

THE STRUCTURE OF SOME CYTOPLASMIC COMPONENTS OF  
PLANT CELLS IN RELATION TO THE BIOCHEMICAL  
PROPERTIES OF ISOLATED PARTICLES

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PLATES 13 AND 14

(Received for publication, September 11, 1956)

In previous publications, Martin and Morton (15-18) have described the cytochromes, and enzymic and chemical properties of the microsomal and mitochondrial fractions isolated from two types of non-photosynthetic plant tissues, namely the white petioles of silver beet and the roots of germinating wheat. These studies have shown that the two types of cytoplasmic particles differ in their biochemical characteristics in a manner analogous to those of the similar components isolated from animal tissues.

In order to understand the function of cellular components in the metabolism of the intact cell, some knowledge is required of their fine structure, as well as of their biochemical properties. The work described here was undertaken to provide details of the structure of the particles in the mitochondrial and microsomal fractions from the beet petioles and wheat roots. Moreover, it appeared essential to investigate the relationship of the isolated particles (as used in biochemical studies) to the components of the cytoplasm of the intact cell. This appeared to be particularly necessary in the case of the microsomal particles. At the time of commencement of the studies described here, this relationship had not previously been investigated by the use of thin sectioning procedures in conjunction with electron microscopy.

Beet petioles and wheat roots as well as mitochondria and microsomes isolated from these tissues have been examined in sections in the electron microscope. The isolated particles were satisfactorily correlated with structures present in the intact cells. It has been found that the mitochondria of both plant tissues show internal membrane structures somewhat resembling those in mitochondria of animal cells, described by Sjöstrand (33) and by Palade (25). However, the isolated plant mitochondria had clearly undergone some change of structure from that of the mitochondria in the intact cell. It has been found that microsomes do not occur as such in the intact wheat root cell, but rather appear to be derived by rupture and transformation of a well defined

cytoplasmic membrane system (endoplasmic reticulum). Structures resembling the "Golgi zones" of animal cells have been observed in wheat root cells.

This work was mainly carried out during 1954 and was briefly reported at the 31st meeting of the Australian and New Zealand Association for the Advancement of Science in August, 1955.

### *Materials and Methods*

#### *Chemicals:*

*Modified Locke's Solution.*—The following were dissolved in 1 liter of glass-distilled water: NaCl, 9 gm.; KCl, 0.42 gm.; NaHCO<sub>3</sub>, 0.2 gm.; NaH<sub>2</sub>PO<sub>4</sub>, 0.05 gm.; and glucose, 0.2 gm.

*Dextrin.*—The Judex Chemical Co. product was used in most cases. A preparation of E. Merck, Germany, designated as "dextrin 'Merck'—extra pure" was also used.

*Veronal Buffer.*—Buffer at pH 7.4 was prepared as described by Michaelis (20), using sodium diethyl barbiturate (Judex Chemical Co.).

*Osmium tetroxide (OsO<sub>4</sub>).*—This was supplied by Judex Chemical Co.

#### *Tissue Preparations:*

*Whole Tissues.*—Roots were obtained from wheat germinated for 48 hours under conditions used for commercial malting. White petioles of silver beet (*Beta vulgaris*) were obtained from plants collected from the garden immediately prior to use.

*Microsomes and Mitochondria.*—These were isolated from silver beet petioles (16) and from wheat roots (18) using the procedures previously described for obtaining cytoplasmic particles for enzymic studies. The particles were not washed but were immediately suspended in 0.2 M sucrose at 0°.

#### *Fixation:*

Generally, the fixative was a 2 per cent (*w/v*) solution of OsO<sub>4</sub> either in veronal buffer, or in modified Locke's solution, and adjusted to pH 7.4 in each case. Except with the whole plant tissues (as indicated below) similar results were obtained with these two solutions.

*Cytoplasmic Particles.*—About 1 ml. of a heavy suspension of the isolated particles in either 0.2 M sucrose or in 30 per cent (*w/v*) dextrin was cooled to 0° in an ice bath, and an equal volume of buffered OsO<sub>4</sub> (at 0°) added slowly with stirring. Fixation was carried out at 0° for 2 to 10 minutes, completion being judged by the darkening of the particles.

*Whole Plant Tissues.*—Sections (about 0.5 to 1 mm. thick) were cut by hand with a razor and transferred to approximately 1 ml. of 30 per cent (*w/v*) dextrin at 0°. An equal volume of 2 per cent (*w/v*) OsO<sub>4</sub> in modified Locke's solution was added, and the sections fixed for 10 to 15 minutes at 0°.

#### *Dehydration and Embedding:*

Excess OsO<sub>4</sub> was removed by washing the fixed material by suspending in 5 to 10 ml. of solution of similar composition to the fixative but without the OsO<sub>4</sub>. The particles were allowed to sediment slowly on standing. This washing was repeated three times, and was followed by a similar washing using 0.15 M NaCl, all operations being carried out at 0–3°. The tissue was then dehydrated by passage through a graded series of ethanol solutions (50, 75, 85, 90, 95, and 99 per cent (*v/v*) in each case) at 0–3°. The particles were allowed to sediment either by standing, or by using the minimum centrifugal force necessary. It has been observed that use of excessive centrifugal force may cause damage to the fixed tissues which become quite brittle during dehydration. After 5 to 10 minutes dehydration the fluid was changed, three passages being used for each ethanol concentration.

Ethanol was then removed by washing the fixed material several times with small quantities (about 0.5 ml.) of *n*-butyl methacrylate. The material was then suspended in about 2 ml. of *n*-butyl methacrylate containing 1 per cent (*w/v*) benzoyl peroxide as catalyst. The suspension was transferred to gelatin capsules, and the plastic polymerised at 47° for 12 to 18 hours.

*Electron Microscopy:*

Sections (10 to 30  $m\mu$  thick) were cut with glass knives on a microtome of the type described by Hodge, Huxley, and Spiro (11) and examined without removal of the embedding medium, using an RCA, model EMU 1, electron microscope. The electron micrographs were taken at original magnifications between 2,000 and 9,000 times, and photographically enlarged. The magnification of the instrument was calibrated using the interferometric method of Farrant and Hodge (8). All micrographs are of thin sections cut through the plant tissue or the fractions isolated from it.

OBSERVATIONS

*Microsomes Isolated from Plant Tissues:*

Fig. 1 shows microsomes isolated from the white petiole of silver beet. It is seen that this fraction consists mostly of vesicular elements, comprising an external membrane enclosing a space containing some aggregated material. Whether more or less of the membrane is seen is determined by the relation of the vesicle to the plane of section. The aggregated material enclosed in the vesicles is possibly protein material precipitated by the  $OsO_4$ . In Fig. 1 may also be recognised some small dense granules (indicated by an arrow) most of which are closely associated with the membranes of individual vesicles. The isolated beet microsomes thus appear to consist of (1) vesicles with well defined membranes, (2) material (possible protein) contained within the vesicles, and (3) some small dense granules (about 10  $m\mu$  diameter), mostly associated with the membranes surfaces.

Fig. 2 shows microsomes isolated from roots of germinating wheat. The vesicular elements are somewhat smaller than those seen in the beet microsomal fraction (Fig. 1). There is a notable absence of precipitated material enclosed in the vesicle, the space being quite uniform and only slightly more dense than the background. The wheat root microsomal fraction contains many more of the small dense granules than does the similar fraction from beet.

Figs. 1 and 2 show that both microsomal fractions contain, in addition to the vesicular structures, occasional dense bodies (*D*) of about 50 to 100  $m\mu$  diameter. Similar bodies occur in intact wheat root cells. Many more of these dense bodies were seen in the "mixed particle" fraction obtained from silver beet by sedimentation at 20,000 *g* for 15 minutes (16).

Fig. 2 includes a mitochondrion (*M*) which was present in the microsomal fraction isolated from wheat roots, while Fig. 3 shows a portion of the mitochondrial fraction isolated from silver beet petioles. In general, the structure of the mitochondria from the two sources is similar, showing an outer membrane enclosing a relatively dense interior containing a number of membranes. The

mitochondrial fractions from both sources contained some vesicular bodies, which may have been derived from mitochondria or which could represent microsomal contamination. The structure of the isolated mitochondria is comparable with that of mitochondria from red beet root as described by Farrant, Potter, Robertson, and Wilkins (9).

*The Fine Structure of Wheat Root Cells:*

Fig. 4 shows portion of a longitudinal section through cells in the meristematic region of a root of germinating wheat. The cell walls (*CW*) are penetrated by protoplasmic strands (plasmodesmata, *P*) which link adjacent cells. The cells were only slightly vacuolated and possessed very dense cytoplasm containing a variety of well defined bodies such as mitochondria (*M*) and plastids (not shown here). It is seen that the cells contain a dense reticular network of membranes, which tend to be obscured by large numbers of small dense granules (about 10 m $\mu$  diameter). This system of reticular membranes with associated small granules is here called the "endoplasmic reticulum," by analogy with structures observed in animal cells (see Discussion). Fig. 5 shows rather better the detail of the endoplasmic reticulum, numerous profiles of which are clearly visible.

Figs. 4 and 5 also show a number of cytoplasmic structures, (*GZ*), consisting of smooth membranes associated with vacuolar spaces. The membranes are free of the small dense granules present in the endoplasmic reticulum. These structures, which were quite abundant in the meristematic cells, are designated as "Golgi zones," since they resemble the Golgi zones of animal cells as described by Dalton and Felix (5).

Fig. 6 shows finer detail of the structure of a mitochondrion in a wheat root cell. From Figs. 4 and 6 it is clear that the mitochondria possess a limiting membrane (which appears to be double), and a number of poorly oriented internal double membranes.

#### DISCUSSION

In previous publications it has been shown that cytoplasmic particles, isolated from petioles of silver beet (16) and from roots of germinating wheat (18), may be separated by differential centrifugation into mitochondrial and microsomal fractions. These are distinguished by their enzymic (16, 18) and chemical (17, 18) properties, and by their distinct cytochrome components (15). In order to translate these results to the whole plant cell, it was necessary to relate the isolated structures to components of the intact cells. This required electron micrographs of thin sections of the isolated cytoplasmic particles and also of the intact parent cells. Only in the case of the wheat roots have both these types of electron micrographs been obtained. Repeated attempts to obtain successful sectioning of fixed beet petiole tissue have been largely unsuccessful, due mainly to the difficulty of obtaining satisfactory fixation and em-

bedding of such highly vacuolated tissue. However, sections cut through vacuolated onion apical stem cells show structures resembling those of the wheat root cells, although less well defined.

Suspending the pieces of wheat root directly in buffered osmium tetroxide containing 15 per cent (*w/v*) dextrin has been found to give as good fixation of the meristematic cells as the method described in this paper and the fine structure of the cells of the developing wheat root will be described in a subsequent paper.

*The Fine Structure of the Cytoplasm of Plant Cells:*

Figs. 4, 5, and 6 show details of the cytoplasm of the wheat root cells. As with all other types of fixed tissue used for cytological study, the possibility of alteration of the cytoplasm due to the action of the fixative must be considered. There is every reason to believe that this problem is less serious where fixation in buffered osmium tetroxide is used (see Palade (24)), especially if the fixation is brief. The electron micrographs themselves indicate that little damage has been done to the cells in fixing and sectioning. Fig. 4 shows that the fine cytoplasmic strands of the plasmodesmata are continuous between cells, and that the cytoplasm is only slightly (if at all) contracted away from the cell wall. Moreover, a considerable degree of order is apparent throughout the cells. For these reasons, it is believed that Figs. 4, 5, and 6 show rather closely the structure of the cytoplasm of the wheat root cell as it existed at the time of fixation.

The electron micrographs of the wheat root cells have shown that these contain several more structures than can be recognised by conventional light microscopy. The nature of these structures will be described in another report.

*Comparison of the Structure of Mitochondria in Intact Plant Cells with that of the Isolated Bodies:*

As shown by Palade (25) and by Sjöstrand (33), the mitochondria of the proximal convoluted tubules of rat kidney have internal membranes or *cristae mitochondriales*, which appear to traverse the mitochondrion perpendicular to the long axis. These membranes are numerous and are arranged quite regularly. The mitochondria of the meristematic cells of the wheat root are quite small by comparison with those of kidney cells and hence their internal structure is less clear. However, Figs. 4 and 6 clearly show that the wheat root mitochondria have internal projections from the outer membrane. These structures are somewhat sinuous in appearance and they are not as well defined as those of kidney mitochondria. They are considered to be true membranes or lamellae, rather than filaments. In some cases they appear to form an acute angle with the long axis of the mitochondria (Fig. 6) although mostly they are oriented at right angles (Fig. 4).

Although the mitochondria of the various animal cells so far investigated

appear to have internal membranes, as pointed out by Palade (25) and by Beams and Tahmisian (2) there may be considerable variation in the arrangements of these in the mitochondria of different animal organs. It is not surprising, therefore, that the structure of wheat root mitochondria is not identical with that of kidney mitochondria. Since the mitochondria from both wheat roots and beet petioles contain mostly lipide (about 39 per cent dry weight) and protein (about 40 per cent) with less than 1 per cent nucleic acid (17, 18) it is reasonable to assume that the membranes revealed by the electron micrographs are essentially lipoprotein structures.

Comparison of Figs. 2 and 3 with Figs. 4 and 6 shows that the isolated mitochondria have undergone some disruption of their fine internal structure. This type of disorganisation is consistent with swelling of the mitochondria due to osmotic effects. Structural damage to chloroplasts of *Nitella* cells, following exposure of the algae to hypotonic solutions, was observed by Mercer, Hodge, Hope, and McLean (19).

The mitochondria isolated from both beet petioles and wheat roots show a number of enzymic activities (16, 18), while mitochondria isolated under somewhat similar conditions from Mung bean oxidise sodium pyruvate completely to carbon dioxide and water (21). In view of the modifications of structure revealed by comparisons of Figs. 2 and 3 with Figs. 4 and 6, the question arises as to the degree of preservation of fine structure that is essential for retention of various enzymic activities. The results suggest that many activities are retained even when lipoprotein membranes have been rather considerably disarranged (*cf.* also 1).

There may however, be important quantitative changes in the activities of mitochondria that have undergone structural alterations. Slater and Cleland (35) demonstrated an increase in  $\alpha$ -ketoglutaric oxidase activity during swelling of heart muscle sarcosomes in hypotonic solutions. Changes in sucrose concentrations from 0.25 M to 0.88 M considerably affects the fine structure and also influences the adenosinetriphosphatase activity of mitochondria isolated from rat liver (36). Therefore, some caution is necessary in using enzymic activities of isolated mitochondria as a source of information on their activity in the whole cell, especially as regards quantitative aspects (*cf.* Schneider, 32).

The slight structural changes revealed here may be quite sufficient to account for the finding of antimycin A-resistant pathways for oxidation of reduced diphosphopyridine nucleotide (16, 18; see also de Duve, Pressman, Gianetto, Wattiaux, and Appelmans (6); and Ernster, Jalling, Löw, and Lindberg (7)) and of sodium succinate (18) in isolated mitochondria. Moreover, since oxidative phosphorylation is destroyed when mitochondria are disrupted mechanically, but not when disrupted by digitonin solutions (4), it is clear that the type of modification of the fine lipoprotein structures seen in Figs. 4 and 6 may profoundly influence enzymic activities. The present investigation indicates a

need for further examination of methods for isolating from plant tissues cytoplasmic particles for enzymic studies.

Very recent observations suggest that the fine structure and the enzymic activities may be well preserved when liver mitochondria are isolated in a sucrose-polyvinyl pyrrolidone medium (22).

*Relationship of the Isolated Microsomes to the Endoplasmic Reticulum of Plant Cells:*

Figs. 4 and 5 show that few, if any, spherical vesicles comparable with those of the isolated microsomes occur in intact wheat root cells. Hence it must be concluded that the microsomes (Figs. 1 and 2) arise by rupture and transformation of some component of the cytoplasm.

The small dense particles seen in association with the vesicles of the isolated microsomes (Figs. 1 and 2) correspond with the similar particles seen in the reticular network of the endoplasmic reticulum of the wheat root cell (Figs. 4 and 5). Hence it appears that the microsomes arise mostly from the endoplasmic reticulum following rupture of the plant cell. Between 75 and 90 per cent of the dry weight of the isolated microsomes is lipide and protein (17, 18). The rupture of lipoprotein membranes would be expected to form vesicles corresponding to microsomes. Direct evidence of this type of transformation has been offered by Branster, Hodge, and Morton (unpublished work) who observed formation of microsomes from the endoplasmic reticulum in intact but swollen liver cells (10). Mercer *et al.* (19) also observed vesicle formation from the lamellar structures of swollen chloroplasts in *Nitella* cells. Vesicles might possibly also arise by rupture of mitochondria. The absence of succinic dehydrogenase activity, which is retained in mitochondrial fragments (3), as well as other criteria, establishes that the isolated plant microsomes do not arise from this source. Some of the vesicles without associated small dense granules observed in the microsomal fraction may have arisen by rupture of the membranes of the Golgi zones.

Palade and Siekevitz (29, 30) have shown that the nucleic acid of liver microsomes is largely localised in the small granules associated with the vesicle membranes, while most of the protein and almost all of the phospholipide, haemochromogen, and reduced diphosphopyridine nucleotide-cytochrome *c* reductase activity is associated with the membranes or the contents of the vesicles. The localisation of the nucleic acid of liver microsomes in the small dense granules is also supported by the findings of Littlefield, Keller, Gross, and Zamecnic (14). The much higher ribonucleic acid content of the microsomes from wheat roots, as compared with those from silver beet (17, 18) correlates with the much higher content of dense granules observed in the wheat root particles (compare Figs. 1 and 2), indicating that the small granules contain most of the nucleic acid of plant microsomes.

Since this work was completed, Kuff, Hogeboom, and Dalton (13) have described the enzymic properties, as well as the appearance in thin sections, of cytoplasmic particles isolated from liver homogenates. Several workers (26-28, 31) have described the endoplasmic reticulum and the intracellular cytoplasmic membranes (34) of different types of animal cells. It is now clear that microsomes from animal cells are also derived mainly by rupture and transformation of this structure (30, 10, 12). In view of the extensive modification of the endoplasmic reticulum during transformation to microsomes (compare Figs. 2 and 5), enzymic activities determined with such isolated structures may differ considerably from those of the cellular counterpart, quantitatively and possibly qualitatively.

Very recently, however, by using relatively dense media of sucrose or sucrose-polyvinyl pyrrolidone mixtures, different workers (30, 23) have isolated from animal cells "microsome" fractions, the constituents of which retain much of the fine structure of the endoplasmic reticulum as observed *in situ*. It is possible that such fractions may retain the enzymic activities of the endoplasmic reticulum rather better than microsomes isolated in less dense media.

#### SUMMARY

1. Electron micrographs of thin sections of material fixed with buffered osmium tetroxide have been used for comparison of the fine structure of isolated cytoplasmic particles from silver beet petioles and roots of germinating wheat with that of the cytoplasm of the intact cells.

2. Mitochondria of wheat roots have an external double membrane and poorly oriented internal double membranes. As compared with the structures seen *in situ*, the isolated mitochondria showed evidence of some disorganisation of the fine internal structure, probably due to osmotic effects. The possible influence of such changes on the enzymic properties of the isolated mitochondria is discussed.

3. The isolated plant microsomes are mainly spherical vesicular structures consisting of (a) an outer membrane enclosing (b) either an homogeneous slightly dense material (wheat root microsomes) or some granular dense material (silver beet microsomes) and (c) small dense particles, mostly associated with the vesicle membranes.

4. The cytoplasm of the wheat root cells does not contain any structures similar to the isolated microsomes but has a very dense reticular network, consisting of membranes with associated small dense particles, here called the endoplasmic reticulum. The observations indicate that the isolated microsomes arise mainly by rupture and transformation of the membranes of this structure. The effects of such extensive changes in the lipoprotein membranes on the enzymic activities of the endoplasmic reticulum, as studied in isolated microsomes, is discussed.



5. Meristematic wheat root cells contain structures which consist of smooth membranes with associated vacuoles and are similar to the Golgi zones of animal cells. The membranes of these zones probably contribute to the microsomal fraction under the conditions of preparation used for the enzymic and chemical studies previously reported.

We wish to thank Barrett's Food Co., Ltd., for the generous gift of wheat malt used for this and the related studies previously reported. One of us (E. M. M.) held a Studentship awarded by the Commonwealth Scientific and Industrial Research Organisation, whose financial assistance is gratefully acknowledged.

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#### EXPLANATION OF PLATES

All figures are electron micrographs of thin sections of material fixed with osmium tetroxide solutions. Figs. 1 to 3 are of cytoplasmic particles isolated from dispersions of plant tissues; Figs. 4 to 6 are of sections through the meristematic regions of roots of germinating wheat, illustrating the fine structure of cytoplasmic components *in situ*.

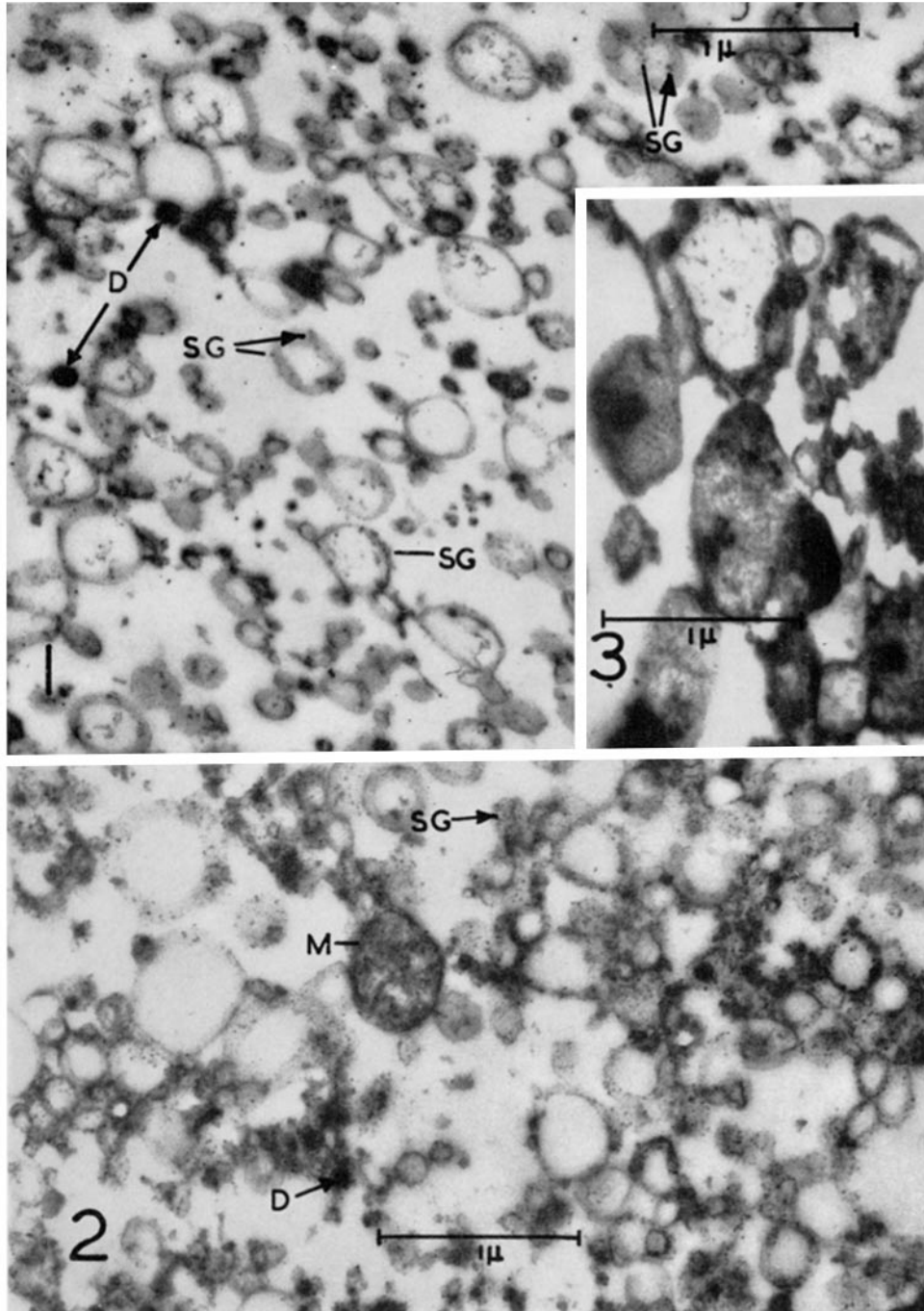
<i>CW</i> , cell wall.	<i>M</i> , mitochondria.
<i>D</i> , dense body.	<i>P</i> , plasmodesmata.
<i>ER</i> , endoplasmic reticulum.	<i>SG</i> , small dense granules.
<i>GZ</i> , "Golgi" zone.	

#### PLATE 13

FIG. 1. Microsomal fraction from petioles of silver beet. Note the vesicles, with some associated small dense granules (*SG*). Dense bodies (*D*) are also evident.  $\times 28,000$ .

FIG. 2. Microsomal fraction from roots of germinating wheat. The large number of small dense granules (*SG*) are mostly associated with the vesicle membranes. This field was selected to include a mitochondrion (*M*) and a dense body (*D*).  $\times 28,000$ .

FIG. 3. Mitochondrial fraction from petioles of silver beet.  $\times 28,000$ .



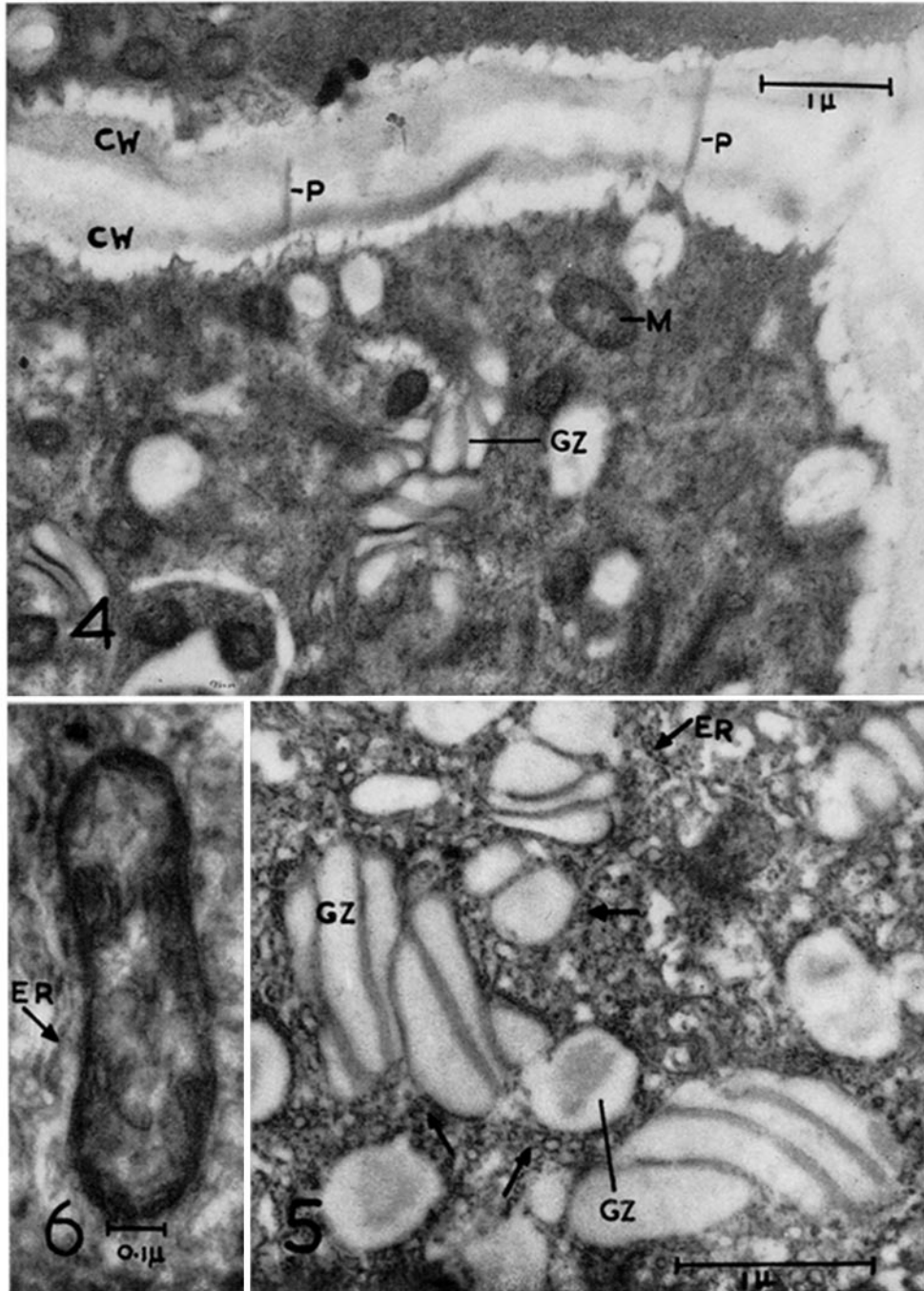
(Hodge, Martin, and Morton: Cytoplasmic components of plant cells)

PLATE 14

FIG. 4. Longitudinal section showing portion of two adjacent cells. The long axis of the cells runs approximately from top to bottom of the micrograph. The cell walls (*CW*) and plasmodesmata (*P*) linking the adjacent cells are clearly shown. The cytoplasm contains the dense endoplasmic reticulum and mitochondria (*M*) and Golgi zones (*GZ*).  $\times 18,000$ .

FIG. 5. Detail of the cytoplasm. Note that the Golgi zones (*GZ*) comprise vacuoles and smooth lamellae, while the numerous profiles of the endoplasmic reticulum (*ER*), indicated by the arrows, consist of membranes with associated small dense granules.  $\times 28,000$ .

FIG. 6. A mitochondrion from a wheat root cell *in situ*, showing the external limiting membranes and internal cristae. Some elements of the endoplasmic reticulum (*ER*) of the cytoplasm are indicated by the arrow.  $\times 74,000$ .



(Hodge, Martin, and Morton: Cytoplasmic components of plant cells)