Acta Bot. Neerl. 25(2), April 1976, p. 129–148.

THE ULTRASTRUCTURE OF PRIMARY TRACHEARY ELEMENTS CONTAINING LARGE AMOUNTS OF STORAGE MATERIAL IN THE HYPOCOTYL OF THE CUCUMBER

LEENTJE GOOSEN-DE ROO and MELISA C. POLSBROEK

Botanisch Laboratorium, Rijksuniversiteit, Leiden.

SUMMARY

Tracheary elements with well-developed cell wall thickenings and containing large amounts of storage material were occasionally found in hypocotyls of the cucumber of certain ages. The storage material, consisting of protein bodies, lipid bodies, and starch grains, was closely packed. The few cell organelles present were disintegrated.

The ultrastructure of the aberrant tracheary elements and procambium elements likely to differentiate into them is described.

The storage material must be regarded as newly formed from material dissolved in neighbouring cells. It is postulated that activities necessary for differentiation suppress the deposition of storage material while the capacity of the cell to store material remains for some time after the termination of the dormancy period of the seed. The new formation of storage material would occur in procambium elements in which differentiation related processes were initiated before the onset of dormancy.

1. INTRODUCTION

During earlier studies (GOOSEN-DE ROO 1973a and 1973b) concerning the role of cell organelles in the formation of cell wall thickenings in primary tracheary elements in the hypocotyl of the cucumber (*Cucumis* 'Sporu-Origineel'), tracheary elements containing large amounts of storage material were occasionally found. The storage material consisted of protein bodies, lipid bodies and starch grains. These materials and the few cell organelles present were usually closely packed in the tracheary elements. The formation of cell wall thickenings seemed to be completed in these elements; that is, they exhibited well-developed thickenings in the shape of rings or spirals.

Tracheary elements do not normally contain much storage material during differentiation. The various kinds of storage material present at an early stage of development are broken down shortly after imbibition of water by the seed and during germination, considering germination as defined by MAYER & POLJAKOFF-MAYBER (1963). Forty-eight hours after the beginning of imbibition, most of the developing tracheary elements contain only some remaining lipid bodies. At the end of differentiation, the protoplast of the tracheary elements disintegrates and the mature elements appear empty under the electron microscope. Obviously, the tracheary elements that have well-developed cell wall thickenings and much storage material as well as some cell organelles do not fit in with this picture.

In this paper, therefore, the ultrastructure of such exceptional tracheary elements is described. Furthermore, there is a discussion on the time of appearance and disappearance of the contents of these tracheary elements in relation to the development of the seedling and the differentiation of the elements concerned.

2. MATERIAL AND METHODS

2.1 Culture of the plants

Seeds of the cucumber (*Cucumis* 'Sporu-Origineel' from de Ruyter and Son; Bleiswijk, Holland) were germinated on moistened filter paper in a controlled culture room. Details of the culturing technique have been described previously (GOOSEN-DE ROO 1973a). Germinating seeds and young seedlings were collected after germination periods of 2, 6, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours. Seedlings of 120, 144, and 168 hours of age had been transferred from the petri dishes to plastic cups at an age of 96 hours. The cups contained gravel and were provided with 100 ml. Hoagland solution every 12 hours. Material from seeds that were soaked for 10 minutes in distilled water was collected as well.

2.2 Light microscopy

Hypocotyls and cotyledons were used for light- and electron microscopical examination. Hypocotyl pieces were cut from the young seedling as described earlier (GOOSEN-DE ROO 1973a). Each hypocotyl piece contained one large vascular bundle and sometimes also a small one. Only the large vascular bundle was examined. Cells of the mesophyll from the middle of the cotyledons were studied. Sections of 1–2 micrometers thickness cut from the material embedded for electron microscopy were used for light microscopical examination. Transverse and radial sections were cut from hypocotyl pieces. Only transverse sections were cut from the cotyledons. The sections were stained with a mixture of 1% toluidine blue and 1% borax in a ratio 1:1, pH 8.6–8.8, at a temperature of 50°C for about 45 seconds.

2.3 Electron microscopy

Hypocotyl pieces were fixed in glutaraldehyde diluted in a sodium cacodylate buffer followed by a post fixation with osmium tetroxide in a sodium veronal buffer or in a sodium cacodylate buffer. Several fixation variations designated 1a, 1b and 2 were used (GOOSEN-DE ROO 1973a). Two different "staining" combinations were used for the ultrathin sections: uranyl acetate followed by lead citrate according to REYNOLDS (1963) or according to VENABLE & COGGESHALL (1965), abbreviated U/R or U/V. The method of fixation and "staining" applied to the material shown in the various photographs is mentioned in the legends of the figures.

Pieces of the cotyledons were fixed only according to method 2. The cotyledon pieces used were taken from seedlings of 36, 48, 72, and 96 hours of age.

3. RESULTS

3.1 General remarks

Tracheary elements with well-developed cell wall thickenings and filled with storage material were found in hypocotyls of 36, 48, 72, and 96 hours of age (*figs. 1* and *2*). The incidence of hypocotyl pieces containing these tracheary elements is given in *table 1*. The aberrant tracheary elements were observed in 25% of the total number of hypocotyls from seedlings of 36 hours of age. For hypocotyls of 48, 72, and 96 hours of age, these percentages were 23, 6, and 3, respectively. Storage material was abundant in all cells in hypocotyls of seedlings younger than 24 hours, the nature and quantity of the storage material differing in different cell types. In such seedlings, however, there appeared to be no tracheary elements with cell wall thickenings. Tracheary elements with early stages of cell wall thickenings but without an abundance of storage material were occasionally found in hypocotyls from seedlings 24 hours of age. In hypocotyls of seedlings older than 96 hours, tracheary elements with cell wall thickenings but lacking storage material were present.

Table 1.
Relationship between the incidence of hypocotyl pieces with tracheary elements filled with storage material and the age of the imbibed seed or of the seedling. Incidence expressed as percentage of the number of hypocotyl pieces investigated.

Age in hours	Number of hypocotyl pieces investigated	Incidence of hypocotyl pieces with tracheary elements with well-developed cell wall thickenings and filled with storage material; incidence expressed as percentage of the number of hypocotyl pieces investigated
0 (10 minutes soaked)	1	0
2	1	0
6	2	0
12	13	0
24	14	0
36	4	25
48	31	23
72	16	6
96	36	3
120	10	0
144	3	0
168	2	0

The tracheary elements filled with storage material were more or less elongated cells with pointed ends (spindle-shaped) (*fig. 1*). These elements had sometimes divided transversely into two cells. The transverse walls of the elements had a single perforation.

The length of the elements as measured from end point to end point was between 150 and 200 μm. The distance from an end point to the nearest transverse wall was about 80–90 μm. These measurements were derived from medianly sectioned tracheary elements.

The tracheary elements filled with storage material had diameters between 5.2 and 11.7 μm with an average of 8.5 μm (standard deviation 2.20; number of measurements 30). Tracheary elements with diameters up to 20 μm could be found in hypocotyl pieces from seedlings of 72 hours and older. In these pieces, the tracheary elements filled with storage material were present in the innermost part of the xylem area in the vascular bundle (*fig. 3*). These elements were also bordered by some intraxylary phloem.

In the vascular bundles containing the tracheary elements filled with storage material as well as wider tracheary elements (diameters between 10 and 20 μm), the formation of cell wall thickenings in the wider elements had apparently just started or was still in progress (see also GOOSEN-DE ROO 1973a and 1973b). The shape of the wider elements varied. Some of them were similar to the tracheary elements filled with storage material; they were spindle-shaped and had transverse, perforated walls. Most of the tracheary elements were cylindrical-shaped, however. The widest elements particularly were cylindrical-shaped. These elements had a single perforation in the transverse walls when mature.

3.2 Electron microscopy: ultrastructure of the tracheary elements filled with storage material

3.2.1 Storage material

Protein bodies in our material were almost black (electron-dense) on the photographs (*fig. 4*). The electron density was not evenly distributed over the body. There were sometimes one or more sites that were more electron-transparent than was the matrix of the protein body. These sites are called soft globoids (LOTT, LARSEN & DARLEY 1971). Globoid crystals were sometimes present within the soft globoid. These globoid crystals cannot be impregnated very wel by Epon. During ultrathin sectioning of the material, the globoid crystals are extruded from the ultrathin sections (LOTT, LARSEN & DARLEY 1971). In the electron micrographs, therefore, only the electron-transparent site where the globoid crystal was present within the soft globoid area can be observed (*figs. 4* and *5*). It depends on the plane of sectioning whether these structures can be observed in the protein bodies. A protein crystalloid has been described by LOTT & VOLLMER (1973) for protein bodies in *Cucurbita maxima* cotyledon cells. There was, however, no protein crystalloid in the present material, which may be due to the difference in preparation technique (fixation against freeze-etching).

The shape of the protein bodies in the tracheary elements filled with storage material was circular to oval in the sections, presumably dependent on the plane of sectioning. Occasionally, the shape was more irregular or even polygonal, probably due to surrounding lipid bodies.

It is difficult to decide from our electron micrographs whether a surrounding membrane is present on the protein bodies. The periphery of the protein bodies was sometimes occupied by ribosomes.

No vacuoles in which protein bodies were broken down were observed in the tracheary elements filled with storage material (GUILLIERMOND 1921, LOTT & VOLLMER 1973). This was in contrast to parenchyma cells of the hypocotyl (*figs. 1* and *6*) and to mesophyll cells of the cotyledons from seedlings of the same age, in which the breakdown of protein bodies could be clearly observed.

The length (i.e., the greatest distance between two points on the circumference of the protein body section) of the protein bodies varied from 0.3 to 4.7 μm, with an average of 1.7 μm (standard deviation 0.99; number of measurements 47).

Considering the morphology of protein bodies from electron micrographs in the literature, they have also been listed under other names for instance: protein grain, aleurone grain and storage protein.

More or less electron-transparent lipid bodies were present in our material. On the electron micrographs, their "colour" varied from white to dark-grey. Sometimes the lipid bodies contained one or more complete electron-transparent spherical or oval sites (*fig. 4*).These sites were also found by WERKER & VAUGHAN (1974) in comparable structures (spherosomes) in cells of the embryo of *Sinapis alba*.

The lipid bodies were surrounded by a clearly distinguishable membrane (*fig. 7*).

The shape of the lipid bodies was mostly circular, sometimes oval or irregular (probably resulting from being closely packed). In some tracheary elements filled with storage material, large masses of lipid material were present, presumably resulting from fusion of lipid bodies (*fig. 4*).

The length of the lipid bodies varied from 0.1 to 1.9 μm, with an average of 0.8 μm (standard deviation 0.43; number of measurements 226).

Judging from the electron micrographs in the literature, the above-mentioned lipid bodies have also been referred to as lipid droplet, lipid vesicle, lipid vacuole, oil body, spherosome and spherosome-like organelle.

In our material, more or less electron-transparent starch grains were found. On electron micrographs, their "colour" varied from white to dark-grey, like the "colour" of the lipid bodies. However, the electron density is not as evenly distributed over the starch grains as it is in the lipid bodies (*fig. 5*).

In the tracheary elements filled with storage material, the starch grains were rarely present in plastids. They appeared to be free in the cytoplasm. In that case, membranes could not be observed around the starch grains.

The shape of the starch grains varied from spherical or oval to irregular. In some cases, polygonal-shaped starch grains were found; they were present

in clusters. The shape of the starch grains was generally more irregular than that of the lipid bodies.

The length of the starch grains varied from 0.4 to 2.6 μm, with an average of 0.9 μm (standard deviation 0.41; number of measurements 61).

3.2.2 Cell organelles

The storage material in the tracheary elements concerned was densely packed. The remaining space was filled with cell organelles. In storage-material-filled tracheary elements, free ribosomes (seldom polyribosomes), vesicles, mitochondria, plastids, microbodies and some vacuoles were observed. Some of these organelles are shown in *figure 5*. A structure resembling a dictyosome with vesicles in the vicinity was once observed (*fig. 5*). Endoplasmic reticulum was not observed with certainty. Nuclei were not observed.

The various cell organelles mentioned above (with the exception of ribosomes) seemed to be disintegrated. The organelles were reminiscent to those observed in our former studies in the disintegrating protoplasts of tracheary elements during the final stages of the formation of cell wall thickenings.

Lipid bodies sometimes seemed to be partly fused with vacuoles (*fig. 8*).

3.2.3 The cell wall

The cell wall thickening in the tracheary elements filled with storage material was in the shape of rings or a spiral. The area of transversely cut cell wall thickenings varied from 0.75 to 2.75 μm^2.

When two tracheary elements filled with storage material lie side-by-side, the primary cell wall between the cell wall thickenings was mostly broken down, except that there was still some fibrillar material between the cell wall thickenings (*fig. 9*). When two end points of the tracheary elements filled with storage material lie more or less end-to-end, the primary cell wall of the elements was broken down where the end points join each other. The breakdown of the primary cell wall between the cell wall thickenings is not specific for the tracheary elements filled with storage material, but generally occurs in differentiated tracheary elements that lie side-by-side (O'Brien & Thimann 1967).

3.3 Electron microscopy: ultrastructure of procambium elements that might represent a previous stage of tracheary elements filled with storage material

In order to study the ultrastructure of procambium elements that are probably differentiating into tracheary elements filled with storage material, hypocotyl pieces from seedlings younger than 24 hours were investigated. The ultrastructure of procambium elements in hypocotyl pieces from seedlings 24 hours old was not studied, because we occasionally found tracheary elements with developing cell wall thickenings in these pieces. The hypocotyl pieces from seedling younger than 24 hours were obtained from a seed that had been soaked for 10 minutes in distilled water and from young seedlings 2, 6, and 12 hours of age. The electron micrographs did not reveal major differences

among the hypocotyls of these ages. The results given below were obtained from 8 hypocotyl pieces from seedlings of 12 hours of age which were studied in more detail.

The position of the procambium elements in the vascular bundle was the only basis for the assumption that they might be destined to develop into tracheary elements filled with storage material. These procambium elements had diameters between 3.3 and 10.0 μm, with an average of 6.6 μm (standard deviation 1.75; number of measurements 28).

3.3.1 Storage material

The procambium elements always contained protein and lipid bodies (*fig. 10*), whereas starch grains were very rarely found. The protein and lipid bodies were clearly smaller than those in the tracheary elements filled with storage material (*figs. 11* and *12*; Mann-Whitney U test, SIEGEL 1956; $p < 0.001$ for the protein bodies as well as for the lipid bodies). The length of the protein bodies in the procambium elements varied from 0.1 to 4.0 μm, with an average of 0.9 μm (standard deviation 0.80; number of measurements 37). The length of the lipid bodies in the procambium elements varied from 0.2 to 0.9 μm, with an average of 0.5 μm (standard deviation 0.16; number of measurements 41). The length of the protein bodies in the tracheary elements filled with storage material shown in *fig. 11* was reported in part 3.2.1. The length of the lipid bodies in the tracheary elements filled with storage material used for *fig. 12* represented a random selection from the 226 measurements mentioned in part 3.2.1. Fifty-seven measurements were selected, varying from 0.1 to 1.9 μm, with an average of 0.8 μm (standard deviation 0.37).

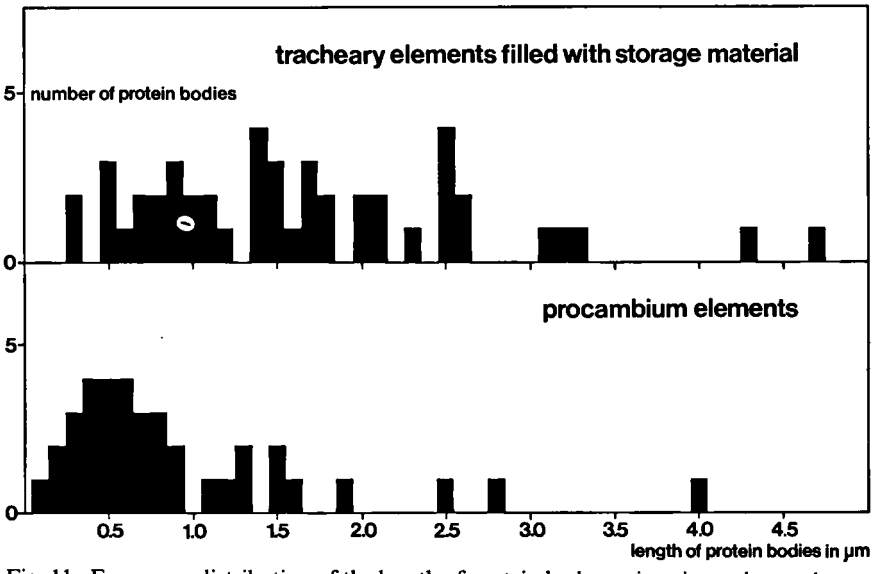


Fig. 11. Frequency distribution of the length of protein body sections in tracheary elements filled with storage material and in procambium elements.

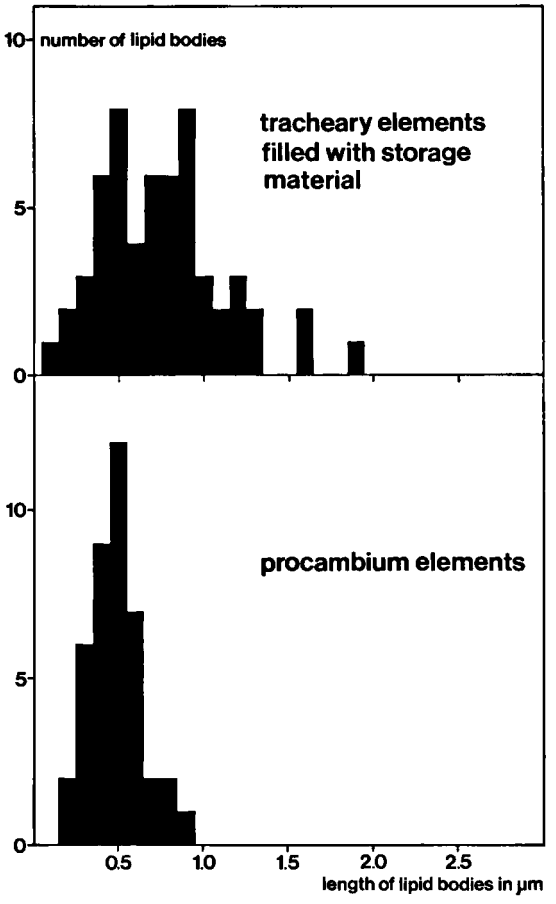


Fig. 12. Frequency distribution of the length of lipid body sections in tracheary elements filled with storage material and in procambium elements.

The electron micrographs suggested that the protein bodies split into fragments (*fig. 13*). The lipid bodies did not contain electron-transparent circular or oval sites.

3.3.2 Cell organelles

In the procambium elements probably differentiating into tracheary elements filled with storage material, the following cell organelles and structures were found: free ribosomes and polyribosomes, dictyosomes (without vesicles attached to them or in their neighbourhood), endoplasmic reticulum (smooth and rough), mitochondria, plastids, peripheral microtubuli, microfilaments, small vacuoles, ring-shaped membrane structures (*fig. 14*) and nuclei. The ring-shaped membrane structure consisted of circlets or crescents of smooth membranes resembling smooth endoplasmic reticulum. The membrane-surrounded ribosomes were sometimes densely packed. The diameter of the structure varied between 0.2 and 0.5 µm.

3.3.3 The cell wall

The cell wall in the procambium elements had a rather loose fibrillar appearance and there was no cell wall thickening.

4. DISCUSSION

The most conspicuous aspect of the tracheary elements filled with storage material was the presence of the storage material, which was partly or wholly digested in all other cells of the young seedling. Storage material was present in the cells of the embryo in an intact condition during the dormancy of the seed and during approximately the first 24 hours of the germination processes. Dormancy refers to an imposed dormancy or "quiescence" (WAREING 1969). The first indications of the degradation of storage material could be observed after 24 hours. The protein bodies disintegrated first, the lipid bodies and starch grains later on. At 72 hours of age, storage material had disappeared in all cells except in a few tracheary elements in which the cell wall thickenings were fully developed.

In the following discussion we have attempted to find the simplest explanation for the origin of the storage material in the tracheary elements concerned, on the basis of the present observations.

The origin of storage material in the tracheary elements concerned could be explained in two different ways:

1. The storage material remained from the dormancy period of the seed, but it was not yet broken down.
2. The storage material from the dormancy period was broken down as it was in the other cells of the young seedling, but new storage material was afterwards formed in some of the tracheary elements.

In either explanation, three possibilities concerning the point of time of the formation of cell wall thickenings in the tracheary elements can be postulated:

1a, 2a. The formation of cell wall thickenings took place before the onset of dormancy. Mature vascular elements have been found in embryos before the germination of the seed (ESAU 1965). Cellular processes which normally take place during seed germination have actually been described as occuring before dormancy by MOLLENHAUER & TOTTEN (1971).

1b, 2b. The formation of cell wall thickenings had begun before dormancy and therefore, occurred partly before and partly after dormancy.

1c, 2c. The formation of cell wall thickenings took place after the termination of dormancy.

Possibilities 1a, 1b, and 1c must be rejected because tracheary elements with well-developed or partly developed cell wall thickenings and containing large amounts of storage material were not found in hypocotyls of 0, 2, 6, 12, and 24 hours of age. The presence of the aberrant tracheary elements in hypocotyl pieces of various ages seems to follow an optimum distribution (see *table 1*), reaching its peak of 23–25% at an age of about 36 to 48 hours. Tracheary elements filled with storage material were not observed in seedlings older than

96 hours. With the expectation that the probability of the aberrant tracheary elements is 23–25% at an age of less than 36 hours, 7 of the 31 pieces of ages younger than 36 hours should have contained tracheary elements with cell wall thickenings as well as abundant storage material. The observed frequency, however, was zero (chi-square test; $p < 0.02$). Tracheary elements with beginning cell wall thickenings were occasionally found in hypocotyl pieces from seedlings of 24 hours of age, but these elements contained very little storage material, if any.

Possibility 2a must also be rejected, because tracheary elements with well-developed cell wall thickenings and without storage material were not found in hypocotyls of 0, 2, 6, 12, and 24 hours of age.

Possibility 2b is not very likely. Tracheary elements with partly developed cell wall thickenings and without large amounts of storage material were occasionally found in hypocotyls from seedlings 24 hours of age and not in younger material.

Therefore, possibility 2c seems to offer the most probable explanation for the aberrant tracheary elements. The course of events would be: termination of the imposed dormancy, breakdown of storage material, formation of cell wall thickenings, formation of new storage material.

Now the question remains as to why storage material would be deposited in a few isolated elements at the same time when storage material is broken down in all other cells. This could be answered by the following hypothesis concerning the regulation of the onset and termination of dormancy. Through some unknown mechanism, the deposition of storage material takes place before the dehydration of the cells in the embryo begins. The accumulation of storage material might be a long-lasting process, but only the deposition of storage material shortly before the onset of dormancy is considered here. Dehydration causes reversible morphological changes in cell organelles (HALLAM 1972, MARINOS & FIFE 1972) stopping the ongoing cellular activities. Dormancy would be terminated after the cells imbibe water and again become active. At this point, synthesis of various cell organelles takes place in most cells while storage material is broken down. In each cell the differentiation related activities are resumed at whatever its stage of development was at the time dehydration sets in. Furthermore, it is postulated that cellular activities necessary for differentiation are largely incompatible with the deposition of storage material; i.e. deposition is somehow suppressed by activities related to differentiation. The capacity of the cell to deposit storage material, however, is present for a certain time after imbibition of water.

The occurrence of the aberrant tracheary elements can now be explained by assuming that procambium elements differentiating into the aberrant tracheary elements were slightly ahead in their differentiation with respect to the other procambium elements already before the onset of dormancy. This assumption is in agreement with the fact that the aberrant tracheary elements are generally the oldest protoxylem elements. In the procambium elements concerned, synthesis of cell organelles necessary for the formation of cell wall thickenings

was already underway before the deposition of storage material was initiated. As a consequence of the ongoing cellular activities, these elements had deposited less storage material than had other elements when dehydration sets in. After dormancy, the small amount of storage material is broken down quickly and the formation of cell wall thickenings can be started at once, due to the relatively large number of well-developed cell organelles. In these procambium elements, the formation of cell wall thickenings was completed even before the cell's capacity to deposit storage material had disappeared. Therefore, the suppression of deposition of storage material is terminated when the formation of cell wall thickenings is completed. Naturally, storage material is deposited before the protoplasts of the tracheary elements disintegrate. The occurrence of tracheary elements with early stages of cell wall thickenings but without an abundance of storage material, as occasionally found in hypocotyls from 24-hours-old seedlings, could be explained as elements in which the formation of new storage material has not yet taken place.

According to this explanation, the material deposited in the aberrant tracheary elements probably originates from other cells. This implies that, for instance, in parenchyma cells of the hypocotyl and in mesophyll cells of the cotyledons, the breakdown of storage material would be more rapid than the use of the dissolved material by the same cells. The formation of new cells leads to a rapid increase in the demands of the young seedling. Therefore, any amount of storage material that seems quite abundant at an early stage may be barely sufficient at a later stage. A rapid breakdown of material results in an ample supply of material for newly-formed cells or for cells that happen to be poor in storage material. It has been postulated that the capacity to deposit storage material remains present to a certain degree some time after imbibition. This might be functionally important in regulating the concentration of the dissolved storage material.

ACKNOWLEDGEMENTS

The authors thank Prof. Dr. W. K. H. Karstens for his critical reading of the manuscript and Miss Pauline van Spronsen for technical assistance.

REFERENCES

ESAU, K. (1965): *Vascular differentiation in plants*. Holt, Rinehart and Winston. New York, Chicago, San Francisco, Toronto, London.

GOOSEN-DE ROO, L. (1973a): The relationship between cell organelles and cell wall thickenings in primary tracheary elements of the cucumber. I. Morphological aspects. *Acta Bot. Neerl.* **22**: 279–300.

— (1973b): The relationship between cell organelles and cell wall thickenings in primary tracheary elements of the cucumber. II. Quantitative aspects. *Acta Bot. Neerl.* **22**: 301–320.

GUILLIERMOND, A. (1921): Origine et évolution des vacuoles dans les cellules végétales et grains d'aleurone. *C. R. Soc. Biol.* **85**: 1033–1036.

HALLAM, N. D. (1972): Embryogenesis and germination in rye (Secale cereale L.). I. Fine structure of the developing embryo. *Planta* **104**: 157–166.

LOTT, J. N. A., P. L. LARSEN & J. J. DARLEY (1971): Protein bodies from the cotyledons of Cucurbita maxima. *Can. J. Bot.* **49**: 1777–1782.
— & C. M. VOLLMER (1973): Changes in the cotyledons of Cucurbita maxima during germination. IV. Protein bodies. *Protoplasma* **78**: 255–271.
MARINOS, N. G. & D. N. FIFE (1972): Ultrastructural changes in wheat embryos during a "presowing drought hardening" treatment. *Protoplasma* **74**: 381–396.
MAYER, A. M. & A. POLJAKOFF-MAYBER (1963): *The germination of seeds*. Pergamon Press. Oxford. London. New York. Paris.
MOLLENHAUER, H. H. & C. TOTTEN (1971): Studies on seeds. II. Origin and degradation of lipid vesicles in pea and bean cotyledons. *J. Cell Biol.* **48**: 395–405.
O'BRIEN, T. P. & K. V. THIMANN (1967): Observations on the fine structure of the oat coleoptile. III. Correlated light and electron microscopy of the vascular tissues. *Protoplasma* **63**: 443–478.
REYNOLDS, E.S. (1963): The use of lead citrate at high pH as an electron opaque stain for electron microscopy. *J. Cell Biol.* **17**: 208–212.
SIEGEL, S. (1956): *Nonparametric statistics for the behavioral sciences*. McGraw-Hill Book Company Inc., New York.
VENABLE, J. H. & R. COGGESHALL (1965): A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**: 407–408.
WAREING, P. F. (1969): Germination and dormancy. In: *Physiology of plant growth and development*. Ed. M. B. WILKINS. McGraw-Hill. London.
WERKER, E. & J. G. VAUGHAN (1974): Anatomical and ultrastructural changes in aleurone and myrosin cells of Sinapis alba during germination. *Planta* **116**: 243–255.

List of abbreviations used in the photographs

Fi	fibrillar material
G?	structure resembling a Golgi body
GC	globoid crystal
Li	lipid body
M	mitochondrion
N	nucleus
PB	protein body
PCW	primary cell wall
R	ribosome
Rs	ring-shaped membrane structure
S	starch grain
SG	soft globoid
T	cell wall thickening
Tr	tracheary element with well-developed cell wall thickenings and filled with storage material
Va	vacuole

Legends for photographs

Unless otherwise stated, the bar represents 1 μm.

Fig. 1. Longitudinal section through a hypocotyl 48 hours of age. Three tracheary elements are visible; two of them are filled with storage material. Note that the protein bodies in the adjacent parenchyma cells are nearly broken down; only remnants of protein bodies are visible in vacuoles. 1a; U/R.

Fig. 2. Transverse section through a hypocotyl 48 hours of age, showing three tracheary elements, two of which are filled with storage material. 2; U/R.

Fig. 3. A light microscopic view of a part of a transversely cut hypocotyl with a vascular bundle (72 hours of age). The arrow indicates the position of tracheary elements filled with storage material. 2; Toluidine blue.

Fig. 4. Detail of the contents of a tracheary element filled with storage material. In one protein body, a soft globoid area and the site of a globoid crystal are visible. Note the electron-transparent sites in the lipid bodies and the fusion of lipid bodies. 48 Hours of age. 1a; U/R.

Fig. 5. Detail of the contents of a tracheary element filled with storage material. Note the difference in structure of lipid bodies and starch grains. Various disintegrated cell organelles can be observed. 48 Hours of age. 1a; U/V.

Fig. 6. The breakdown of protein bodies in parenchyma cells of the hypocotyl, resulting in the appearance of vacuoles. 48 Hours of age. 2; U/R.

Fig. 7. Lipid bodies. An arrow indicates the membrane around a lipid body. The black spots on the lipid bodies represent contamination from "staining" on the ultrathin section. 48 Hours of age. 1a; U/R.

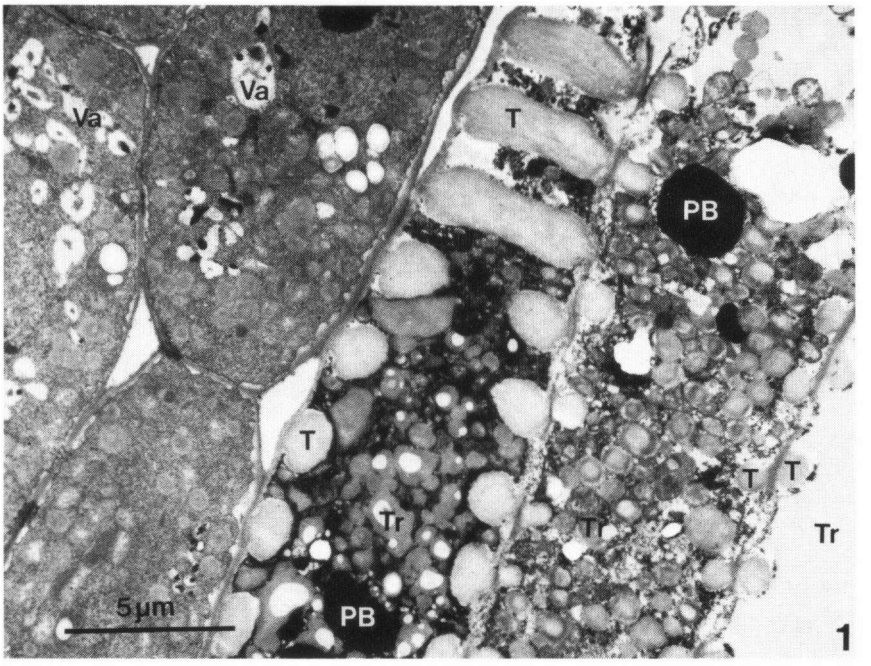
Fig. 8. Lipid bodies and a vacuole which seemed to be partially fused. 48 Hours of age. 2; U/R.

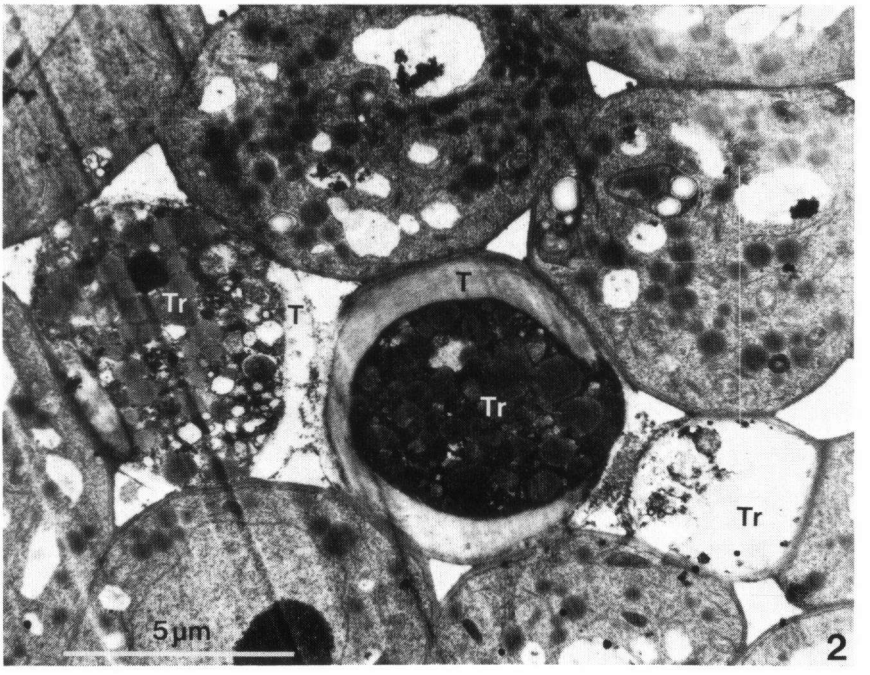
Fig. 9. Detail of two neighbouring tracheary elements filled with storage material. The left element is sectioned tangentially, the right element is sectioned medianly. The primary cell wall between the cell wall thickenings is broken down: note the remaining fibrillar material (arrow). 48 Hours of age. 1a; U/R.

Fig. 10. Contents of a procambium element 12 hours of age. 1a; U/R.

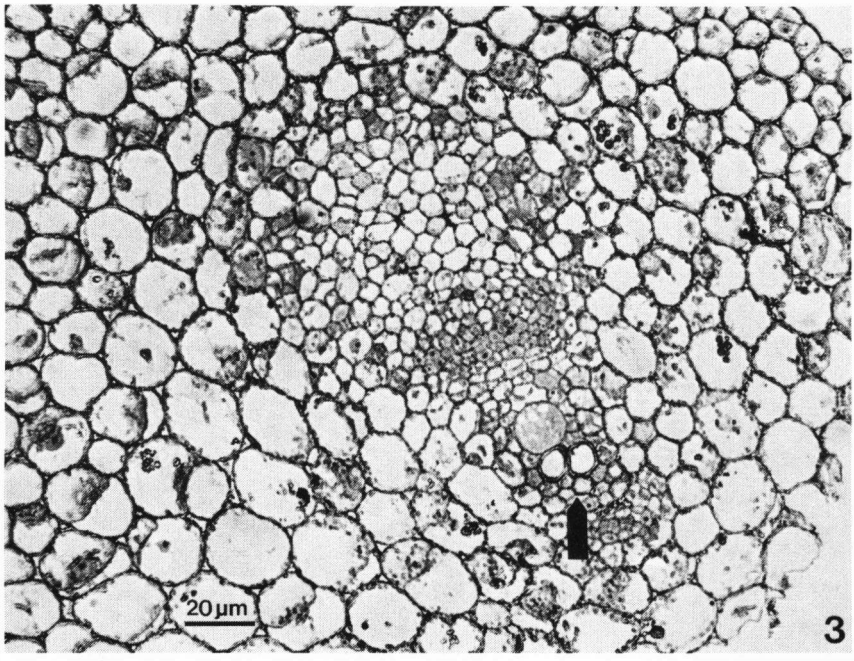
Fig 13. Part of a procambium element. Note the shape of the protein bodies. 12 Hours of age. 1a; U/R.

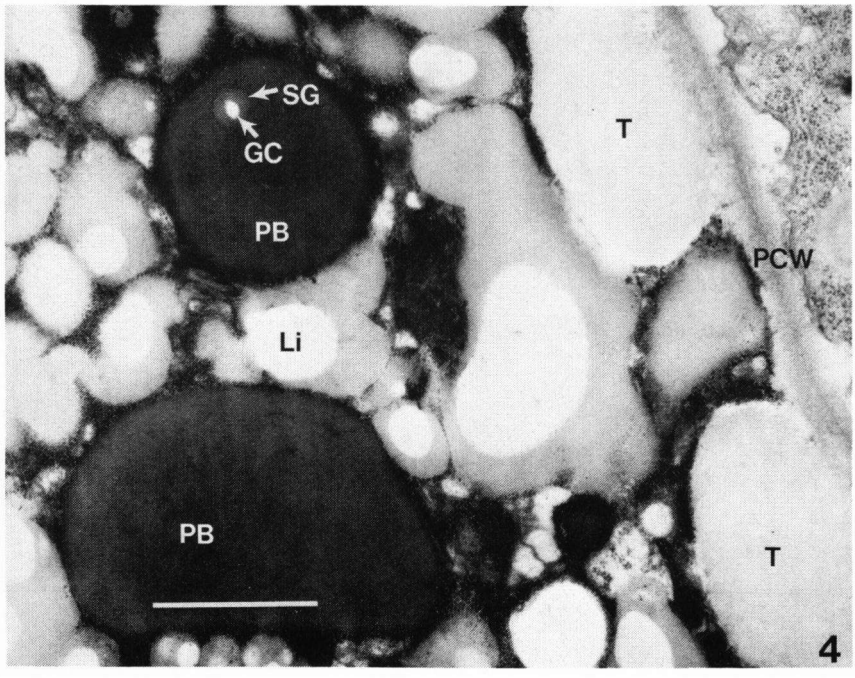
Fig. 14. Detail of the contents of a procambium element 12 hours of age. 1a; U/R.



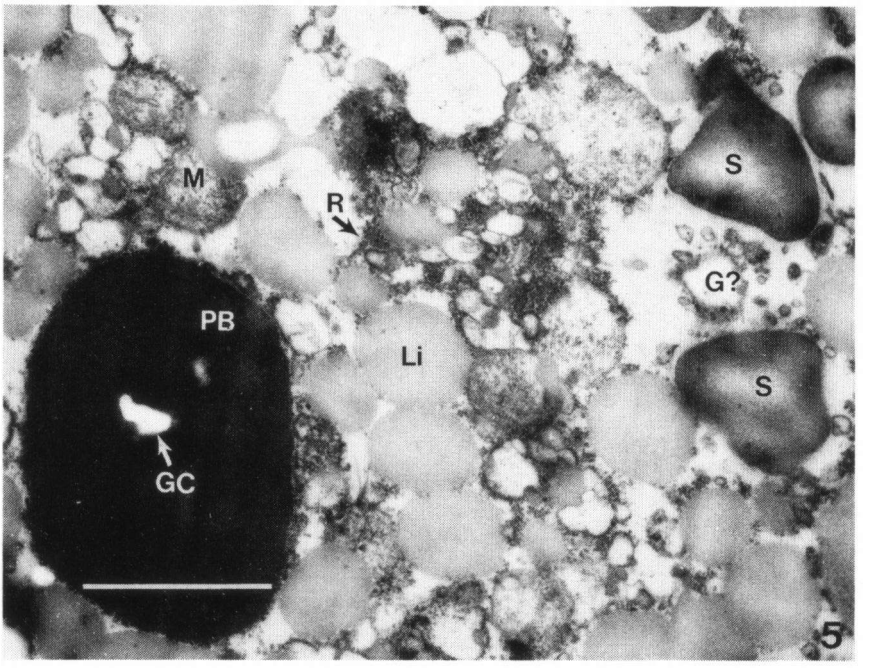


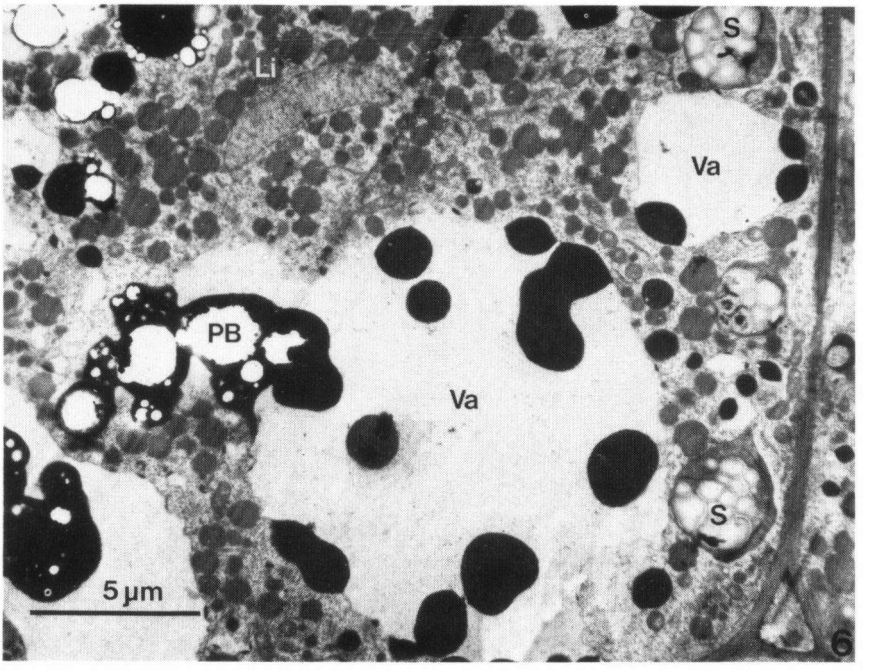
Figs. 1 and 2.



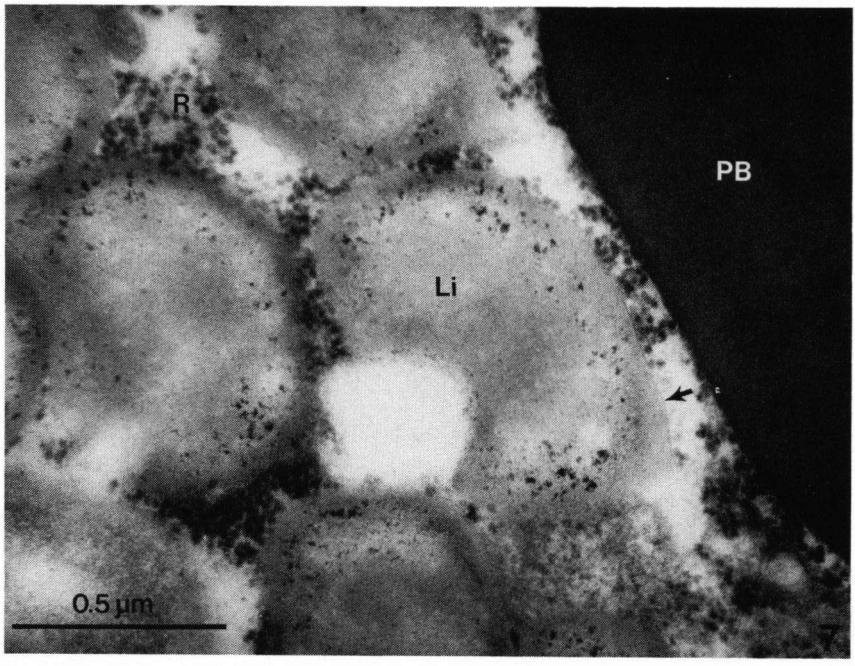


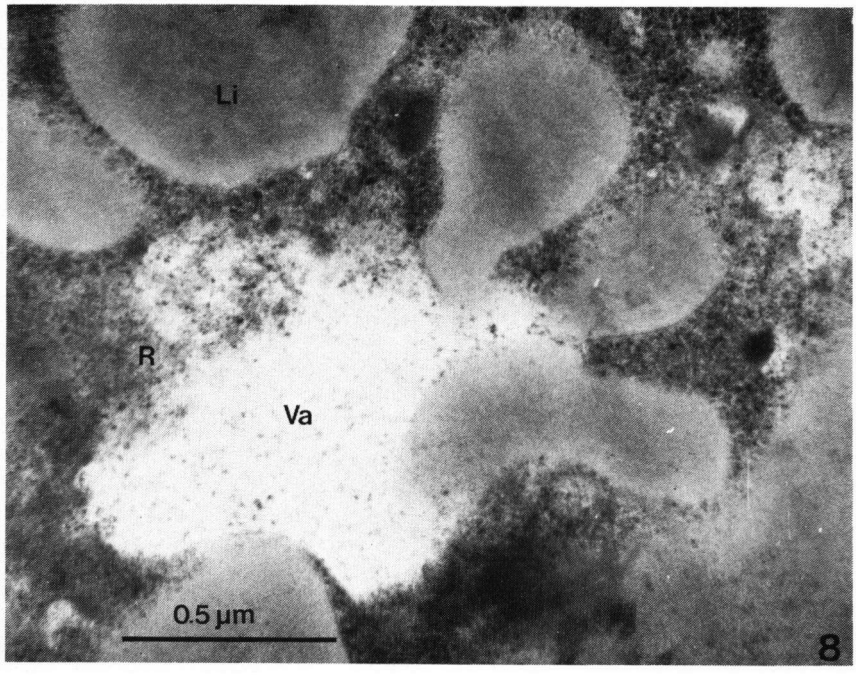
Figs. 3 and 4.



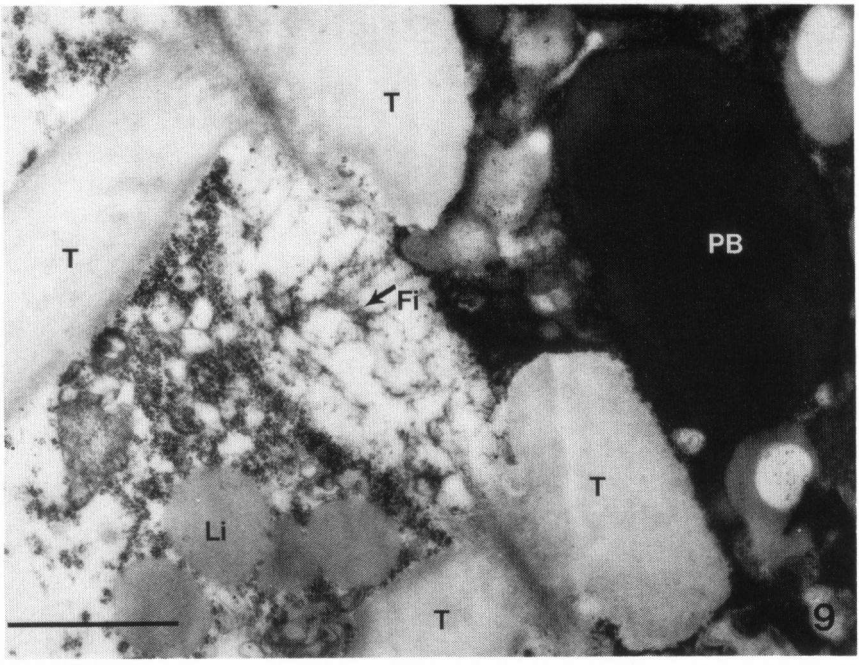


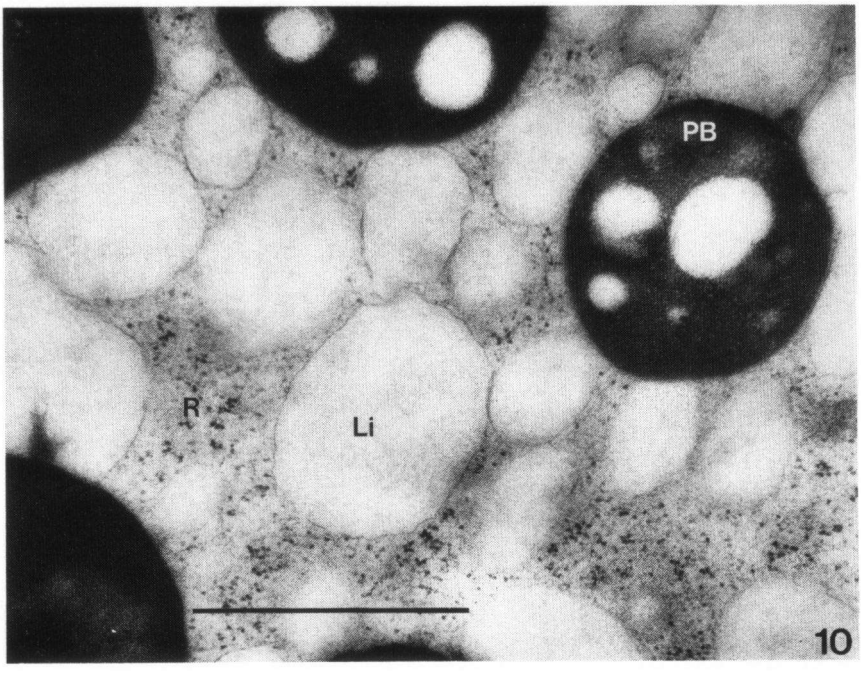
Figs. 5 and 6.



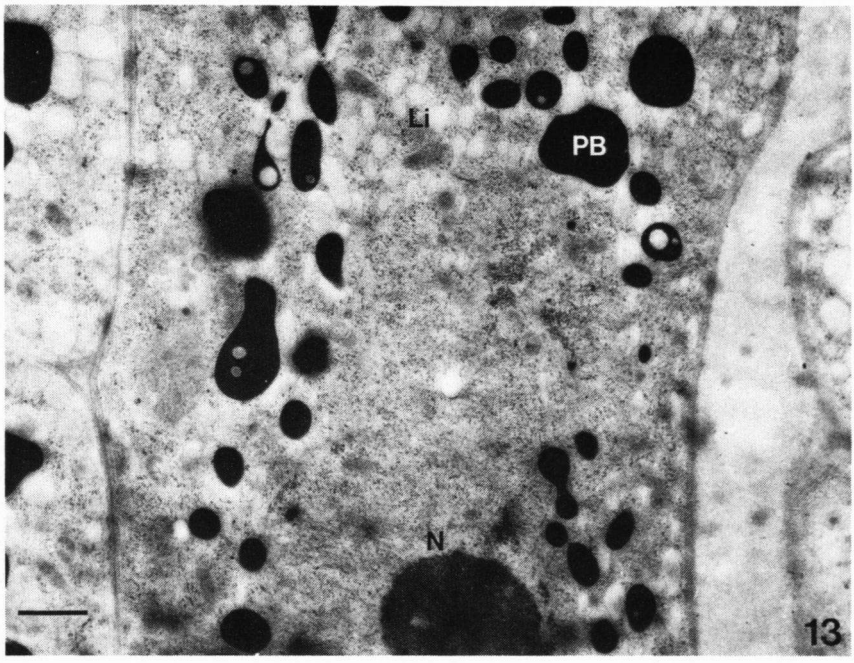


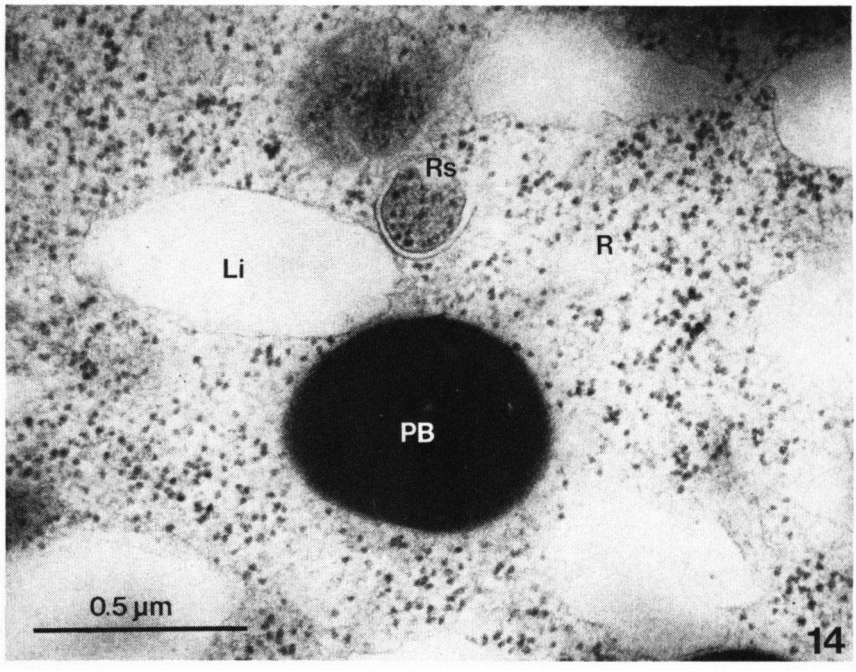
Figs. 7 and 8.





Figs. 9 and 10.





Figs. 13 and 14.