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The Morphology, Occurrence, and Distribution of Dilated Cisternae of the Endoplasmic Reticulum in Tissues of Plants of the *Cruciferae*

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With 7 Figures

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Summary

Morphology, occurrence, and distribution of dilated cisternae of the endoplasmic reticulum (ER) were studied by electron microscopy. The cisternae which contained an electron-dense matrix were intimately associated with the granular ER membranes appearing as tubular necks at the edges of the ER profiles. After budding off from the ER the cisternae still had ribosomes attached to the outside of the bounding membranes. The accumulations were variable in shape, being 0.4 to 1.5 μ in width and 4 to 5 μ in length.

The cisternae were found to be unique for plants of the *Cruciferae* and could not be observed in species from related families such as *Papaveraceae* and *Resedaceae*.

The dilated cisternae were a common component of the cytoplasm in root tips, stems, and leaves. In meristematic cells the number of accumulations was small but increased in older differentiating cells of the root cap. The similarity to "microbodies" described by previous authors from other plants is discussed.

1. Introduction

The term "microbody" as used by Mollenhauer, Morré, and Kelley (1966) and Frederick, Newcomb, Vigil, and Wergin (1968) is restricted to distinctive organelles which are bounded by a single membrane and closely associated with the endoplasmic reticulum. Membrane continuity between the microbody and the ER, however, is never clearly defined. In contrast to this, results presented in a previous paper on dilated cisternae from the ER membranes in cress seedlings (Iversen and Flood 1969) indicated a continuity between the ER and the cisternae. The cisternae consisted of a finely granular, electron-dense material and seemed to be identical with the cisternal swellings from the granular ER of cells in the root hair zone of radish as described by Bonnett and Newcomb (1965). In cress seedlings the profiles were present in the cells of the root cap, the outermost cortical layers, and the epidermis. From reconstructions based on series of ultrathin sections, the three-dimensional

structure and the interrelationships of the cisternal accumulations were depicted. It was suggested that the cisternal accumulations might be unique for plants of the *Cruciferae* and that the cisternae contained proteins (IVERSEN and FLOOD 1969).

The occurrence of microbodies within plant cells is of interest since these intracellular components may represent enzyme concentration sites (*e.g.*, FREDERICK and NEWCOMB 1969). In connection with studies to prove the proteinaceous nature of the ER cisternae by cytochemical methods (IVERSEN 1971), an extended study of the distribution of the cisternae within cruciferous plants was performed. In the present paper observations are presented on the structure, frequency, and distribution of the ER cisternae within 50 different species of *Papaveraceae*, *Cruciferae*, and *Resedaceae*.

2. Materials and Methods

2.1. Seed Germination

Seeds of the different plant species were sterilized with 3% hypochlorite for 15 minutes and germinated in the dark for 2 to 4 days in Petri dishes on filter paper moistened with sterilized distilled water. Germinated seeds were then transferred to a green-house and continuously illuminated until the seedlings had attained a well developed leaf and root system. Tissues from the selected seedlings were then fixed for electron microscopic examination.

2.2. Routine Fixation, Embedding, and Electron Microscopical Procedures

Tissue from different parts of the plants were cut into small (1 to 2 mm) pieces and fixed according to one of the following procedures:

a) One hour at 4° C in 2% potassium permanganate in veronal-acetate buffer (pH 6.8) containing 1.5% sucrose and 0.014% calcium chloride.

b) Two hours at 4° C in 2% osmium tetroxide in veronal-acetate buffer (pH 6.8) containing 0.014% calcium chloride.

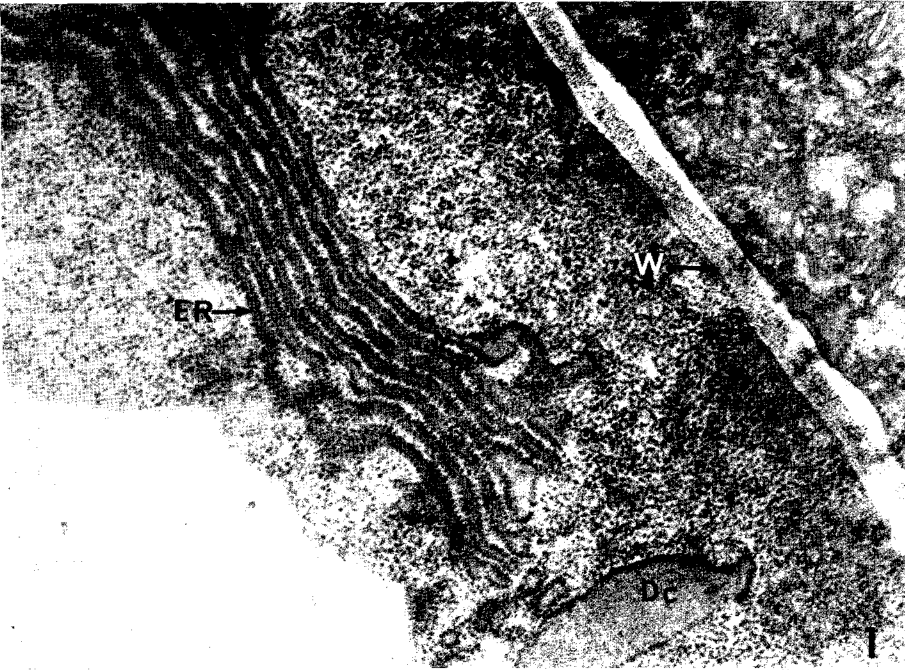
c) Two hours at 21° C in 3% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8). The material was washed for 2 hours in four changes of the same buffer and postfixed for 2 hours at 4° C in phosphate-buffered 2% osmium tetroxide.

The last method was used for routine fixation. Dehydration was carried out in ethanol of increasing concentrations at room temperature and the tissue was embedded in Epon. Sections were cut on an LKB Ultrotome I and a Reichert Om U 2 ultramicrotome fitted with glass knives. The sections were stained for 15 minutes with aqueous 1% uranyl acetate followed by lead citrate (REYNOLDS 1963) for the same time. A Siemens Elmiskop I and 100 and an AEI 6 B electron microscope were used at 80 kV.

3. Results

3.1. General Observations on Morphological Structure of Dilated Cisternae

The dilated cisternae observed in the cruciferous species represent localized dilations of the endoplasmic reticulum. This conclusion was confirmed by observations of profiles such as that illustrated in Fig. 1. At the ends of stacks



Figs. 1 and 2. Dilated cisternae in root tip cells of *Allysoides utriculata*. Fig. 1. A dilated cisterna (*Dc*) possibly budded off from the flattened sacs of the endoplasmic reticulum (*ER*). The edges of the ER profiles are apparently dilated to produce further cisternae. *W* = cell wall. ×37,700. Fig. 2. Mature accumulations. A long, dilated cisterna (*Dc*), the rough endoplasmic reticulum (*ER*), and the nuclear membrane (*M*) are indicated. ×43,300

of flattened sacs of ER membranes the dilated cisternae can be seen as tubular necks. A dilated cisterna possibly derived from the same ER membrane system is indicated.

The cisternal accumulations were approximately $0.4–1.5 \times 4–5\ \mu$ but longer cisternae (up to $12\ \mu$) have been observed (Fig. 2). The accumulations were bounded by a single membrane with attached ribosomes identical with the granular ER membranes (Figs. 2, 3, and 4). The cisternae were still covered with ribosomes after budding off from the ER membranes. When fixed in permanganate, however, the ribosomes were absent from the cisternae membranes.

The cisternae appeared as elongated slender rods or irregularly-shaped profiles in longitudinal sections; in transverse section they appeared circular (Figs. 2 and 3). They did not seem to be oriented in any special way with reference to the axes of the cells of the root.

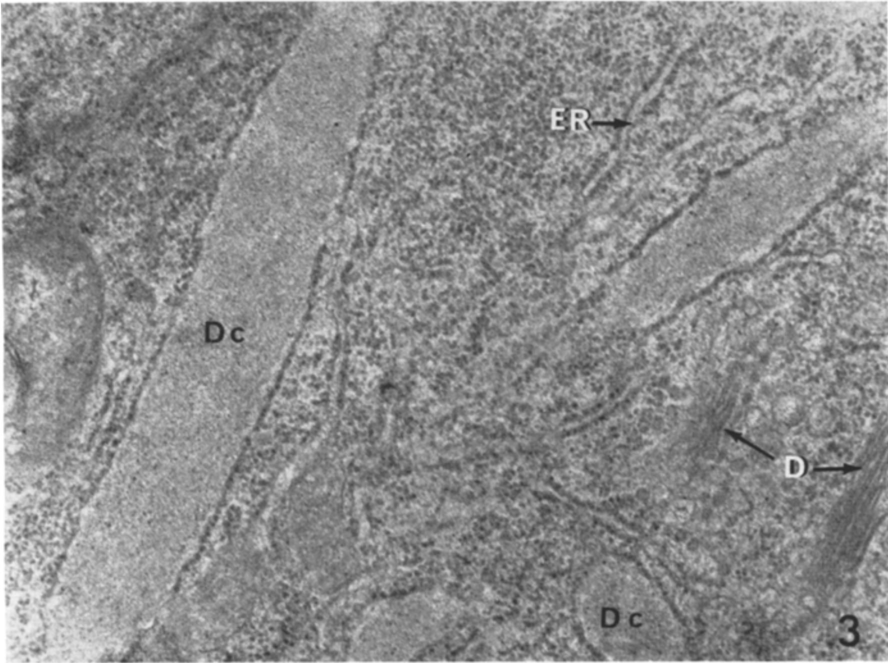
The morphological characteristics of the cisternae were the same when tissue was fixed in osmium tetroxide alone or in glutaraldehyde followed by osmium. The cisternae contained a granular or occasionally fibrous material (Fig. 7). After permanganate fixation the matrix was more electron-dense and finely granular. Crystalloid inclusions have never been found in the cisternae but protein-crystalloid-like organelles have been observed in the same plants (Fig. 6).

3.2. General Observations on the Distribution of the Cisternae within Different Tissue

The cisternae were not restricted to any particular region of the plants under study, being present in the root, stem and leaves. In the root tip they were present in the root cap, the outermost cortical layers, and the epidermis. In cress they were not present in the apical meristem where the ER was only slightly developed, but in other species they were observed in the apical meristems which had developed a fairly extensive ER. In the more vacuolated and differentiated cells in the elongation and root hair zone, the cisternae were a common component of the restricted cytoplasm adjoining the cell wall in cells of the epidermis and cortex. Present observations on the distribution of the cisternae in stems and leaves are limited. The profiles were found in surface cell layers of the stems and in the pallisade cells of the leaves.

3.3. General Observations on the Frequency and Distribution of the Cisternal Accumulations within the Cell

In meristematic cells and the various types of differentiating cells the cisternae were relatively small. They had a lower electron-density than those in more differentiated cells. The association with the ER was intimate and extensive and the dilations were spherical in shape. The numbers of cisternae were



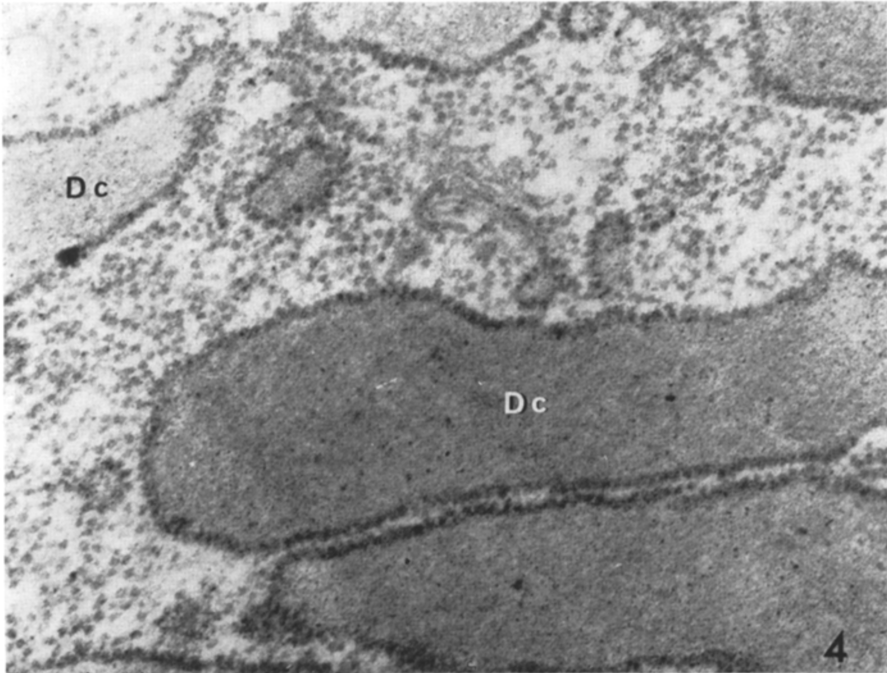


Fig. 3. Dilated cisternae (*Dc*) in a root tip cell of *Thlaspi arvense*. The dilated cisternae are shown in longitudinal and transverse section. Dictyosomes (*D*) and the endoplasmic reticulum (*ER*) are indicated. ×40,000

Fig. 4. Dilated cisternae (*Dc*) in a root tip cell of *Allysoides utriculata* at high magnification. The electron density varies in different cisternae; the limiting membranes are coated with ribosomes. ×84,000

Table 1. *Distribution of ER Cisternae in Different Species of Rhoeadales.* (Time after soaking the seeds is indicated. Plus signs indicate the existence of cisternae; minus signs that cisternae have not been observed)

a)

Species	Time after soaking (days)	Existence of ER cisternae in roots	Species	Time after soaking (days)	Existence of ER cisternae in roots
Papaveraceae			*Cardamine hirsuta* L.	7	+
Papaver argemone L.	7	—	*Cardaminopsis petraea* (L.)	8	+
P. orientale L.	7	—	*Arabis alpina* L.	9	+
P. radicatum Rottb.	7	—	*Allysoides utriculata* (L.)	9	+
P. album Mill.	7	—	*Alyssum alyssoides* (L.)	8	+
Meconopsis dhwosii	6	—	*Schivereckia podolica* Andrz.	9	—
M. cambrica	6	—	*Draba aizoides* L.	8	+
Argemone mexicana L.	9	—	*D. nivalis* Liljeblad	8	+
A. platyceras Link and Otto	9	—	*D. carinthiaca* Hoppe	8	+
Roemeria refracta D.C.	6	—	*Draba hirta* (*daurica*) auct., vix. L.	8	+
Glaucium flavum Crantz.	8	—	*D. cinera* Adams	8	+
G. corniculatum (L.)	8	—	*Cochlearia officinalis* L.	8	+
G. squamigerum Kar. & Kir.	8	—	*Thlaspi arvense* L.	9	+
G. vitellinum Boiss and Buhse	8	—	*Iberis saxatilis* L.	9	+
G. oxylobum Boiss and Buhse	8	—	*I. amara* L.	9	+
Eschscholtzia californica	7	—	*Lepidium sativum* L.	3	+
E. lobbi Greene	7	—	*Brassica oleracea*	4	+
E. tenuifolia Benth	7	—	*B. nigra*	4	+
E. douglasii Walp.	7	+	*Sinapis alba* L.	4	+
Dicranostigma franchetianum	8	—	*Raphanus sativus* L.	4	+
Hunnemannia fumariifolia Sweet	8	—			
			Resedaceae		
Cruciferae			*Reseda lutea* L.	8	—
Sisymbrium officinale (L.)	7	+	*R. alba* L.	8	—
S. tanacetifolium (L.)	7	+	*R. phyteuma* L.	8	—
Descurainia sophia (L.)	7	+	*R. complicata* Bory	8	—

correlated to the occurrence of ER membranes, but the accumulations were not restricted to specific parts of the meristematic cells.

In older differentiating cells of the root cap the accumulations assumed the more distinctive form described above. They were found in the cytoplasmic layer along the cell wall and in the cytoplasmic trabeculae of highly vacuolated cells. The numbers of cisternae in these cells varied, but the mean number in the root cap cells exceeded the number in cells from other parts of the plant. In any one cell, there were approximately as many cisternal accumulations as there were mitochondria, and more cisternae than dictyosomes.

b)

Species	Time after soaking (days)	Existence of ER cisternae in stems	leaves
Papaveraceae			
Papaver radicatum Rottb.	10	—	—
Glaucium flavum Crantz.	10	—	—
Eschscholtzia douglasii Walp.	9	+	+
Cruciferae			
Sisymbrium officinale (L.)	10	+	
S. tanacetifolium (L.)	10	+	
Descurainia sophia (L.)	9	+	
Cardamine hirsuta L.	11	+	+
Cardaminopsis petraea (L.)	10	+	
Arabis alpina L.	11	+	
Alyssum moellendorfianum	10	+	
Draba carinthiaca Hoppe	11	+	
D. cinera Adams	12		—
Iberis amara L.	12	+	
Lepidium sativum L.	5	+	+
Resedaceae			
Reseda lutea L.	11	—	—
R. alba L.	11	—	—

In the highly vacuolated cells of the elongation and root hair zone the profiles were found in the cytoplasm around the large central vacuole. Their frequency was low and the total number in each cell was less than in the cortical and epidermal cells of the root cap.

3.4. Occurrence of the Dilated Cisternae within *Rhoeadales*

Results from a detailed study on the occurrence of the defined ER cisternae within three different families are shown in Table 1. The different seedlings have been examined 3 to 9 days after soaking the seeds, depending upon the ability of the seeds to imbibe water and germinate. Separate studies on the development of the different organelles in cress radicles during germination had revealed small ER-cisternae when the radicles were 1 mm long, 24 hours after soaking. It is suggested that the cisternae pre-exist in the dormant seeds and undergo developmental changes during soaking and germination. Therefore, to get roots at approximately the same developmental stage, seedlings from the different species were selected after various periods of time.
As can be seen from Table 1 the cisternae were unique for plants of the *Cruciferae*. The few exceptions within this family where accumulations were

not revealed, *e.g.*, in the root tip of *Schivereckia podolica* and in the leaves of *Draba cinera* can either be explained as a technical error (*e.g.*, different seeds have been mixed) or, more likely, as a cytotaxonomical problem. Studies along cytotaxonomical lines are in progress. The cisternae have never been found within species of the *Resedaceae* and only in one species of *Papaveraceae*, *Eschscholtzia douglasii* (Fig. 5).

When accumulations could be observed in the roots they were frequently also found in the other parts of the same plant (Table 1 *b*); this table is incomplete where the plant organ has not been examined in a particular species.

The ultrastructure of the 49 different species will not be described in detail, but a few common organelles may be mentioned. Microbodies other than the dilated ER-cisternae have been identified in most of the species examined. They were usually spherical and approximately 1 μ in diameter. The electron density varied within the different families. The morphological features of the microbodies were quite distinctive and they could be distinguished readily from the dilated cisternae.

Both smooth and rough ER occured in the cytoplasm of the root tips of species from *Papaveraceae* and *Resedaceae*. However, even in species with extensive rough ER, *e.g.*, *Glaucium vitellinum*, dilated cisternae of the cruciferous type have never been found.

4. Discussion

The organelles described in this paper have been observed previously in radish root hairs (BONNETT and NEWCOMB 1965) and in the root tip of cress seedlings (IVERSEN and FLOOD 1969). In the same papers the organelles were reported to be absent in *Melilotus albus*, *Phaseolus vulgaris*, *Helianthus annuus*, and *Zea mays*. On the basis of their morphological characteristics, the cisternae seemed to be a separate type of cellular inclusion distinguishable from other cytoplasmic organelles. As both radish and cress belong to the *Cruciferae*, thorough systematic investigation on the occurrence of these organelles is best started with plants from this and related families.

4.1. Comparison of Microbodies and Dilated Cisternae

In their paper on fine-structural characterization of plant microbodies, FREDERICK *et al.* (1968) distinguished between microbodies and the cisternal dilations described by BONNETT and NEWCOMB (1965). The term "microbody" has been used to describe membrane-limited organelles both in animal and plant cells. The organelles described and identified as plant microbodies by MOLLENHAUER *et al.* (1966) have structural similarity to the animal microbodies observed in liver and kidney of vertebrates (reviewed by DE DUVE and

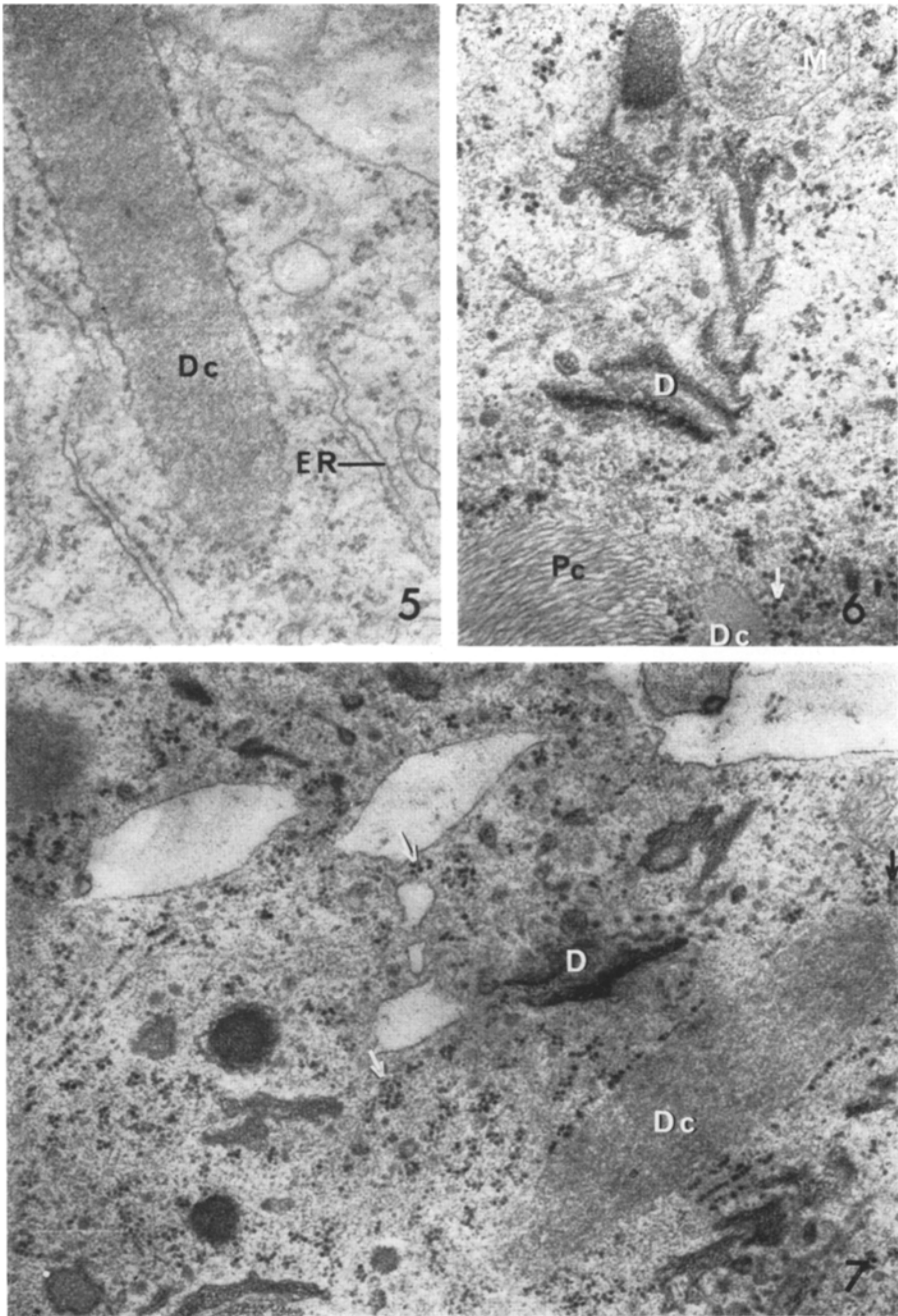


Fig. 5. A long dilated cisterna (*Dc*) from a root tip cell of *Eschscholtzia douglasii* (*Papaveraceae*). The endoplasmic reticulum (*ER*) is of the rough and smooth (arrow) forms. ×50,000

Figs. 6 and 7. Dilated cisternae in root tip cells of *Raphanus sativus*. Fig. 6. A dictyosome (*D*) adjacent to a mitochondrion (*M*), a dilated cisterna (*Dc*), and a proteinaceous crystalloid (*Pc*). A cluster of polysomes near the cisterna is indicated (arrow). ×34,500. Fig. 7. A dilated cisterna (*Dc*) with attached polysomes at one end (dark arrow). Clusters of polysomes are scattered in the cytoplasm (arrows), and a dictyosome (*D*) is indicated. ×27,000

BAUDHUIN 1966). The plant microbodies are morphologically characterized as spherical, elongate or irregularly-shaped single membrane-bounded profiles with a matrix of varying electron density (FREDERICK *et al.* 1968) without a definitive internal structure. They can be distinguished from the dilated cisternae by their size and structure. The microbodies are usually only a few microns long compared with the dilated cisternae which can attain a length of 12 μ. Also, as observed by BONNETT and NEWCOMB (1965), the cisternal accumulations are coated by polysomes (Fig. 7) which suggest that there may be protein synthesis occurring at or near their surfaces. Polysomes are not attached to microbodies in radish roots in the later stage of development, in contrast to the dilated cisternae in the same plant.

There is convincing evidence that animal microbodies may arise from the ER (HRUBAN, SWIFT, and WISSLER 1963, ESSNER 1967). However, continuity between the interior of the ER and that of the microbody has never been observed, in contrast to what has been found in, *e.g.*, *Allysoides utriculata* (Fig. 1) where the dilated cisternae can be seen as tubular necks at the edges of the ER profiles. Particles of approximately the same size and appearance as the microbodies have been given more functional names by certain authors, *e.g.*, "spherosomes" (FREY-WYSSLING, GRIESHABER, and MÜHLETHALER 1963), "phragmosomes" (PORTER and CAULFIELD 1958), and "lysosomes" (SCHNEPF 1964). Organelles similar to these can be found in different species of the *Cruciferae* but they are easily distinguished from the dilated cisternae.

In cotyledon parenchyma cells of *Vicia faba* seedlings, BRIARTY, COULT, and BOULTER (1970) occasionally found ER cisternae with electron-dense contents. The electron-dense material was confined to profiles with rough ER membranes and was absent in regions where ribosomes were not present on the membranes. From their micrographs it seems as if these ER cisternae were less developed and morphologically defined than the accumulations described in this paper.

4.2. Distribution and Function of the ER Cisternae

Dilated ER cisternae with the morphological features found in plants of *Cruciferae* have not, to the present author's knowledge, been described in other plant families. In a previous article (IVERSEN and FLOOD 1969) it was concluded that the cisternal accumulations showed a topographical relation to the superficial cell layers of cress roots, *i.e.*, to the older more vacuolated and differentiated cells separated from each other by thick cell walls. It was suggested that the contents of the cisternae represented protein precursors for the formation of cell walls.

On the basis of the present results on distribution of the cisternae, and the fact that the enzyme myrosinase (β-thioglucosidase) is quite specific for the *Cruciferae* (*e.g.*, NAGASHIMA 1959), experiments have been undertaken to test the idea that this enzyme might be localized in the cisternae. The possible

function of the enzyme may be production of precursors for formation of cell walls. A detailed report on these cytochemical localization experiments will be given in a later paper (IVERSEN 1971).

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