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Liisa Kaarina Simola: The ultrastructure of dry and germinating seeds of Pinus sylvestris L.

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THE ULTRASTRUCTURE OF DRY AND GERMINATING SEEDS OF PINUS SYLVESTRIS L.

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Abstract

SIMOLA, LIISA KAARINA (Dept. Bot., Univ. Helsinki): The ultrastructure of dry and germinating seeds of Pinus sylvestris L. — Acta Bot. Fennica 103: 1—31. 1974.

The ultrastructure of dry and germinating seeds of *Pinus sylvestris L.* has been studied. The endosperm and cotyledon and rootlet cells of the dry seeds are very similar in structure. The cells are packed with spherosomes and protein bodies, but amyloplasts, proplastids, mitochondria, dictyosomes, endoplasmic reticulum and ribosomes are not recognizable. Proteolysis is more rapid than lipolysis during germination. These processes and the development of new cell organelles are seen first in the rootlet cells and last in the endosperm. The rootlets are characterized by rapid development of compound amyloplasts, mitochondria, and tannin vacuoles.

During chloroplast development the cytoplasm in the cotyledon cells is rich in ribosomes. Proplastids concentrate round the nucleus but no nuclear budding is seen. Young chloroplasts may divide and they already synthesize starch. Small plastoglobuli are also frequent. Glyoxysomes, mitochondria and proplastids can be seen in endosperm cells after 8 days' germination.

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Contents

I. INTRODUCTION

Morphologically, the seeds of the family Pinaceae are composed of a rather thin outer seed coat surrouding a middle thick haploid endosperm (female gametophyte), within which lies a relatively small embryo. The seeds of *Pseudotsuga menziesii* contain both lipid (35 %) and protein (32 %) as storage materials. The rest is mainly fibres (29 %) especially in the seed coat, and only 1.7 % is starch and sugars (CHING 1966). In *Pinus banksiana,* as revealed by electron micrographs, the dormant cotyledon cells consist mainly of spherosomes (fat bodies), protein bodies and a nucleus. Mitochondria, dictyosomes and proplastids are not recognizable (DURZAN et al. 1971). The dormant endosperm cells of *Pseudotsuga menziesii* have about the same structure as the cotyledon cells (CHING 1965).

Histochemical studies on *Pinus sylvestris* have indicated that lipase activity localized around the cell wall and between the protein bodies (NYMAN 1965). In dry seeds of the Douglas fir both acid and neutral lipases are mainly located in heavy fat bodies and in the soluble fraction. The highest specific activity of both enzyme systems is associated with these fractions during germination (CHING 1968).

Despite BURGERSTEIN's (1900) claims to the contrary, the seeds of *Pinus sylvestris* do have a low starch content (NYMAN 1966). But, even in the dry state, their content of amylase is relatively high (NYMAN 1969), and TESCHE (1965) has demonstrated amylase activity and some starch in both endosperm and embryo in this and in several other conifers.

The germination of gymnosperm seeds *(Pseudotsuga menziesii)* is accompanied by the following physiological changes (CHING 1959). Imbibition of water is coupled with linearly increasing respiratory activity and after this the meristematic cells begin to divide. At the following stage the activity of several hydrolysing enzymes increases and the cells begin differentiate. Translocation of organic material to the growing cells is a characteristic feature of the next stage and the cells differentiate much further.

The aim of this study is to clarify the ultrastructural changes in the endosperm, rootlet and cotyledons of *Pinus sylvestris* during germination. Basic evidence of this kind is also needed for comparison when ageing processes in seeds are studied at ultrastructural level (SIMOLA, in press).

Abbreviations used in the figures:

A	amyloplast	IS	intercellular space	Pp	proplastid
C	chloroplast	Iv	invagination	Ps	polysome
Cr	crystalloid	M	mitochondrion	Pt	phytate
CW	cell wall	Mb	microbody	R	ribosome
D	dictyosome	N	nucleus	S	spherosome
ER	endoplasmic reticulum	Nu	nucleolus	St	starch
G	globoid	PB	protein body	T	tonoplast
Gs	glyoxysome	PC	protein clump	Tl	thylakoid
I	initial	Pg	plastoglobulus	V	vacuole
Ic	inclusion	Pl	plasmalemma	Ve	vesicle

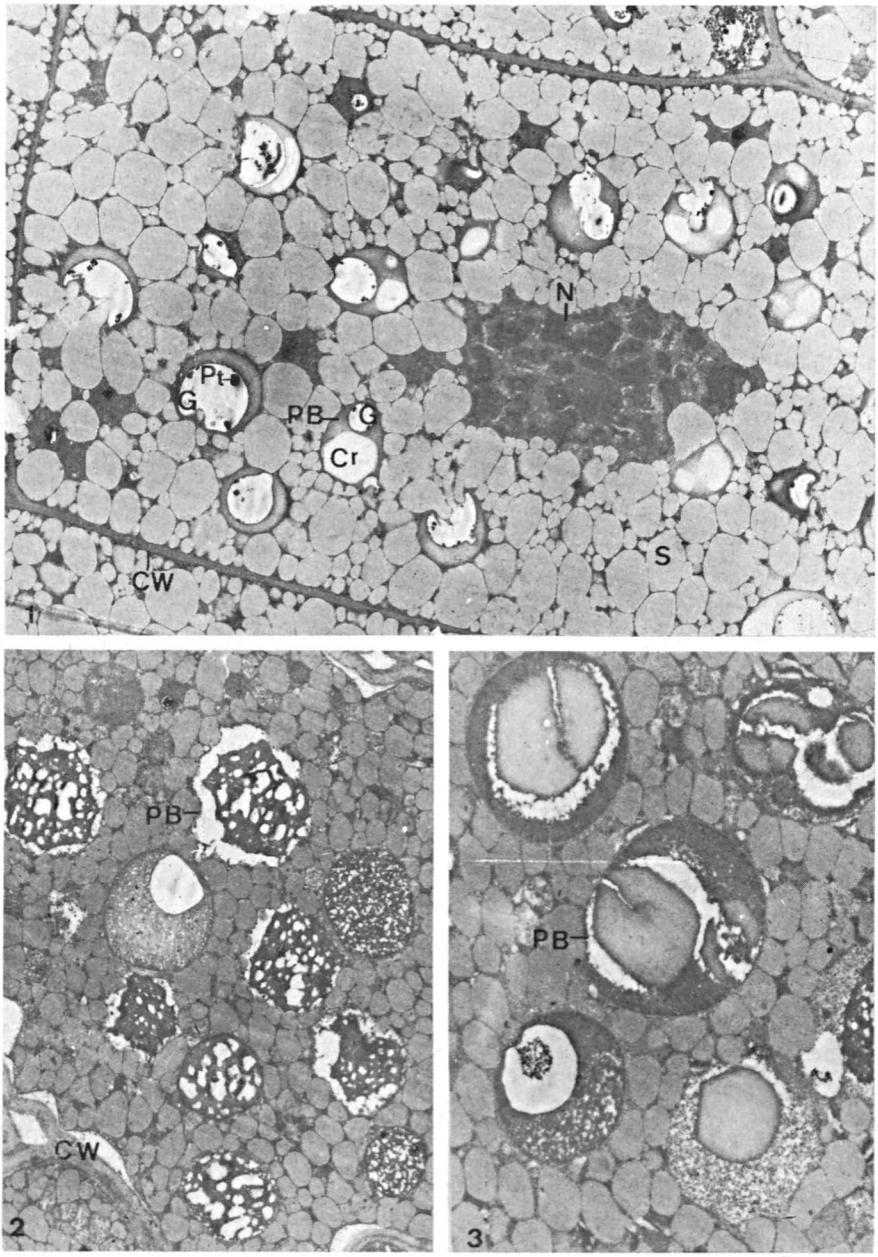
II. MATERIAL AND METHODS

Seeds of *Pinus sylvestris L.* (collected in Västraby, Kronobergslän, Sweden) were germinated under a bell jar on filter paper discs moistened with tap water at 25°C in continuous weak light. Material for fixation was removed daily for five days. Rootlets, cotyledons and the micropylar end of the endosperm were fixed separately. Dry seeds were placed in the fixative for half an hour, then dissected out in it, and fixed for another three hours at 4°C. Imbibed seeds were dissected out before fixation. The material used was as follows:

Dormant seeds
Day 1, imbibed seeds
Day 2, imbibed seeds
Day 3, rootlet about half of length of the seed, cotyledons yellow
Day 4, rootlets about 0.9 cm long, cotyledons green
Day 5, rootlets about 2 cm long, cotyledons green

Imbibed material was immersed in Karnovsky's fixative in cacodylate buffer (pH 7.2, 0.2 M) for 1.5 hours or in 3 per cent glutaraldehyde in phosphate buffer (pH 7.2, 0.1 M). The material was postfixed with 1 per cent osmium tetroxide in phosphate buffer and after dehydration via acetone and propylene oxide embedded either in Spurr or in Epon.

The sections were cut with a diamond knife, stained with lead citrate (Reynolds 1963) and viewed with a Philips 200 electron microscope. Semi-thin sections stained with toluidine blue (Trump et al. 1961) were used for localization of cells. Periodic acid Schiff (PAS), Sudan III and Sudan Black (Jensen 1962) were used for histochemical characterization of the cell contents.



FIGS. 1—3. Cells of a dormant embryo of *Pinus sylvestris*. Karnovsky — OsO_4 fixation. — FIG. 1. Cotyledon cell. Protein bodies (PB) with large globoids (G) and electron-translucent crystalloids (Cr) and traces of phytate (Pt). 2800 x. — FIG. 2. Rootlet cell. Protein bodies (PB) with several irregular electron-translucent areas. Plasmalemma loosened from the cell wall (CW). 4800 x. — FIG. 3. Rootlet cell. Protein bodies (PB) with irregularly shaped cavities and large roundish bodies. 8800 x.

III. RESULTS

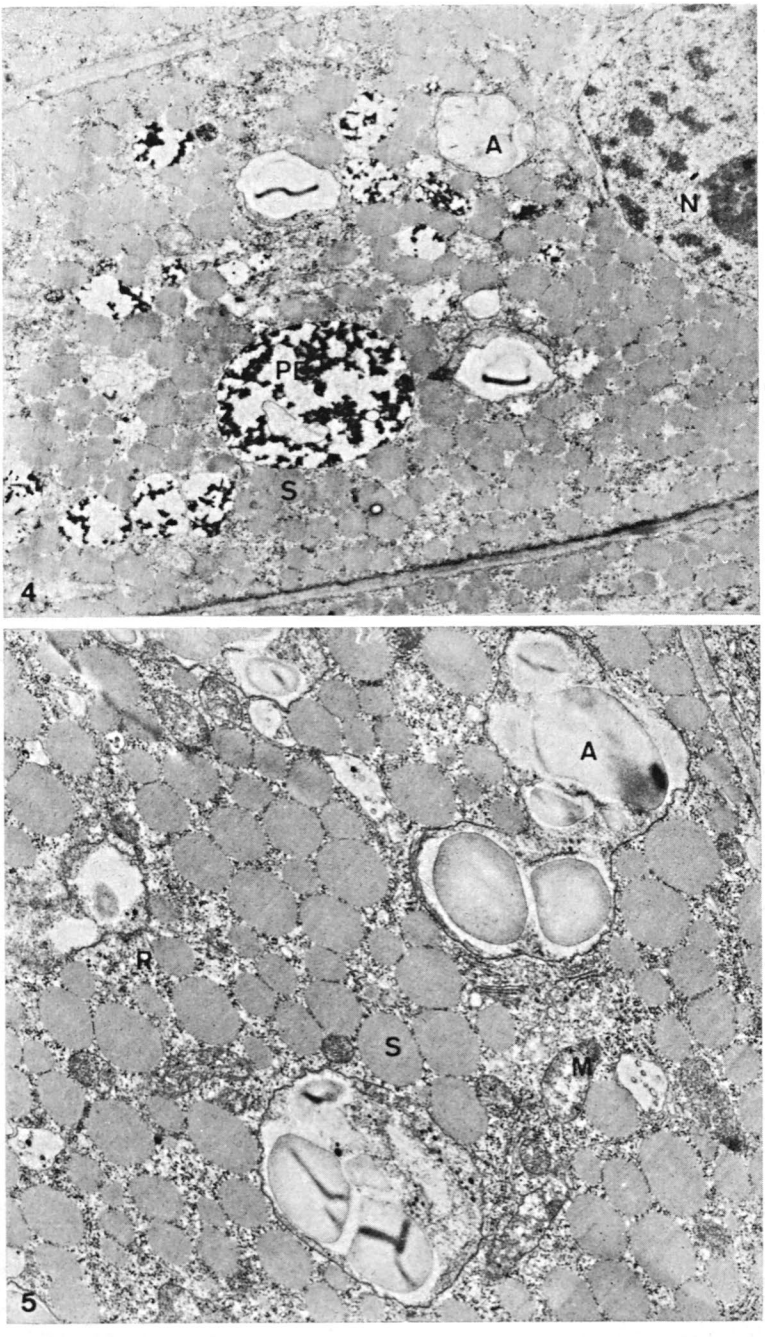
1. Rootlet cells

The structure of rootlet cells of dry seeds corresponds very closely to that of this part of the seeds of *Picea abies* (SIMOLA, in preparation). The protein bodies usually have several irregular electron-translucent areas (Fig. 2) but some of them may have an eccentric globoid. Some of the protein bodies have roundish inclusions (Fig. 3). The plasmalemma has become separated from the cell wall. This is apparently due to desiccation and not to the high osmotic pressure of the fixative. It is not easy to make out any initials or ribosomes in the cytoplasm between the numerous spherosomes as in the rootlets of *Picea.*

After one day's imbibition great changes are seen in the structure of the rootlet cells (Figs. 4 and 5). The protein bodies have formed vacuoles and compound amyloplasts are abundant. Mitochondria, dictyosomes and some endoplasmic reticulum (ER) have already developed and the cytoplasm contains a great number of ribosomes.

By the second day the amount of cytoplasm has increased, ribosomes occur as polysomes, and the spherosomes have become more osmiophilic than before. Rough ER runs parallel with the cell wall and dictyosomes are situated near the plasmalemma, which is not easily recognizable (Fig. 6). The vacuoles contain some electron-dense material, and microbodies may be seen occasionally.

On the third day the different parts of the root tip are already dissimilar in structure. The cells near the calyptra contain compound amyloplasts and osmiophilic spherosomes (Figs. 7 and 8). The number of ribosomes has decreased, but dictyosomes and the vesicles they liberate are frequent, as well as mitochondria with a rather well-developed internal structure (Figs. 8 and 9). ER lines the plasmalemma and is also present in the endosperm. There were several small electron-translucent vacuoles in the cells (Fig. 8). In the root cortex cell the vacuoles fill with tannin material and fuse together to form a large central vacuole (Fig. 11). When tannin formation begins the starch grains diminish and the spherosomes become less electron-dense (Figs. 7, 10 and 11). Ribosomes are very sparse. In the meristematic cells (day 4) the vacuoles are very small and lie between the spherosomes, which tend to occur in groups (Figs. 12 and 13). Dividing plastid initials are very abundant. Some ER and very small initials are rather frequent, but no starch or tannin is seen in the cells. Some of the root cortex cells (day 5) store relatively large amounts of starch, and tannin is seen as flocculent material at the edges of the vacuoles (Fig. 14).



FIGS. 4—5. Rootlet cells (day 1). — FIG. 4. Dissolving protein bodies (PB). 6800 x. — FIG. 5. Multiple amyloplasts (A). Developing mitochrondria (M). 13 600 x.

2. Cotyledon cells

The cells of dry cotyledons are packed with spherosomes and protein bodies like the other parts of the seeds studied (Fig. 1). The protein bodies, however, are rather different in structure from those in the cotyledon cells of *Picea abies* (SIMOLA, in preparation) and *Pinus banksiana* (DURZAN et al. 1971). They usually have one large globoid cavity with some phytate and one or two electron-translucent crystalloids. The protein bodies do not stain with silver hexamine, and observations with the light microscope indicate that they do not stain with PAS. They are therefore believed to be composed chiefly of protein material, unlike the protein bodies of *Picea abies,* which also contain carbohydrates. The smaller, irregular, rather electron-dense bodies are apparently protein bodies transectioned at another level. No amyloplasts are visible, as in the cotyledon cells of *Pinus banksiana* (DURZAN et al. 1971). It is not possible to detect any organelle initials or ribosomes in the cytoplasm between the spherosomes.

After one day's imbibition inclusions have disappeared from the protein bodies and some of the globoid cavities contain abundant phytate (Fig. 16). It seems possible, however, that relatively great variation exist between different cells as regards their phytate content, as in the embryo of *Lactuca* (PAULSON & SRIVASTAVA 1968). Small initials and some proplastids with electron-translucent starch grains are visible, especially near the nucleus (Fig. 15). Their membrane corresponds more nearly to a single than to a double membrane, such as is characteristic of plastids. Ribosomes and small, very electron-dense bodies are visible between the spherosomes. These structures are also found in the cotyledon cells of *P. banksiana* (DURZAN et al. 1971).

Breakdown of protein material begins after two days' germination (Figs. 18 and 19). Possibly, some of the globoids are surrounded by a membrane, because a membrane circle may be visible in the globoid cavity (Fig. 19). The fact that the globoid cavities contain only vestiges of phytate suggests that phytase is rapidly activated or formed during germination. Lipolysis has not begun, but small microbodies with a single membrane and rather electro-dense stroma are visible in the cytoplasm (Fig. 20). They also contain granules of a size roughly corresponding to that of the ribosomes, which are abundant in the cytoplasm between the spherosomes (Fig. 21). Similar structures in the cotyledon of *Pinus banksiana* (DURZAN et al. 1971) have been interpreted as microbodies and are supposed to be initials of plastids. Small, rather weakly developed mitochondria are visible but their early stages cannot be recognized. The

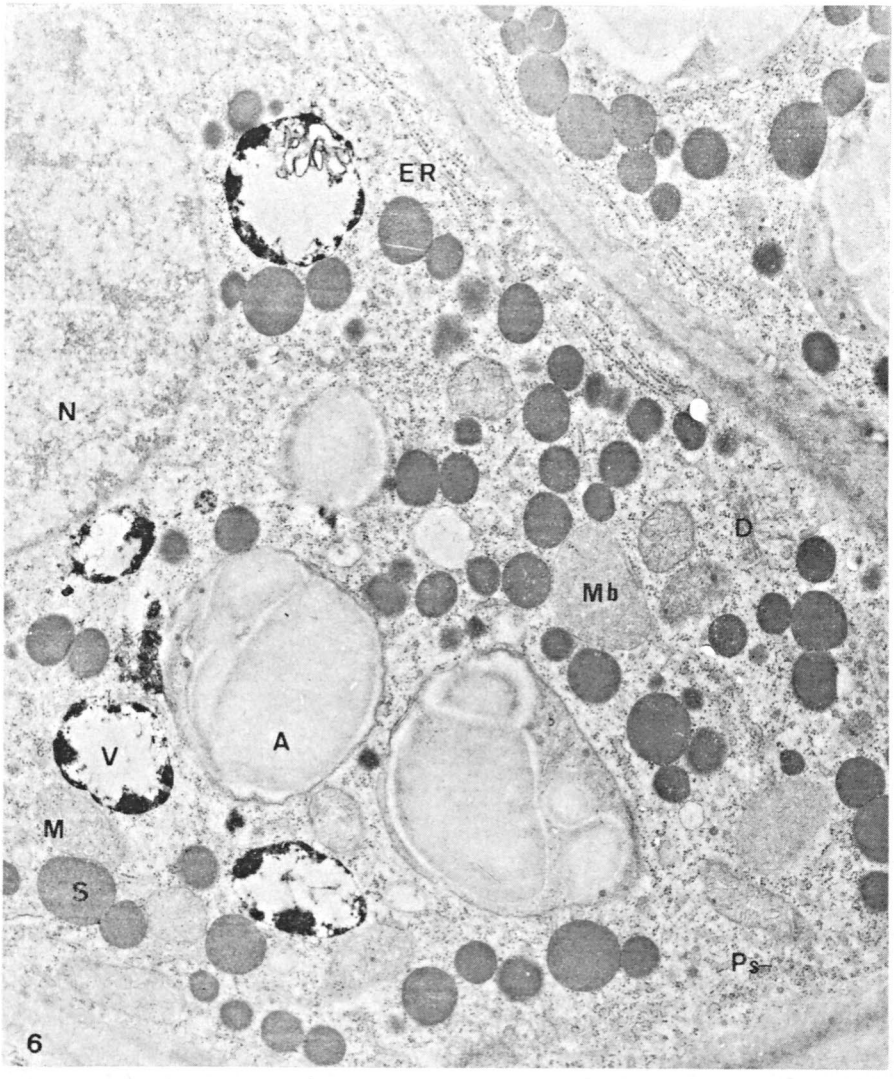
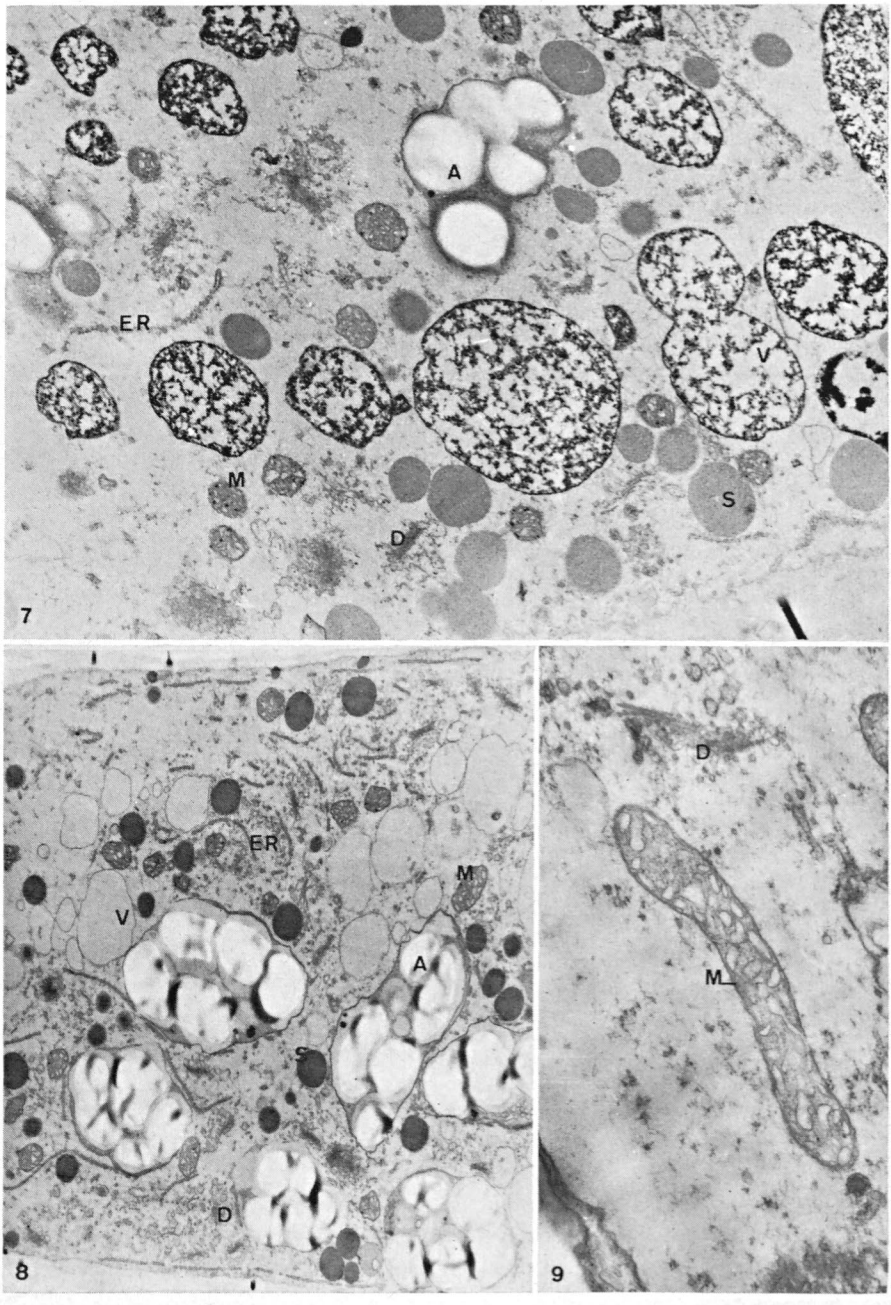
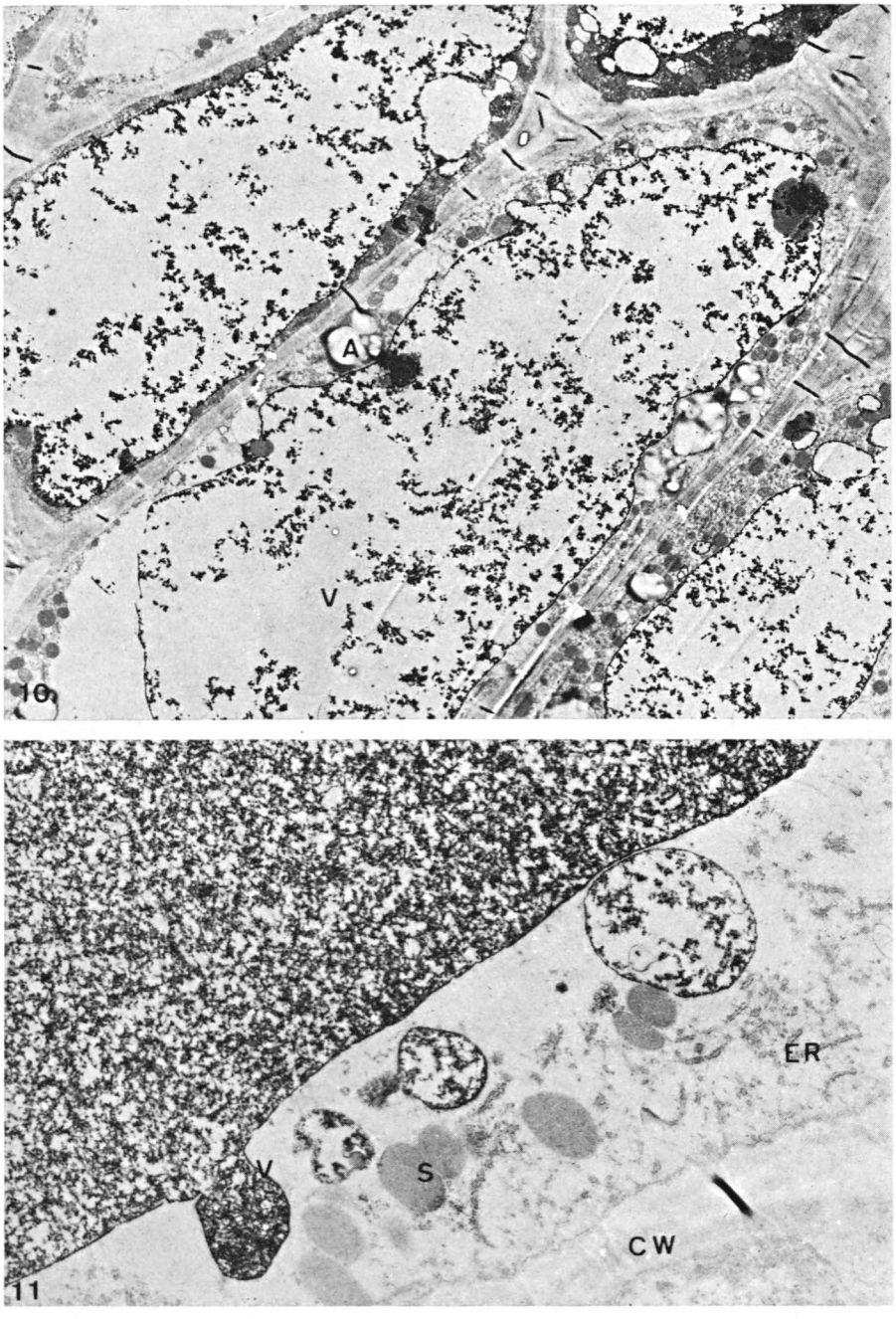


FIG. 6. Rootlet cells (day 2). Amyloplasts (A). Osmiophilic spherosomes (S). Mitochondria (M) containing some ribosomes. Rough ER. Developing vacuoles (V) with some tannin. 13 000 x.

proplastids contain some starch, and a few short thylakoids and small plastoglobuli have formed in the stroma (Figs. 17—19). The structure of the proplastids is very different in *Pinus banksiana* (DURZAN et al. 1971). In this plant the proplastids are very electron-dense and usually elongated, and their internal structure is not well defined.



Figs. 7—9. Rootlet cells near the calyptra (day 3). — Fig. 7. Small vacuoles (V), some of them fusing together. 3200 x. — Fig. 8. Amyloplasts (A). Small electron-translucent vacuoles (V). 7000 x. — Fig. 9. Mitochondrion (M) with well-developed cristae. 11400 x.



FIGS. 10—11. Root cortex cells (day 3). — FIG. 10. Large central vacuoles (V) containing tannin. 3 000 x. — FIG 11. Small vacuole fusing with the large central vacuole. 11 000 x.

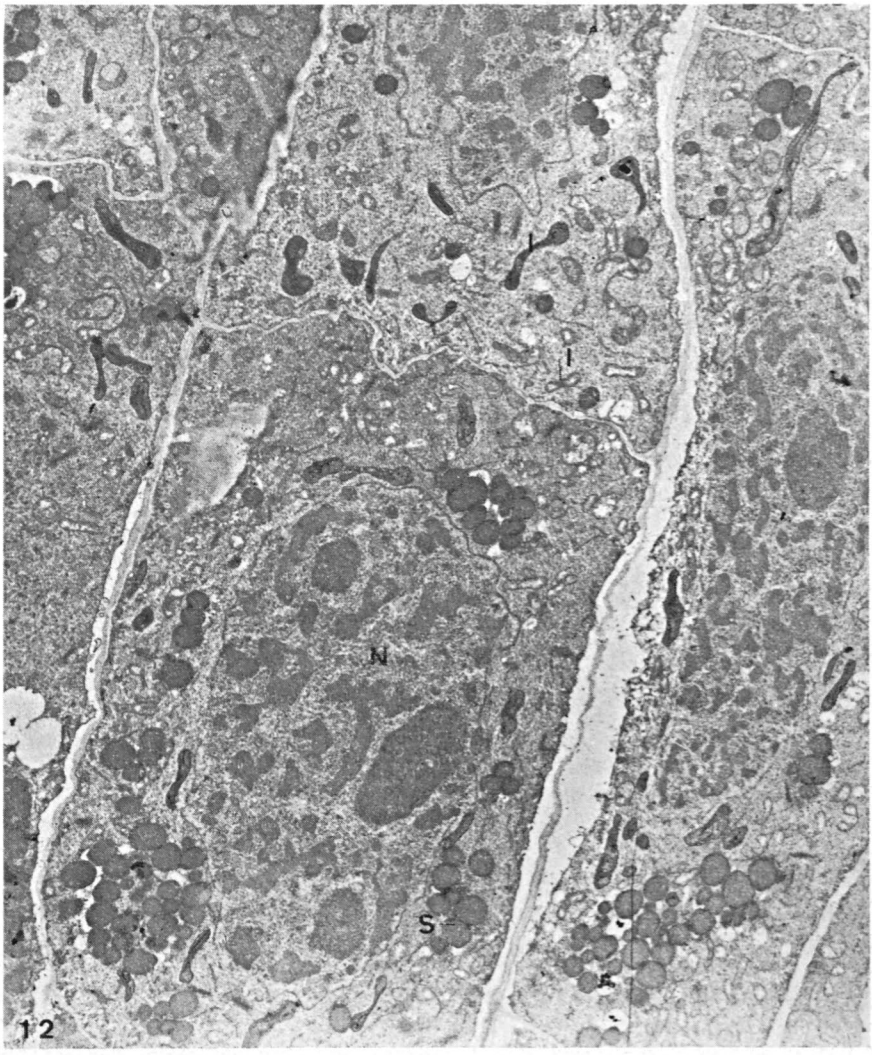
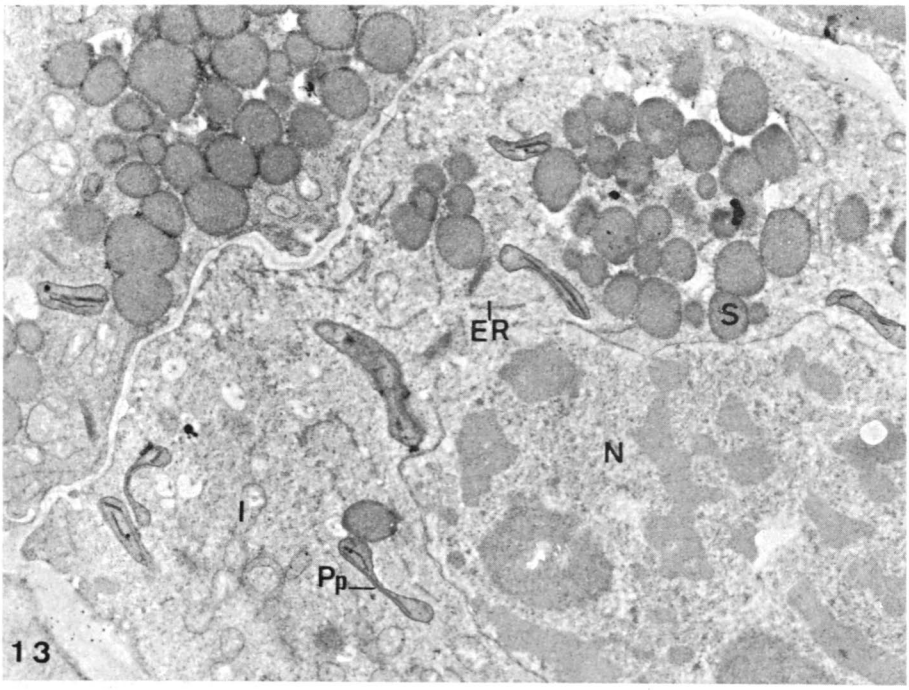


FIG. 12. Root tip cells near the meristem (day 4). Groups of spherosomes (S). Organelle initials (I). 11 000 x.

After three days' germination the vacuoles have enlarged and may contain some osmiophilic granules attached to the tonoplast (Figs. 23 and 24). The mitochondria have a better defined internal structure and the ribosomes are grouped into polysomes. The chloroplasts seem able to divide (Fig. 23). The number of thylakoids increases on the 4th and 5th days, and some small grana are formed (Figs. 25 and 26). The cytoplasm



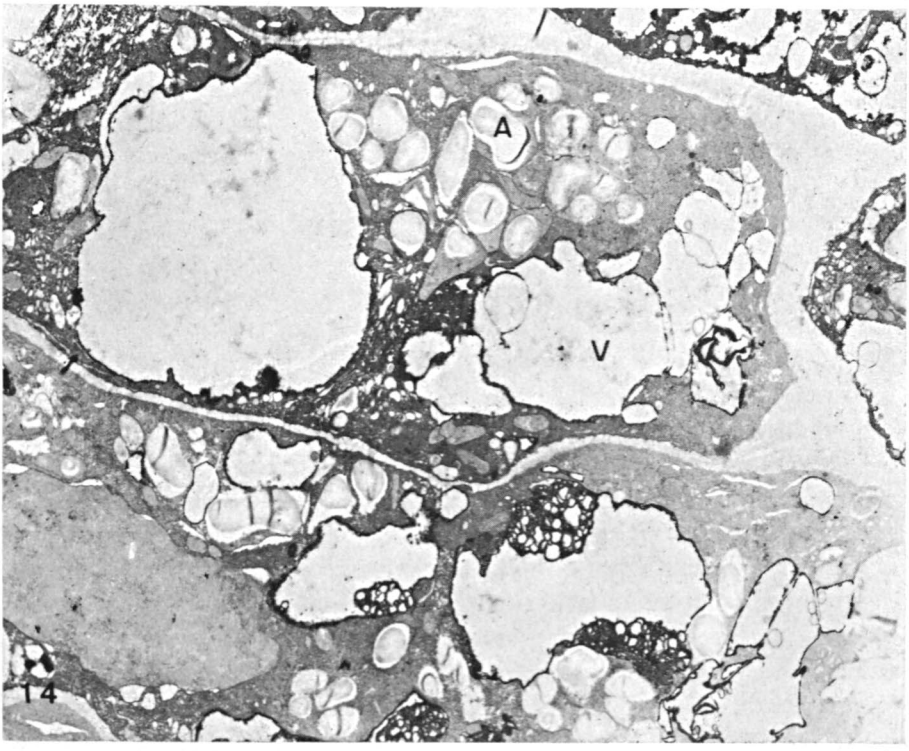


FIG. 13. Root tip cells near the meristem (day 4). Proplastids (Pp). Organelle initials (I). 8 800 x.

FIG. 14. Root cortex cells (day 5). Vacuoles (V) with flocculent tannin material. Amyloplasts (A). 6 800 x.

has a dense background of ribosomes and some rough ER and dictyosomes are visible. No ribosomes are recognisable in the chloroplasts, and the plastoglobuli are small, as in the fullgrown needles of this plant (WALLES et al. 1973). Lipolysis is not effective and the size of the spherosomes varies greatly.

3. Endosperm cells

The haploid endosperm tissue has about the same structure as the rootlets and cotyledons at the beginning of germination. The structure of the protein bodies may vary, as is clearly seen after imbibition before the breakdown of the storage material has begun (day 4). The number of globoid cavities and the electron-density of the protein mass may vary (Figs. 27—29). The globoids may contain osmiophilic grains, apparently phytate, and some membrane circles. An electron-translucent inclusion may be visible in the protein mass (Fig. 27). The spherosomes are electron-translucent.

Breakdown of storage material begins later in the endosperm cells than in the embryos of *Pinus sylvestris*. In view of this, endosperm material was also studied after 8 and 13 day's germination. After five days' germination the protein bodies were often almost intact but disorganization of the spherosomes had begun (Fig. 30). Endosperm cells begin to supply nutrients for the growing embryo when this has used up the bulk of its own protein reserves. After five days' germination numerous spherosomes are seen in the cytoplasm of the cotyledon cells, starch is abundant in the chloroplasts and amyloplasts are abundant in the root cortex cells.

It might perhaps be supposed that only different stages in the breakdown of the endosperm cells occur during germination, but actually they are followed by a stage of reorganization of the cell structure (day 8, Figs. 33 and 34). At this stage the cells have a rather well-organized structure with new formation of cell organelles (mitochondria and proplastids) and several other structures typical of living cells (ER and ribosomes).

After 8 days' germination the endosperm cells of *Pinus sylvestris* contain abundant microbodies (Figs. 32 and 33). These are interpreted as glyoxysomes in the light of earlier observations on the megagametophyte of *Pinus ponderosa* (CHING 1970). These structures are surrounded by a single membrane and may contain an invagination. Glyoxysomes are a form of microbody containing enzymes of the glyoxylate cycle. They are sites of fatty acid oxidation in cotyledons of many oil-containing seeds (castor bean, COOPER & BEEVERS 1969; watermelon, KAGAWA &

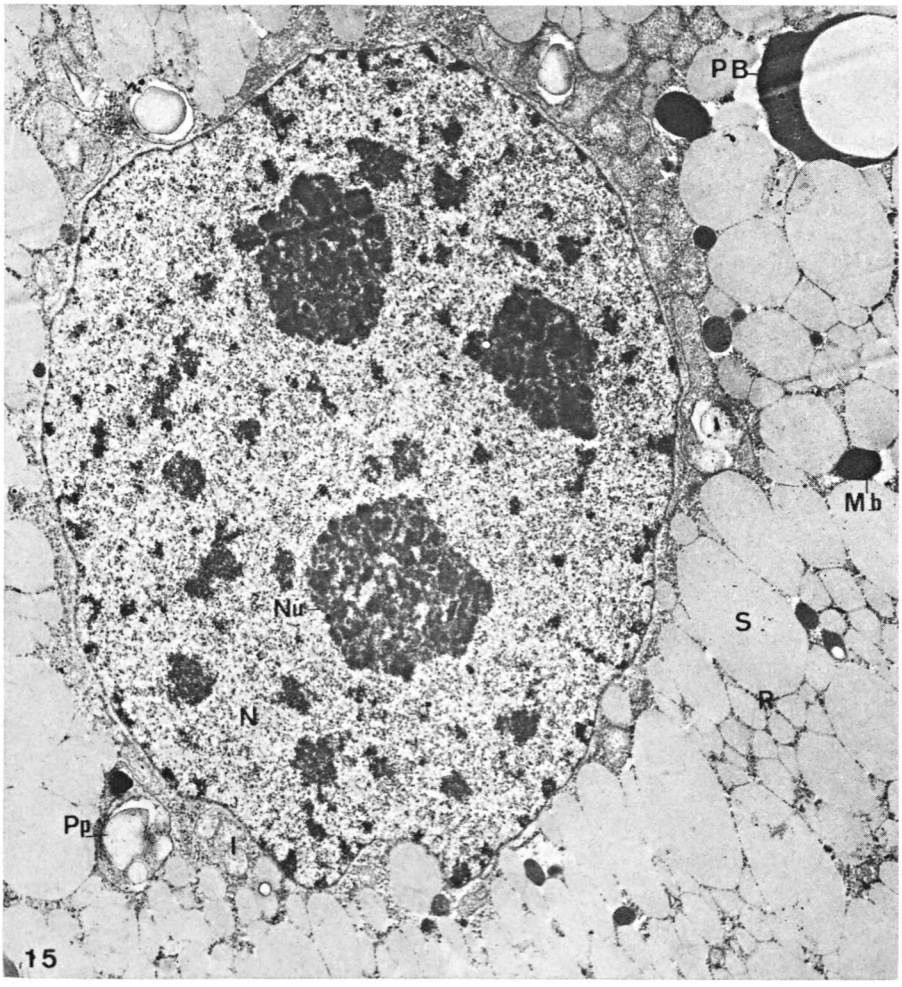


FIG. 15. Cotyledon cell (day 2). Nucleus (N). Three nucleoli (Nu). Proplastids (Pp) with some starch near the nucleus. Organelle initials (I). Microbody (Mb). 6 000 x.

BEEVERS 1970; peanut, LONGO & LONGO 1970; sunflower, GRUBER et al. 1970).

After 13 days the endosperm cells are quite empty and only a little lipid material is seen in the intercellular spaces. How long the endosperm cells preserve their semipermeability is not known.

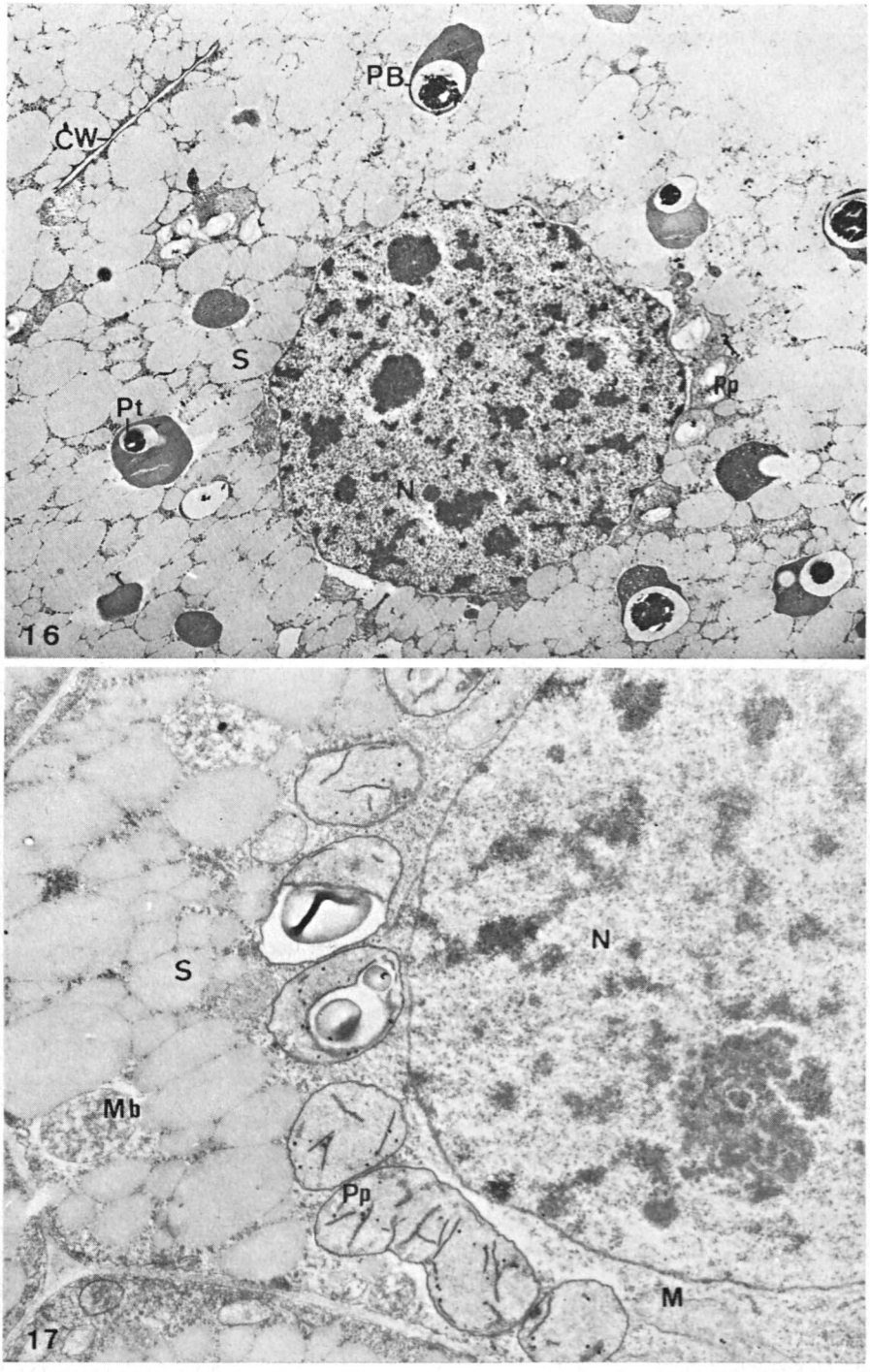
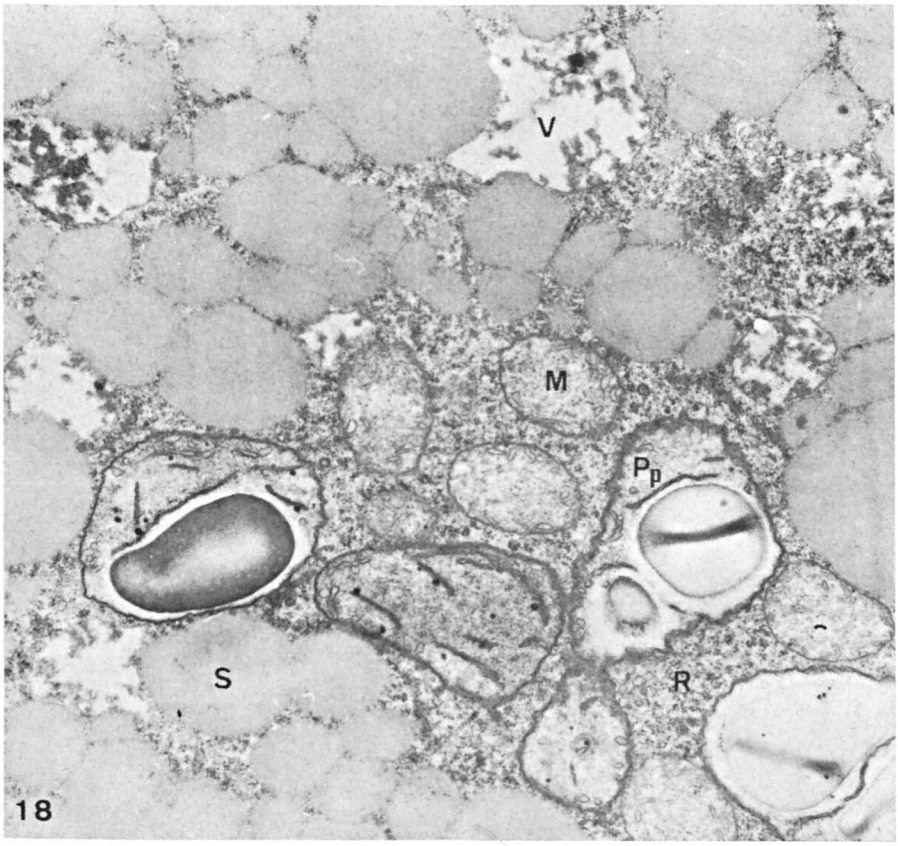
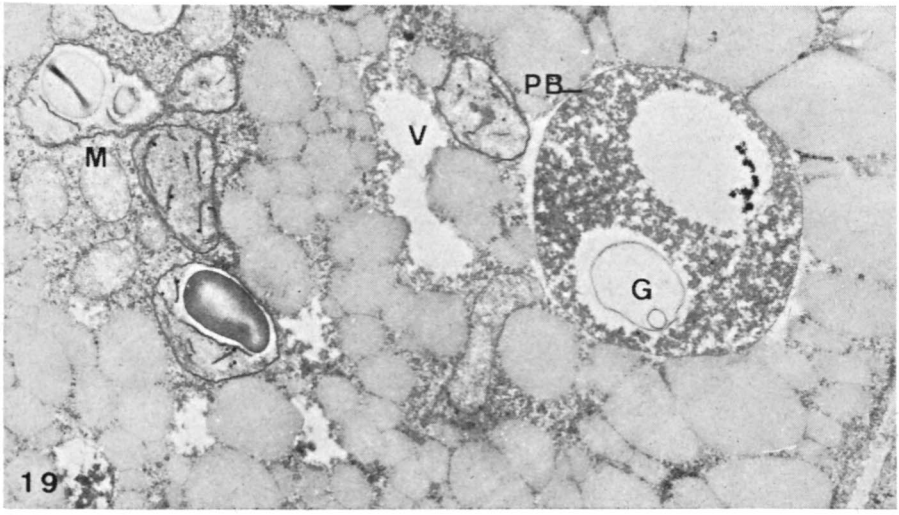


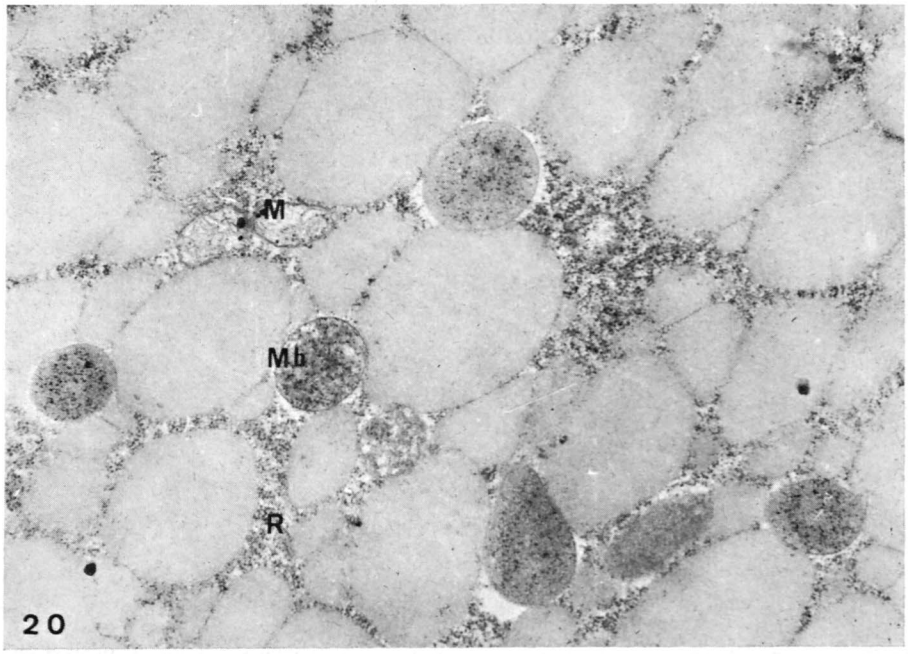
FIG. 16. Cotyledon cells (day 1). Protein bodies (PB) with phytate (Pt) in the globoid cavity (G). 2 800 x.

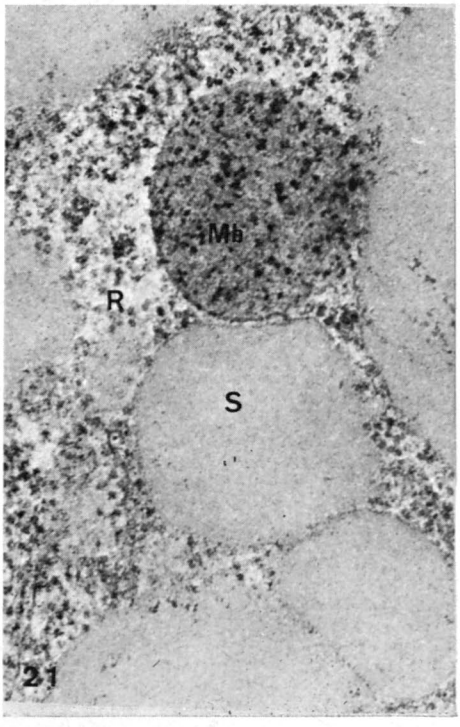
FIG. 17. Cotyledon cells (day 2). Proplastids (Pp) with some thylakoids and small plastoglobuli. 10 400 x.

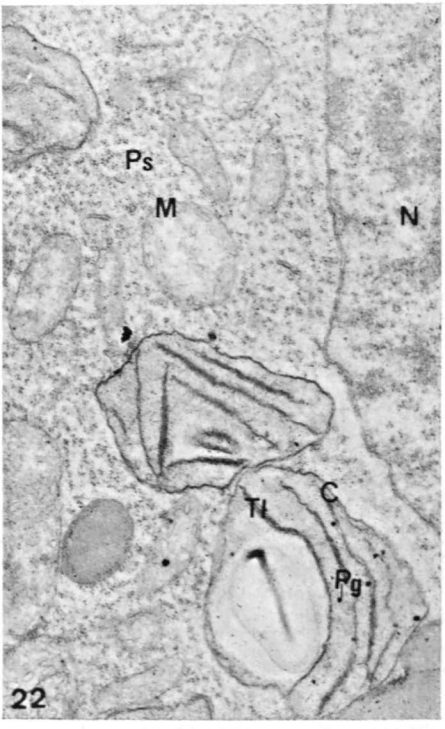




FIGS. 18—19. Cotyledon cells (day 2). Glutaraldehyde — OsO_4 fixation. — FIG. 18. Degradating protein bodies forming vacuoles (V). Proplastids (Pp) with starch, some thylakoids and plastoglobuli. 20 000 x. — FIG. 19. Protein body (PB) with a membrane circle in the globoid cavity (G). 10 400 x.







Figs. 20—21. Fig. 20. Spherosomes surrounded by a single membrane. Microbodies (Mb). 30 400 x. — Fig. 21. Microbody (Mb) containing particles corresponding in shape to ribosomes. 84 000 x.

Fig. 22. Cotyledon cell (day 3). Developing chloroplasts (C) with some thylakoids (Tl) and small plastoglobuli (Pg). 11 000 x.

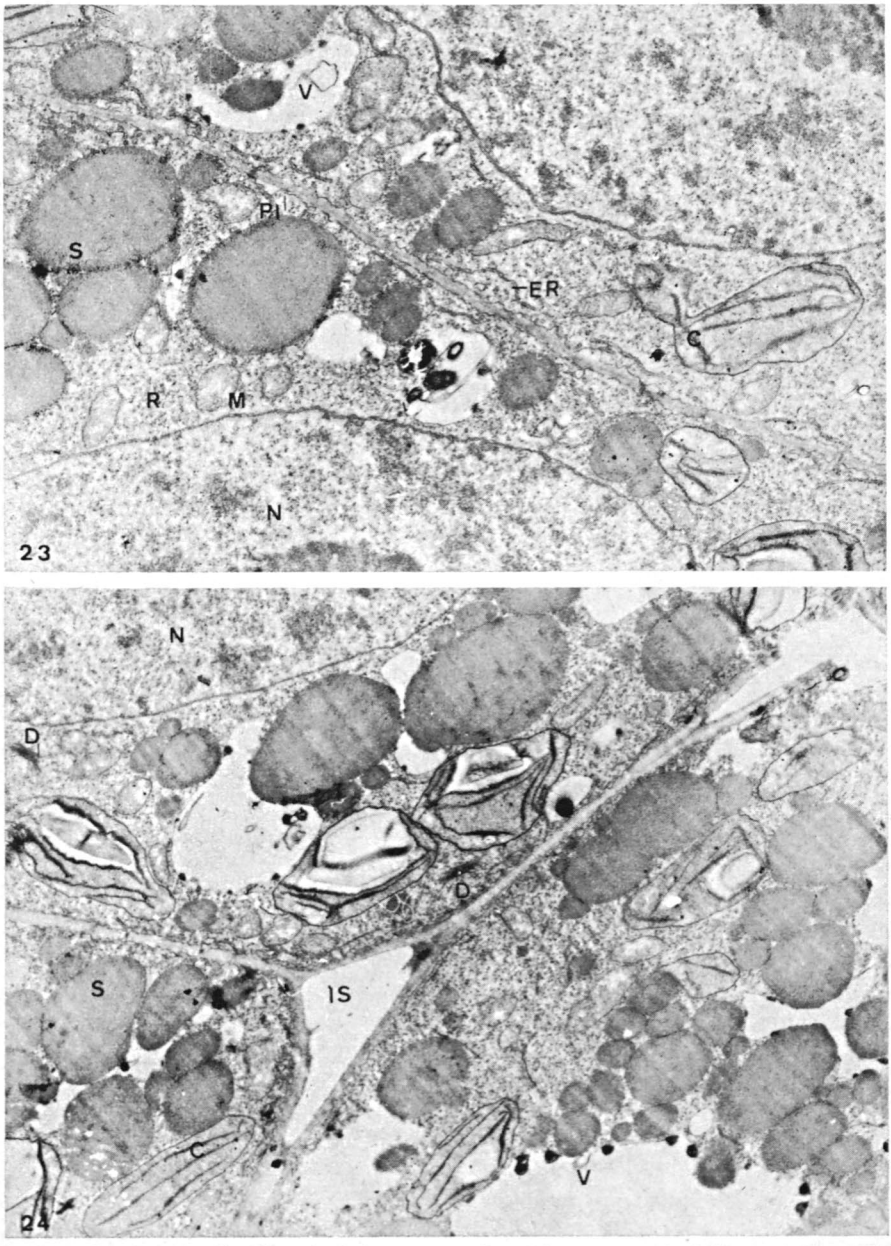


FIG. 23. Cotyledon cells (day 3). Spherosomes (S) with some osmiophilic material at the edges. Dividing chloroplasts (C). 10 400 x.

FIG. 24. Cotyledon cells (day 3). Vacuoles (V) with osmiophilic granules at the edges. Chloroplasts (C). 11 000 x.

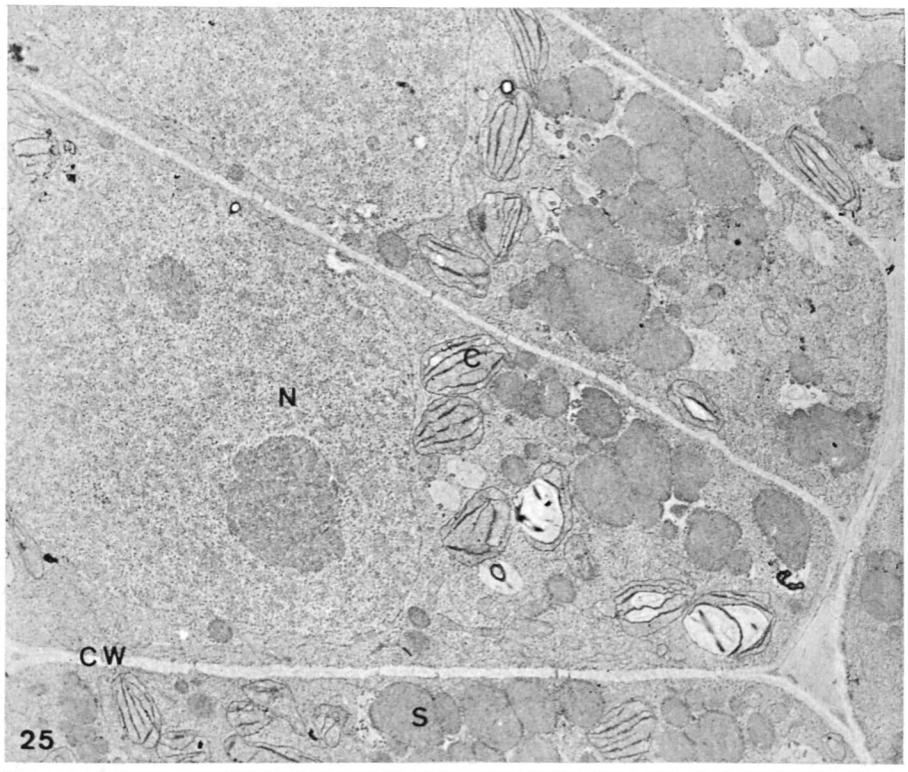
IV. DISCUSSION

1. Structure of dry seeds

Little is known about the ultrastructure of dry seeds owing to the technical difficulties of preparing and sectioning desiccated storage tissue for the electron microscope. Osmium tetroxide vapour has been used for fixing dry seed of *Pisum* but fixation took a very long time, even months (PERNER 1965). Such fixation was not satisfactory for *Gossypium* for which an aqueous solution of $LiMnO_4$ gave good results (YATSU 1965). Most authors have soaked their material in water for varying lengths of time, assuming that at low temperatures the structural changes will be insignificant (BAGLEY et al. 1963). In the present work glutaraldehyde and glutaraldehyde-formaldehyde fixatives followed by osmium tetroxide gave rather good results if a longer fixation time was used for desiccated material than for imbibed seeds. The form of the nucleus and the structure of the protein bodies shows that they were not imbibed before fixation. PAULSON & SRIVASTAVA (1968) have also used aqueous glutaraldehyde and osmium tetroxide for fixing dormant embryos of *Lactuca.*

In structure, the resting cells of the endosperm, rootlets and cotyledons of *Pinus sylvestris* resemble the cotyledon cells of *Pinus banksiana* (DURZAN et al. 1971) and *Picea abies* (SIMOLA, in preparation). In some details, however, there are slight differences. Amyloplasts are not visible in resting cells of *Pinus sylvestris* but are found in *P. banksiana.* Proplastids storing starch become recognizable near the nucleus after one day's germination in both the cotyledon and rootlet cells of *P. sylvestris.* Larger amounts of phytate are seen only occasionally in the globoid cavities of this species (cotyledon cells, day 1) but in the dormant cotyledon cells of *Pinus banksiana* phytate is very abundant (DURZAN et al. 1971). In dry seeds of *P. sylvestris* electron-translucent crystalloid inclusions are characteristic of the protein bodies of the cotyledons. Inclusions of less regular shape are also found in the protein bodies of rootlet cells (day 1, Fig. 3) but even after imbibition may be visible in the endosperm. The electron micrographs of DURZAN et al. (1971) show several inclusions in the protein bodies of *P. banksiana,* but the authors do not comment on these structures, although they are clearly visible.

A well-formed, usually eccentric globoid cavity is found in the protein bodies of cotyledon cells in the dry seeds. In rootlet cells there are several electron-translucent areas of irregular shape in the protein material. Corresponding structures were seen in dry aged embryos of *P. sylvestris*



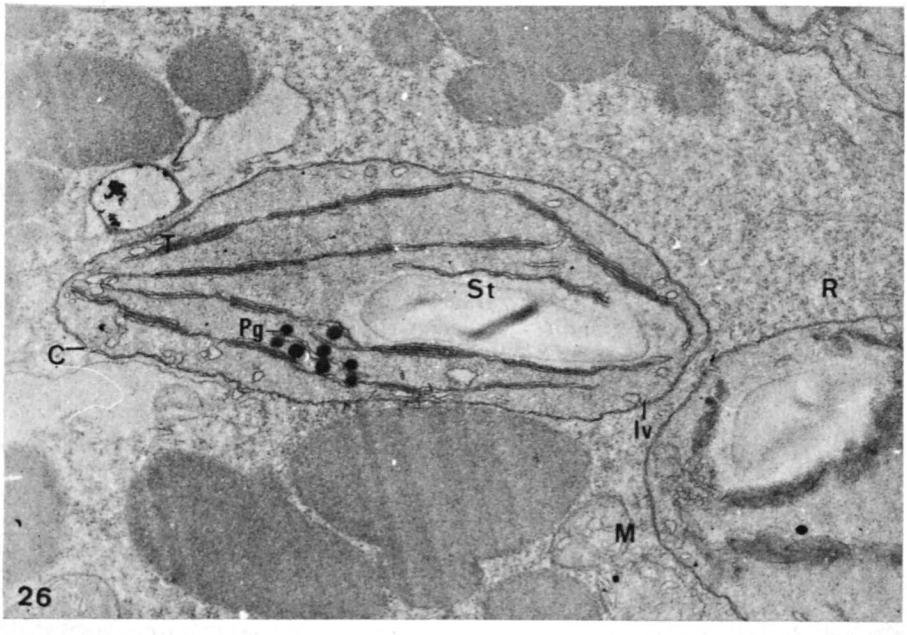
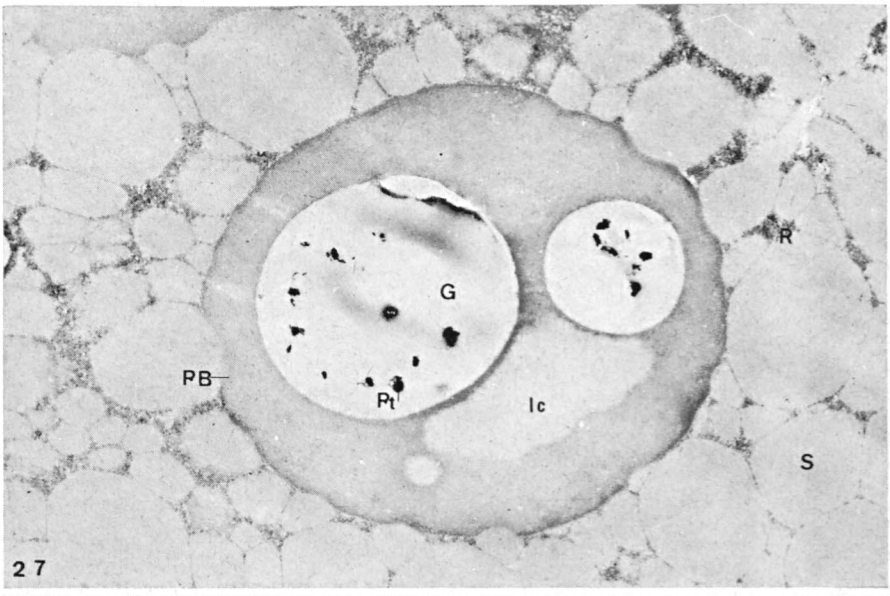
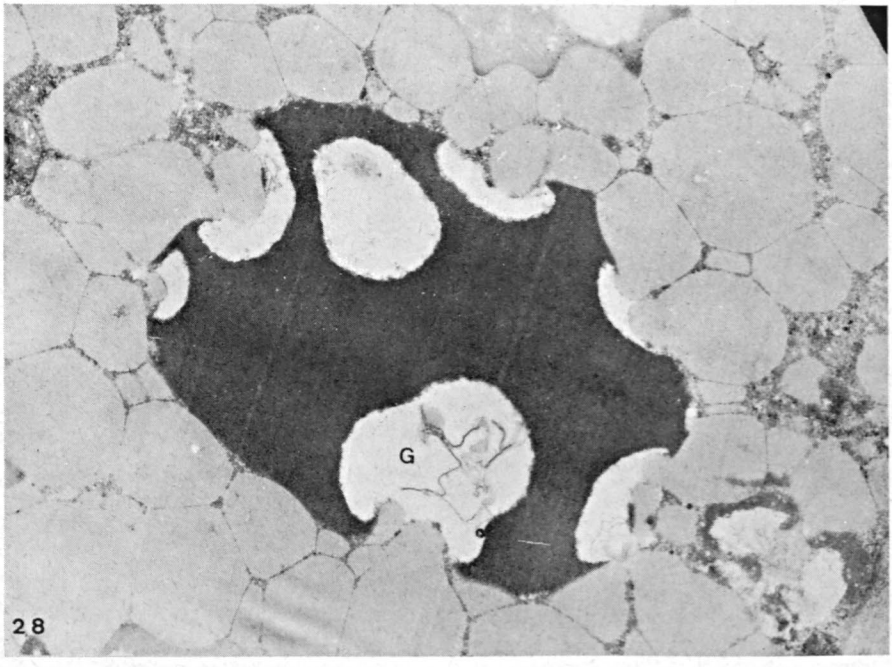


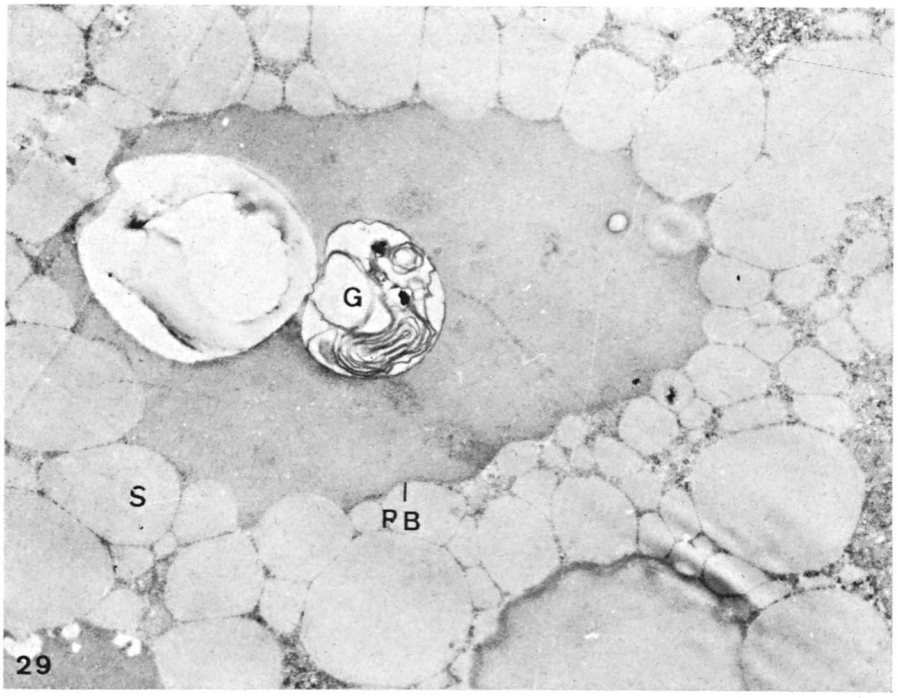
FIG. 25. Cotyledon cells (day 4). Cell walls (CW) straight. Chloroplasts (C). 4 000 x.

FIG. 26. Cotyledon cell (day 5). Chloroplasts (C) with weakly developed stroma and grana thylakoids. Invaginations (Iv) developing from the inner membrane. Starch (St) and plastoglobuli (Pg). 25 000 x.





FIGS. 27—28. Endosperm cells (day 4). — FIG. 27. Protein body (PB) with two globoid cavities (G) with some phytate (Pt). A rather electron-translucent inclusion (Ic). 8 000 x. — FIG. 28. A electron-dense protein body with several globoid cavities (G) at the edges. The cavities may contain membrane material. Proteolysis and lipolysis have not begun. 10 000 x.



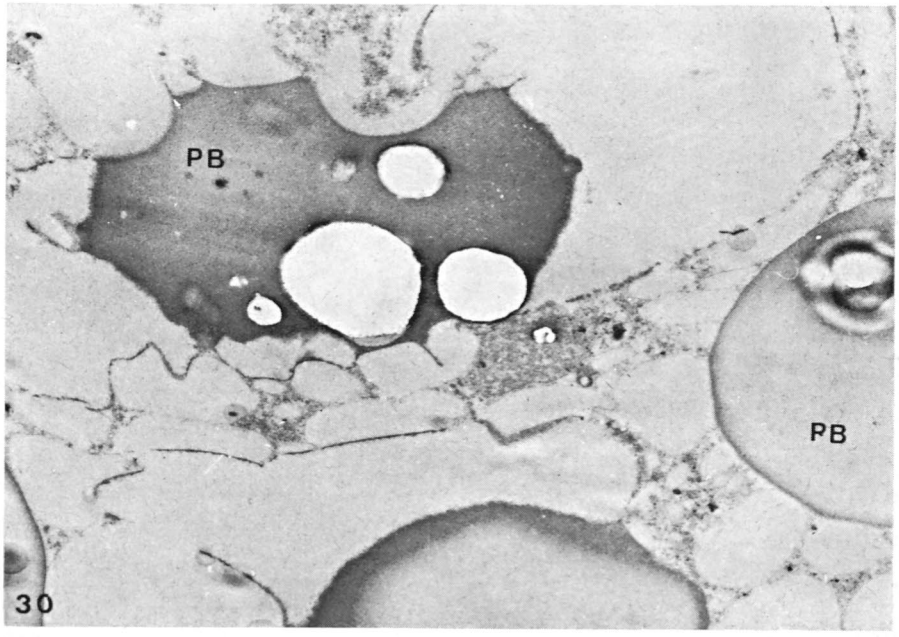
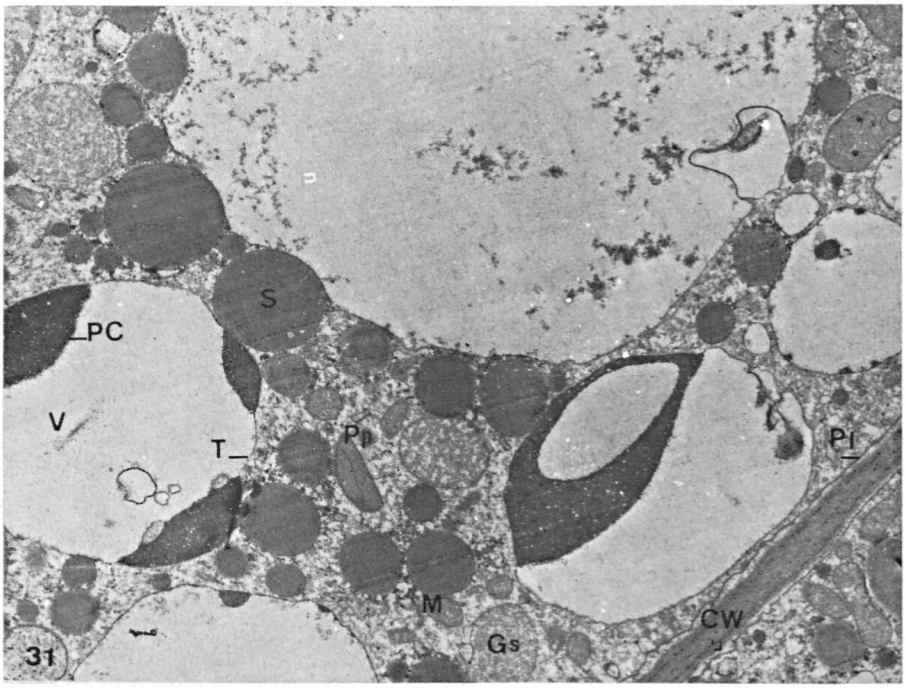
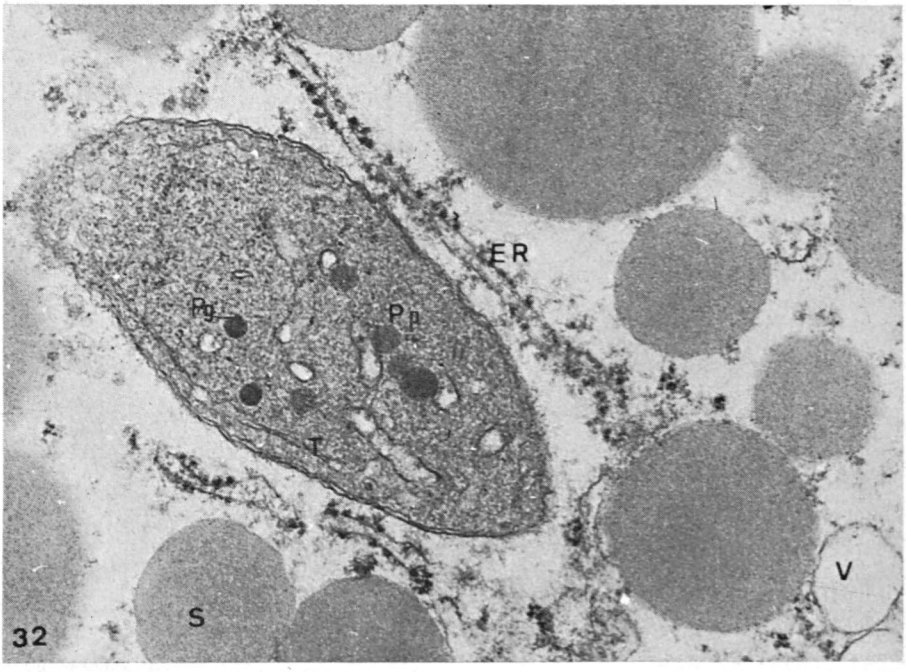
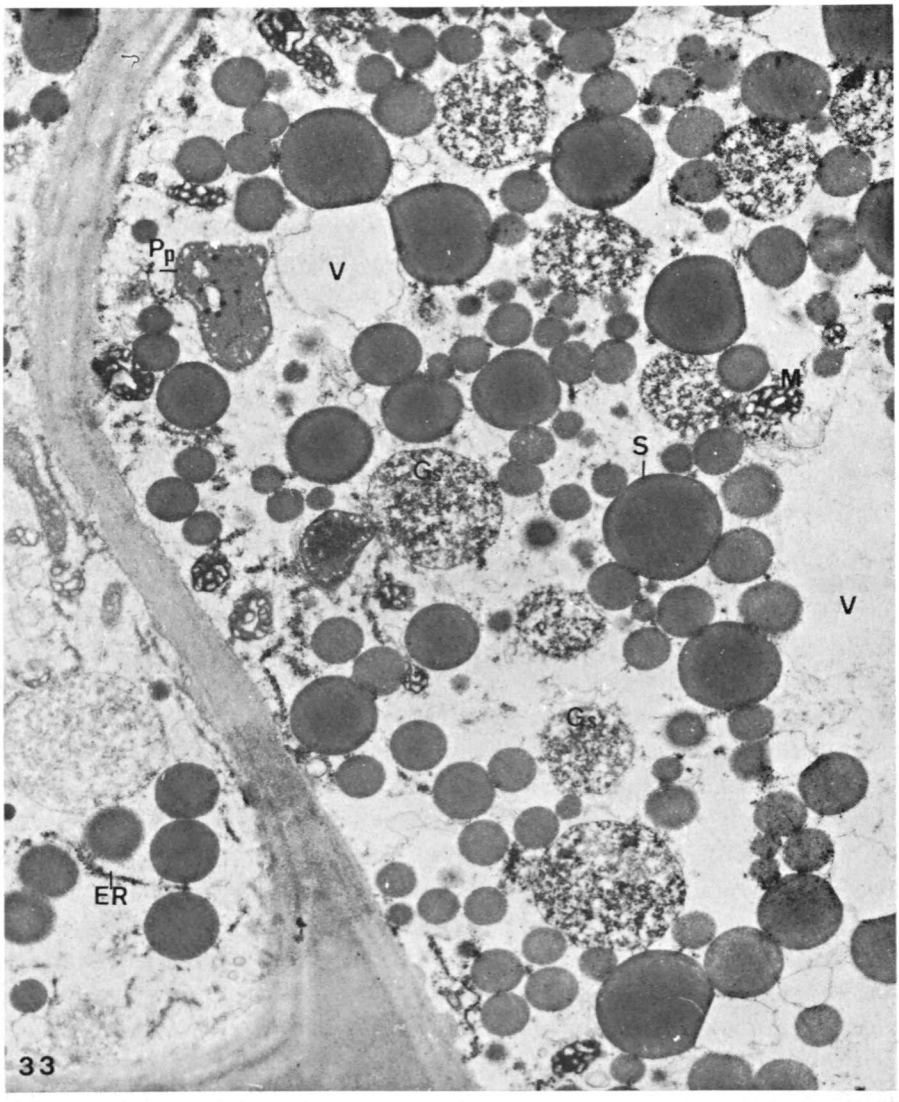


FIG. 29. Protein body (PB) with a myelin-like structure in the globoid cavity (G). 4 800 x.
FIG. 30. Endosperm cell (day 5). The electron-density of protein bodies (PB) varies. Spherosomal membranes disrupted. 11 000 x.





FIGS. 31—32. Endosperm cells (day 8). — FIG. 31. Degradating protein bodies forming vacuoles. Protein clumps (PC). Spherosomes (S) electron-dense. 8 500 x. — FIG. 32. Proplastids (Pp) with some thylakoids and plastoglobuli (Pg). Rough ER. 65 000 x.



FIGS. 33—34. Endosperm cells (day 8). — FIG. 33. Glyoxysomes (Gs). Proplastids (Pp). Mitochondria (M). ER. Vacuole (V). Electron-dense spherosomes (S). 6 000 x. —

and an eccentric globoid cavity was also visible in a cross-section. It seems obvious that these small irregular areas result from desiccation and contain only air. During imbibition the air does not all dissolve but form bubbles which may be visible especially in endosperm cells after imbibition (Figs. 28 and 30). The globoid cavities of *Crambe* seem to be surrounded by a

membrane (cf. v. HOFSTEN 1973). Some material resembling a membrane may be seen in the globoid cavities of cotyledon and endosperm cells of *P. sylvestris* (Figs. 19 and 29).

2. Mobilization of storage material and development of vacuoles

The cellular reorganization and degradation of storage material is more rapid in rootlet cells than in cotyledons and slowest in the endosperm. Proteolysis starts in the endosperm cells when the protein reserves of the cotyledons have been used up. Proteolysis precedes lipolysis in all these parts, as in some other oily seeds (SIMOLA 1973). In aged seeds of *P. sylvestris* lipolysis begins earlier than proteolysis (SIMOLA, in press). In living growing cells amino acids resulting from breakdown of storage proteins are rapidly used for the formation of new cell structures, and the fusion of small vacuoles into a large central vacuole is well documented (Figs. 7 and 11).

The spherosomes of dry seeds of *P. sylvestris* are relatively electron-translucent (Figs. 1—3), as in *Picea abies* (SIMOLA, in preparation) and *Pinus banksiana* (DURZAN et al. 1971). During germination the spherosomes become more osmiophilic, especially in the endosperm. The thin membrane surrounding the spherosomes is sometimes clearly visible (Figs. 20 and 21). This seems to correspond to the half unit-membrane seen around some spherosomes (GRIESHABER 1964, SCHWARZENBACH 1971, YATSU & JACKS 1972, SIMOLA 1973).

During early germination, however, these cells specialize structurally for quite different roles. Proplastids develop to amyloplasts in the rootlets and to chloroplasts in the cotyledons but fail to develop further in the haploid degenerating endosperm tissue. A large proportion of the storage material in roots is used for tannin synthesis.

Lipid is apparently metabolized in different ways in the embryo, where it can be reutilized within a cell, and in the endosperm, where glyoxysomes play an important role and material is transported to the embryo. The

No special structures for tannin synthesis and transport are visible, but the spherosomes become very osmiophilic and the cytoplasm contains rough ER, polysomes and some dictyosomes at the same time (Fig. 6). The same cells synthesize starch effectively, unlike the tannin-containing cells of *Picea glauca* in suspension culture (CHAFE & DURZAN 1973). In the cotyledon cells of *Picea abies* tannin seems to be attached to the spherosomes and sometimes corresponding material is seen inside the spherosome

itself. A small vacuole is usually located close to such spherosomes (SIMOLA, in preparation).

After three days' germination the root-cortex cells have used up most of their storage material and a large central vacuole is formed, but the meristematic cells contain large groups of spherosomes and the proplastids do not store any starch. Storage of starch is characteristic of the cells of both rootlet cortex and cotyledons but vacuolization is much slower in the cotyledons. The proplastids of endosperm cells do not store starch, but small plastoglobuli may be visible. Some microchemical tests indicate that starch is synthesized during the initial stages of germination in both the embryo and endosperm of *Pinus densiflora* and *P. thunbergii* (GOO & FURUSAWA 1955).

3. Development of new cell organelles, especially mitochondria, plastids and glyoxysomes

In the dormant cells no organelle initials, ER, dictyosomes or ribosomes are visible but after one day's germination these are already recognizable in root tip cells. How most of these structures are formed during germination remains obscure, because no intermediate stages could be detected in this species or in other embryos examined earlier (SRIVASTAVA & PAULSON 1968, DURZAN et al. 1971, SASAKI & BROWN 1971). Intact ribosomes present as monomer units may be isolated from dormant embryos of *Pinus resinosa,* and polysome formation begins less than 4 hours after imbibition starts (SASAKI & BROWN 1971). The young rootlet cells have well-developed mitochondria (Figs. 6—9). Their cristae are distinct, and ribosomes as well as some osmiophilic grains may be visible. In the endosperm cells the mitochondria vary in structure. Some are very electron-dense, with electron-translucent intracristal spaces (Fig. 33). A corresponding structure is characteristic of ageing cotyledon cells of *Phaseolus vulgaris* (ŎPIK 1965). Dividing mitochondria of *P. sylvestris* have numerous narrow cristae (microvilli) and some of these organelles have a cavity (Fig. 34).

After eight days' germination the number of mitochondria in the endosperm of *Pinus* is high. The oily cotyledons of *Cucurbita* and *Cucumis* (LOTT & CASTELFRANCO 1970, TRELEASE et al. 1971) also contain great numbers of these organelles. Although the ß-oxidation of fatty acids in oily seeds appears to be localized to the glyoxysomes (COOPER & BEEVERS 1969, HUTTON & STUMPF 1969, CHING 1970, TRELEASE et al. 1971), the mitochondria play an important role in the metabolism of storage lipids.

The conversion of succinate to oxaloacetate takes place in the mitochondria, as does the production of ATP, which is needed for the conversion of acetyl-CoA to sucrose (BREIDENBACH et al. 1968).

Development of a great number of glyoxysomes is characteristic of endosperm cells during effective lipolysis (8th day, Figs. 33 and 34). These structures seem to play no part in the mobilization of lipids in the embryo, in which they are seen only occasionally and have a different structure. In the endosperm of the castor bean glyoxysomes are found throughout seed development (CARPENTER & BEEVERS 1966), but in *P. sylvestris* they develop at a relatively late stage of germination.

Cytoplasmic invaginations containing ribosomes were seen in the microbodies of endosperm cells of *Pinus sylvestris*. Corresponding structures have been seen in the microbodies of *Cucumis* cotyledons during greening (TRELEASE et al. 1971). They have been interpreted as morphological manifestations of the mechanism by which the microbodies lose or gain enzymes. Some invaginations are also seen in the glyoxysomes of the megagametophyte of germinating *Pinus ponderosa* (CHING 1970) and in some epidermal cells of the spadix appendices in *Sauromatum* during flowering (BERGER & SCHNEPF 1970). In *Cucumis* the frequency of invaginations correlates strikingly with increases in the activity of glycolate oxidase (TRELEASE et al. 1971). The glyoxysomes in the endosperm of *Pinus ponderosa* contain both DNA and RNA and are capable of protein synthesis (CHING 1970). The glyoxysomes of castor bean endosperm cells also contain RNA (GERHARDT & BEEVERS 1969). Cytoplasmic DNA different from mitochondrial DNA has also been found in the microsomes of mouse liver (BOND et al. 1969).

4. Concluding remarks

The cells of the different parts of the dry seeds of *P. sylvestris* have the same ultrastructural components and nothing points to their forthcoming physiological role in the development of the young seedling. endosperm is not a mere degenerating storage tissue, however, but its cells undergo remarkable structural changes which may be prerequisite for effective and balanced transport of organic substances to the developing embryo. That the haploid endosperm cells are able to form all the structural components of a normal plant cell suggest that it may be possible to induce successful differentiation of shoots and roots from cultured endosperm cells of *Pinus sylvestris*.

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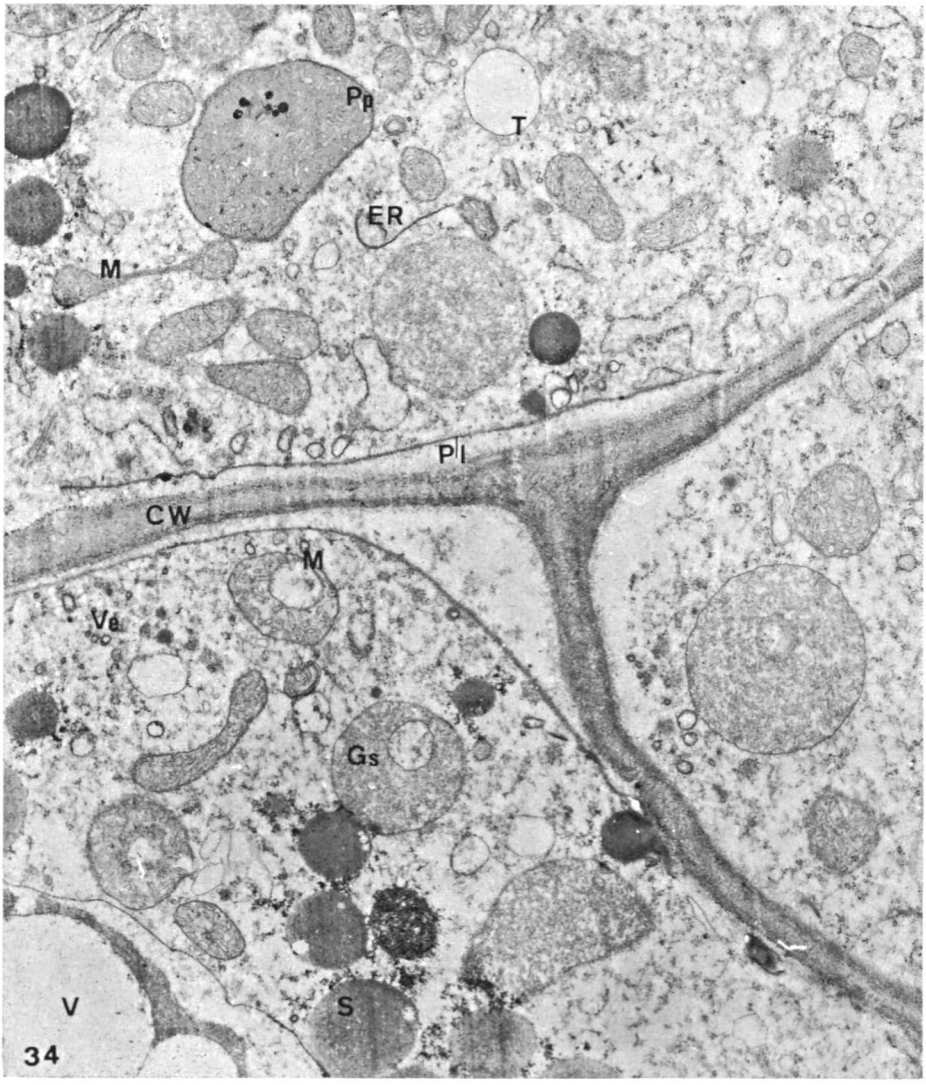

FIG. 34. Glyoxysomes (Gs) some of them with a cavity. Spherosomes (S) with small electron-translucent cavities. Cell wall (CW). Plasmalemma (Pl) disrupting. Vesicles (Ve). 13 000 x.

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