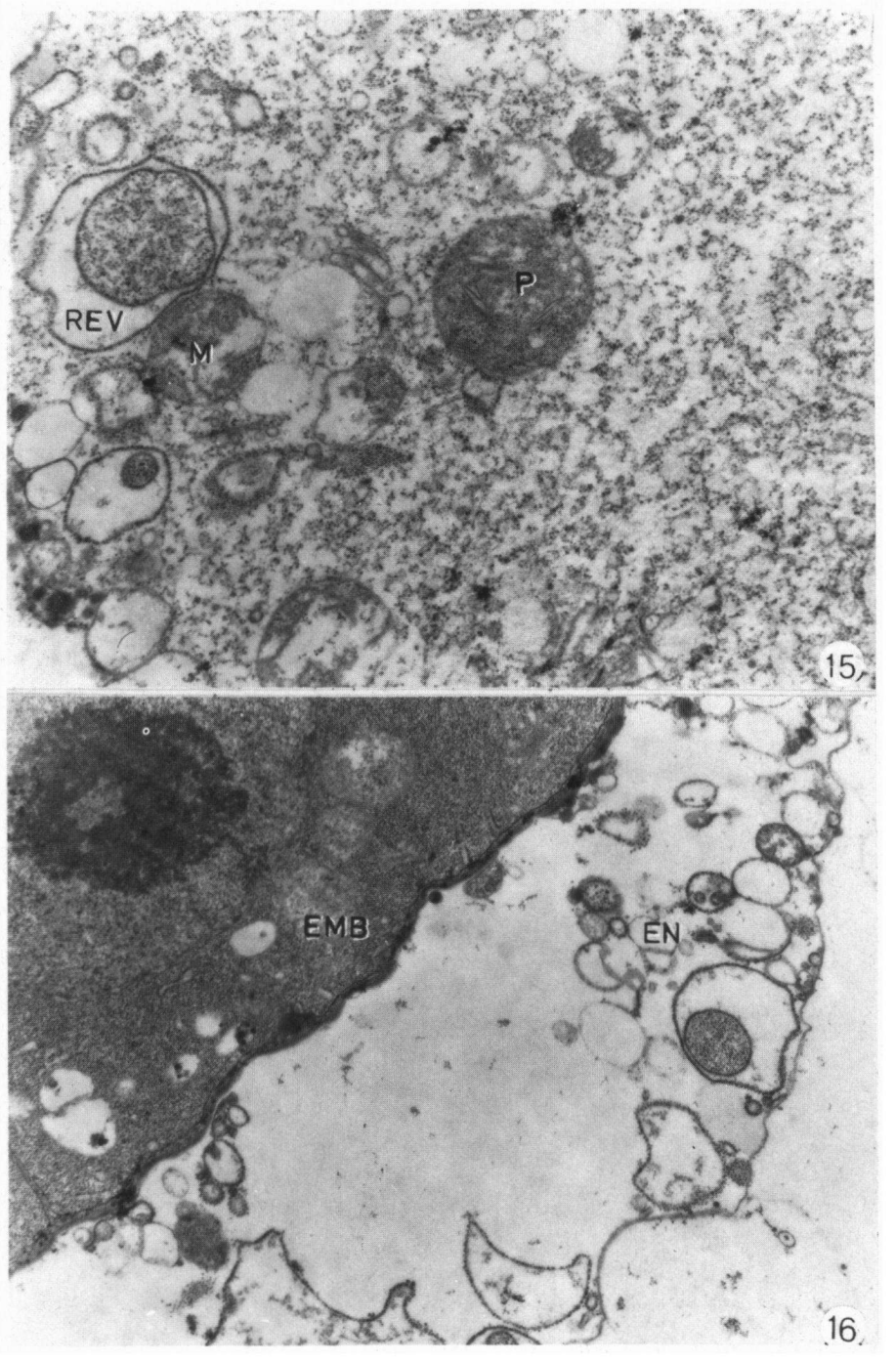

Plate 4, fig. 15. Cytoplasm of micropylar endosperm shows an abundance of ribosomes and polysomes. Mitochondria (M) and plastids (P) are present. Rough endoplasmic reticulum cisterns (REV) enclosing cytoplasm are observed, × 12,000. Fig. 16. Portion of endosperm (EN) near embryonal cells (EMB). The ground matrix lacks ribosomes; ribosomes are attached to – dilated – ER membranes, × 12.000.

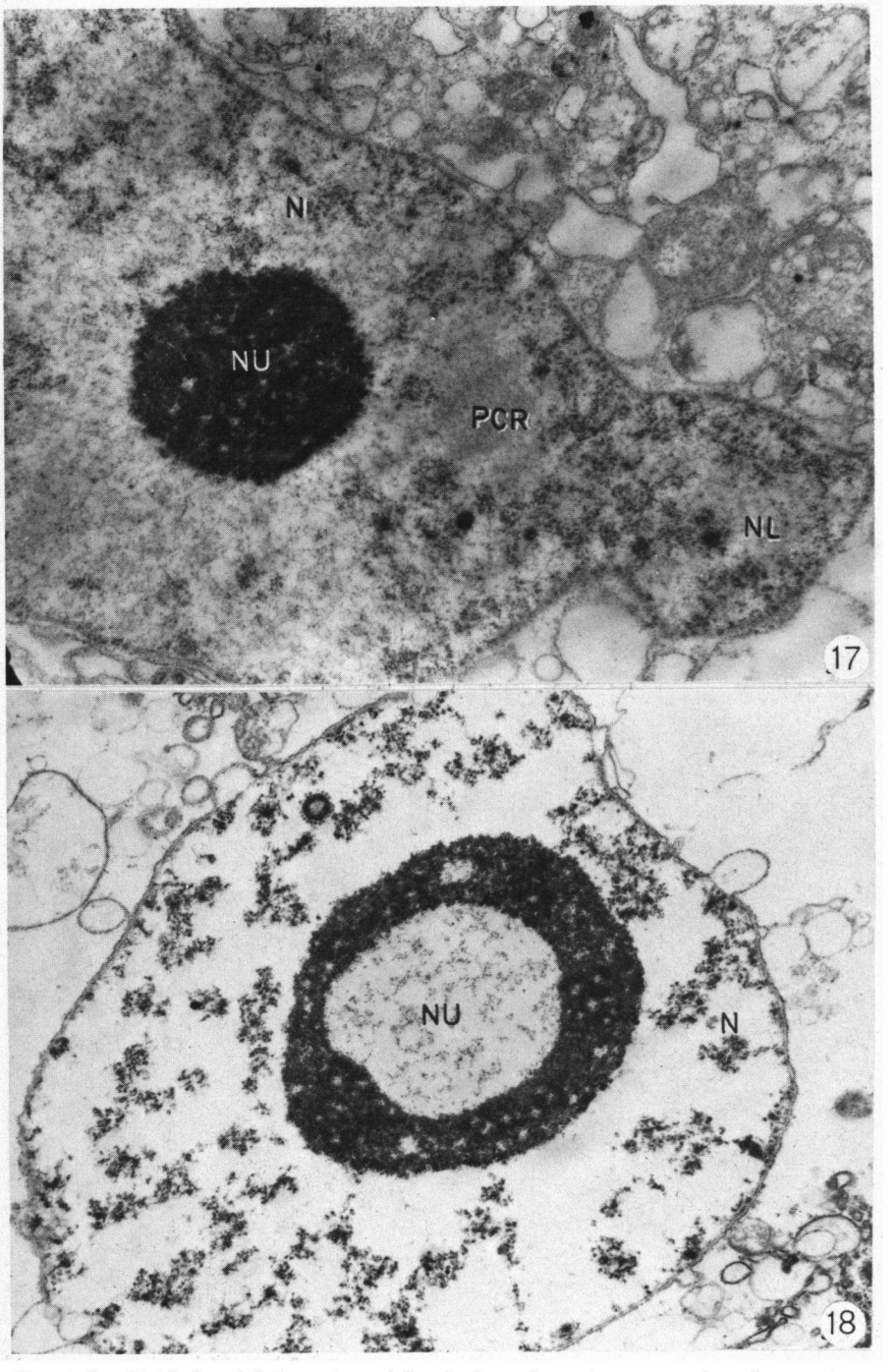

Plate 5, fig. 17. Nucleus (N) from the peripheral micropylar endosperm to show the prominent nuclear lobe (NL). A paracrystalline inclusion (PCR) is present near the dense nucleolus (NU), × 6,000. Fig. 18. Another nucleus (N) from the micropylar portion of the endosperm showing a nucleolus (NU) with distinct pars granulosa and pars amorpha, and blebbing of the nuclear envelope, × 8.000.

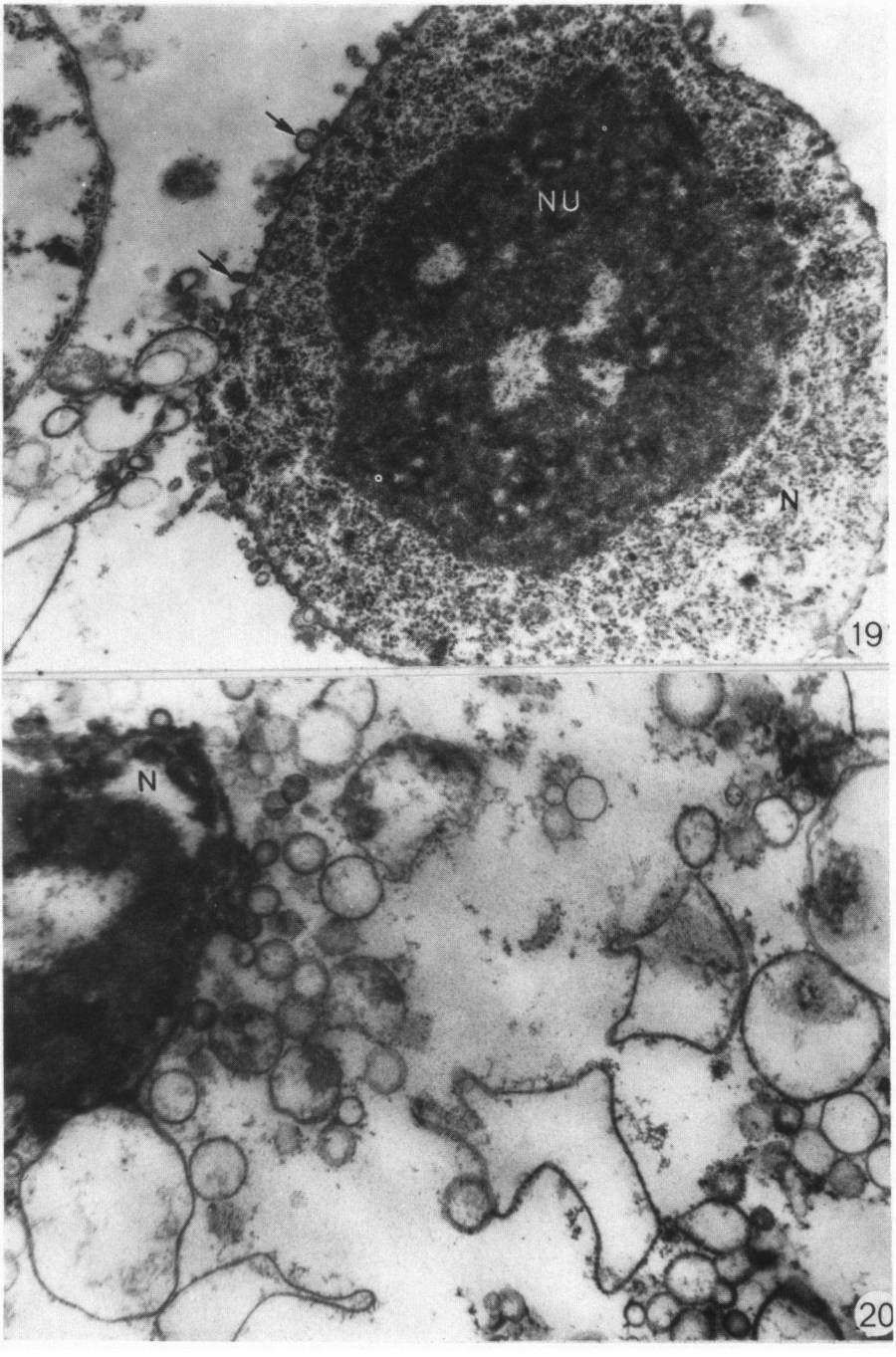

Plate 6, fig. 19. Nucleus (N) of micropylar endosperm showing a compact reticulate nucleolus (NU). Many vesicles (arrows) are pinched off from the outer nuclear membrane, × 12,000. Fig. 20. Portion of chalazal endosperm with part of a lobed nucleus (N). Blebbing of the outer nuclear membrane is very prominent. Dilated ER cisternae and vesicles of different sizes are present in abundance, × 12,000.