THE FINE STRUCTURE OF THE WHEAT SCUTELLUM DURING GERMINATION

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Abstract

The cytological changes that take place in the scutellar epithelium and parenchyma during the first 5 days of germination are described by light and electron microscopy. Within 6 hr small starch grains appear in the plastids of both cell types and the size and number of starch grains increase gradually as germination proceeds. Later in germination starch disappears again from the plastids in the epithelial cells, but large starch grains still remain in the parenchyma cells. The reserves of the protein bodies are hydrolysed and the residual vacuoles undergo extensive coalescence. Modifications in the appearance of the wall material of the epithelial cells as these cells elongate are illustrated and possible functional bases for these changes are suggested. The cells of the scutellar epithelium show no cytological evidence for their known functions of diastase secretion and nutrient absorption.

I. Introduction

The structure of the wheat scutellum during the course of germination is of interest in several connections. Firstly, it has been accepted widely since the early work of Brown and Morris (1890) on barley that this organ plays a role in endosperm digestion. Brown and Morris (1890) showed that the cells of the scutellar epithelium in barley secreted a diastase that would digest starch paste and that the cells of the scutellar parenchyma lacked this activity. Secondly, since the scutellum lies between the reserves stored in the endosperm and the developing shoot and root system of the embryo, it is evident that the products of endosperm hydrolysis must cross the cells of the scutellar epithelium and parenchyma *en route* to the developing embryo. Although the vascular system of the scutellum in the mature grain and the differentiation of these tissues after germination have been described recently (Swift and O'Brien 1970, 1971), the possible role of the cells of the scutellar epithelium and parenchyma in the absorption of the products of endosperm digestion has received scant attention. Thirdly, it is known that the cells of the epithelium and parenchyma store an abundance of "protein bodies" or "aleurone grains" that contain reserves of protein which is degraded during germination. The fine structure of cells engaged in such a process (which we may call proteolysis unaccompanied by general autolysis) is of considerable interest in its own right, and has received some attention (*Arachis hypogea*, Bagley *et al.* 1963; *Tropaeolum majus*, Nougarède 1963; barley, Nieuwdorp and Buys 1964; wheat, Nougarède and Pilet 1964; *Pisum sativum*, Bain and Mercer 1966*b*; Opik 1966; Yoo 1970; *Vicia faba*, Briarty, Coult, and Boulter 1970). Many of these studies and especially those that deal with cereals, suffer from a lack of adequate correlated light microscopy and from poor specimen preservation. Fourthly,

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it is known that the scutellum of wheat lacks starch prior to germination and forms it after germination, apparently from pools of glucose that are *not* derived from endosperm digestion (see Edelman, Shibko, and Keys 1959). However, there is no cytological account of this post-germinative synthesis of starch in the scutellum. Finally, the scutellum, like the aleurone layer, is exposed, especially on its epithelial surface, to the action of the digestive enzymes which attack the endosperm. It is well known that the cells of the scutellar epithelium elongate and separate from one another during germination (Torrey 1902; Reed 1904; Sargant and Robertson 1905; Toole 1924; O'Brien 1942), but the fine structure of this process of cell separation has not been studied previously.

The fine structure of the cells of the epithelium and parenchyma in the dry state and after 3 hr water imbibition (both studied after aqueous fixation), have been described recently (Swift and O'Brien 1972). In this paper we have concentrated on the changes that occur during germination in the protein bodies, starch deposits, and cell walls.

II. Materials and Methods

Wheat grains (*Triticum aesitvum* L. cv Heron) were surface-sterilized with silver nitrate, soaked for 3 hr in distilled water, then set to germinate on 1·2% agar in the dark at 24°C. Seeds were selected at 6, 12, 24, and 30 hr after the beginning of soaking and thereafter at coleoptile lengths of 10, 20, 30, and 40 mm. The techniques of tissue preparation for light and electron microscopy are given in detail in Swift and O'Brien (1972). Briefly, for light microscopy, specimens were fixed in acrolein–glutaraldehyde mixtures and embedded in glycol methacrylate. For electron microscopy, specimens were fixed either in glutaraldehyde followed by OsO_4, or in $KMnO_4$, and embedded in low-viscosity epoxy resin (Spurr 1969).

III. Results

(*a*) *Light Microscopy* (Figs. 1–11)

(i) *Starch Grains.*—By 6 hr a few very small starch grains have appeared in the cytoplasm of the epithelial and parenchyma cells (Figs. 1, 6). The size and number of grains per cell increase gradually as germination proceeds (Figs. 2, 7). By 24 hr numerous small starch granules are found in the epithelium, while in the parenchyma the granules are much larger and more abundant (Figs. 3, 8, 9). The starch grains may occur singly or in groups of a few. There is only a slight increase in the size and number of grains after 24 hr (Fig. 10). When the coleoptile is 20 mm long there are fewer granules in the epithelial cells and many lack starch completely, but numerous

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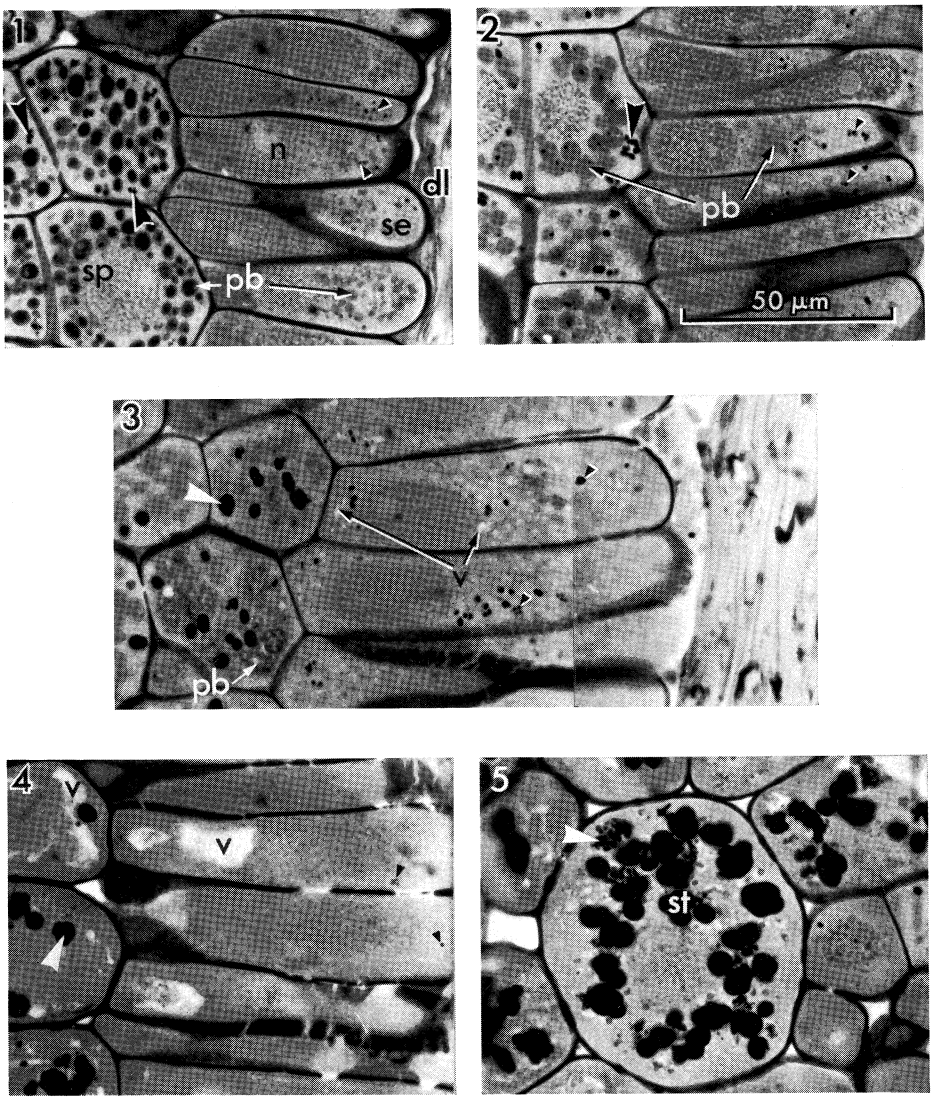
Fig. 3.—24 hr after soaking. Large starch grains are found in the parenchyma, but in the epithelium the starch grains remain very small. Small vacuoles have replaced the protein bodies in the epithelial cells. In the parenchyma cells fusion and partial hydrolysis of the protein bodies have produced large, granular masses of protein. The epithelial cells have elongated and swollen slightly and some adjacent cells have separated from each other along their lateral walls. Some walls show unstained gaps, which are believed to be cracks.

Fig. 4.—40-mm coleoptile stage. The parenchyma contains large starch grains, but starch is rare in the epithelium. Large vacuoles, resulting from hydrolysis and fusion of protein bodies, have appeared in both cell types.

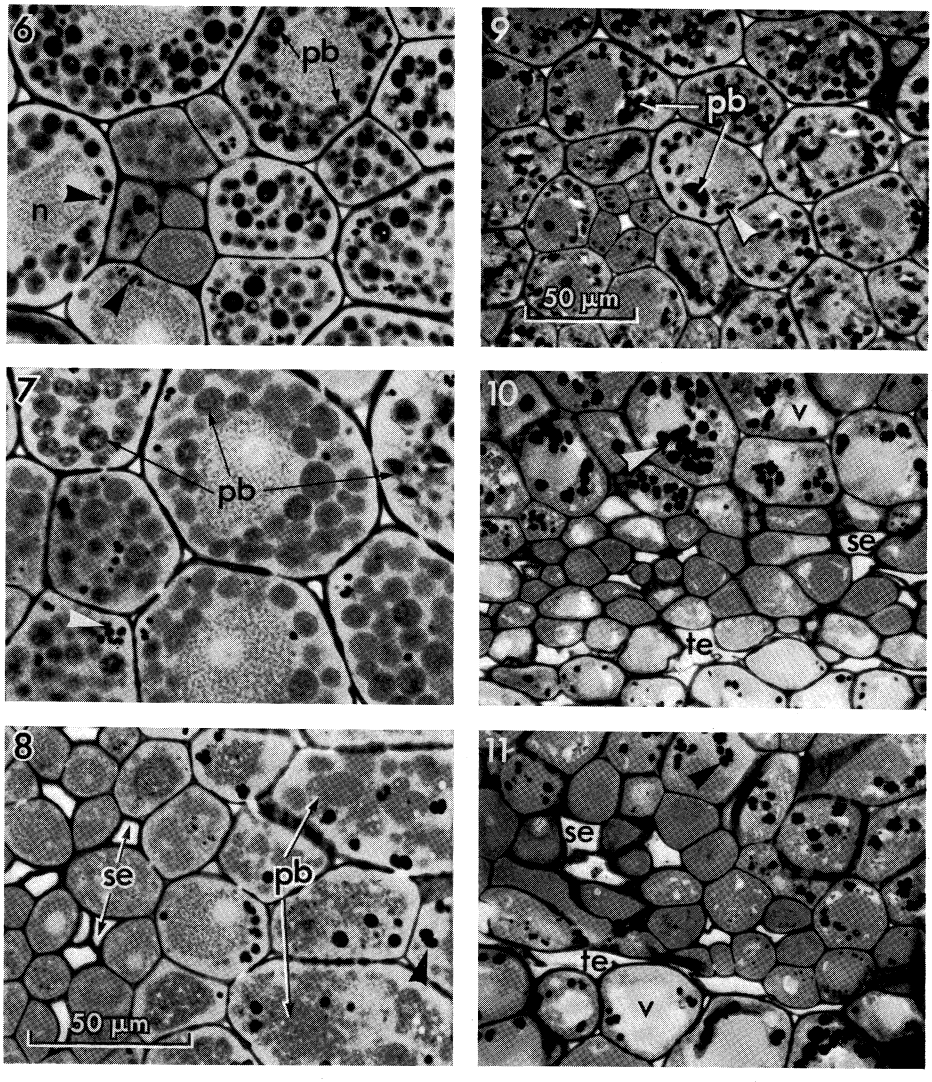
Fig. 5.—40-mm coleoptile stage. Clusters of very small starch grains (arrowhead) lie in the vicinity of the large, intact starch grains (*st*).



Figures 1–5 are light micrographs of transverse sections of the scutellum, showing the changes that take place in the epithelial cells and adjacent parenchyma cells during germination. *dl*, depleted layer of endosperm; *n*, nucleus; *pb*, protein body; *se*, scutellar epithelium; *sp*, scutellar parenchyma; *v*, vacuole; large arrowheads, starch grains in parenchyma cells; small arrowheads, starch grains in epithelial cells. PAS–fast green FCF–toluidine blue O. All at same magnification.

Fig. 1.—6 hr after soaking. Very small starch grains are present in the epithelium and parenchyma. Protein bodies in the epithelium are indistinct, but in the parenchyma they are strongly stained (cf. Figs. 1 and 3, Swift and O'Brien 1972).

Fig. 2.—12 hr after soaking. Starch grains have increased slightly in size and number. The protein bodies in the epithelium appear to be merging, and in the parenchyma the protein bodies stain less intensely than at 6 hr.



Figures 6–11 are transverse sections of the scutellum, illustrating the changes during germination in the large parenchyma cells a short distance away from the epithelium and around the vascular tissues Large arrowheads, starch grains; *pb*, protein body; *se*, sieve element; *te*, tracheary element; *v*, vacuole. Magnification of Figures 6 and 7 as for Figure 2; Figures 10 and 11 as for Figure 9. All PAS–fast green FCF–toluidine blue O.

Fig. 6.—6 hr after soaking. A few starch granules are found amongst the protein bodies, many of which stain very faintly. The distinction between starch (stained red by the PAS reaction) and protein (stained green by fast green FCF) is quite distinct in the original slides though they may appear somewhat similar in black and white.

Fig. 7.—12-hr after soaking. Note the gradual increase in the size and abundance of starch grains by this stage and in the later stages illustrated in Figures 5 and 8–11. By 12 hr hydrolysis of the protein bodies has commenced and they have a granular appearance.

Fig. 8.—24 hr after soaking. Fusion of partly hydrolysed protein bodies has produced large masses of granular protein.

large aggregates of starch grains are still present in the parenchyma. At 40 mm starch grains are absent from most epithelial cells although one or two very small granules may be seen occasionally (Fig. 4). The parenchyma cells still contain abundant starch grains, although smaller fragments of the large granules are seen, suggesting some starch breakdown (Figs. 5, 11).

(ii) *Protein Bodies.*—At 6 hr the protein bodies of the epithelial cells are less discrete and stain more faintly than in the dry grain; they appear to be merging with each other. There is no definite evidence of hydrolysis in the large protein bodies of the parenchyma, but bodies that stain less intensely may represent those in which protein breakdown has commenced (Figs. 1, 6). By 24 hr most of the protein bodies in the epithelial cells have been hydrolysed, leaving small vacuoles which are starting to coalesce (Fig. 3). Vacuolar fusion continues as germination proceeds and by the 40-mm coleoptile stage only one or two large vacuoles lacking protein are found in the epithelial cells (Fig. 4).

Hydrolysis of the protein bodies in the parenchyma cells is slower, but follows the same pattern. Protein breakdown can be detected in some cells 12 hr after soaking (Fig. 7). Hydrolysis in the parenchyma is initiated first not in the cells closest to the epithelium, but rather in the larger parenchyma cells in the central region of the scutellum. Breakdown of the protein bodies is well-advanced throughout the parenchyma by 24 hr and extensive coalescence of the bodies has produced large masses of partly hydrolysed protein (Figs. 8, 9). Dissolution of protein bodies is slower in the parenchyma cells close to the epithelium. Later in germination most of the protein reserves have disappeared and only a few very large vacuoles remain in the cells (Figs. 10, 11).

(iii) *Epithelial Cell Walls.*—By 24 hr the epithelial cells have elongated, swollen slightly at their tips and the walls of some adjacent cells have separated from each other (compare Figs. 1, 2, 3). The cell walls and the cytoplasm stain very faintly with toluidine blue, compared with earlier stages of germination. The walls are stained strongly by the PAS reaction, but there are unstained gaps, which may be cracks in the side walls between adjacent cells and at the tips of the cells. Similar gaps occur in the walls of parenchyma cells. The depleted layer of the endosperm stains more faintly with PAS–toluidine blue than in the preceding stages and starch breakdown has commenced in the endosperm close to the epithelial layer. By the 40-mm coleoptile stage the epithelial cells have elongated to their maximum length.

(*b*) *Electron Microscopy* (Figs. 12–31)

(i) *Endoplasmic Reticulum (ER) and Ribosomes.*—At 6 hr free ribosomes are very abundant, although occasionally short parallel rows of ribosomes are seen, suggesting that some rough ER is present. Membranes generally are indistinct (Fig. 19). By 24–30 hr the electron opacity of the cytoplasmic ground substance has declined, but

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Fig. 9.—Another view of the parenchyma at 24 hr. The remains of the protein bodies are located in vacuoles.

Fig. 10.—10-mm coleoptile. Most of the reserve proteins have disappeared and the small vacuoles are starting to coalesce.

Fig. 11.—40-mm coleoptile. Fusion of the small vacuoles often produces a very large, central vacuole.

membranes are still not very clear after glutaraldehyde–OsO_4. Numerous ribosomes are present, especially in the epithelium; many ribosomes now appear to be associated with cisternae of ER or in small aggregates and there are fewer free ribosomes. All membranes are clearly defined after $KMnO_4$ fixation and a qualitative comparison of the dry and 24-hr stages reveals that the ER has increased considerably in length and abundance. Later in germination the cytoplasmic ground substance becomes less electron dense and the number of ribosomes appears to decline. Most of the ribosomes are attached to short segments of the ER or in small groups of polysomes (Figs. 26, 29). While the plastid membranes are still clear in material fixed in $KMnO_4$, membranes of the mitochondria, dictyosomes, and ER are less distinct, although these organelles are just as numerous as at 24 hr (Fig. 30).

(ii) *Mitochondria.*—The mitochondrial cristae increase gradually from 6 hr to 24 hr, and by 24 hr the cristae are well developed (Figs. 19, 23). In later stages the stroma of the mitochondria becomes more electron-dense (Figs. 24, 26) and some larger, elongated mitochondria are found.

(iii) *Nucleus.*—By 6 hr the chromatin is distributed evenly throughout the nucleus in small clumps (compare Fig. 20 with Figs. 6 and 7 in Swift and O'Brien 1972).

(iv) *Plastids.*—Some of the plastids contain one or a few very small starch grains by 6 hr (Fig. 19), and the size and number of starch grains increase as germination proceeds. 24–30 hr after soaking, the plastid stroma is more electron-dense and a few internal membranes that often partly or completely encircle the starch grains are seen (Figs. 23, 24, 27). At the 20-mm coleoptile stage starch grains have disappeared again from most of the plastids in the epithelial cells, and internal membranes are more numerous, forming concentric rings within the plastid (Fig. 29). Osmiophilic

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Fig. 12.—6 hr after soaking. The wall is composed of a few layers of varying electron contrast. Fibrillar material fills the space between the tips of the two adjacent cells and the depleted layer of the endosperm.

Fig. 13.—12 hr after soaking. Some of the fibrillar material has disappeared and there appears to be a loosening of the structure of the wall in the region of the middle lamella between the neighbouring cells (arrowhead). The electron density of the inner wall material appears to have increased.

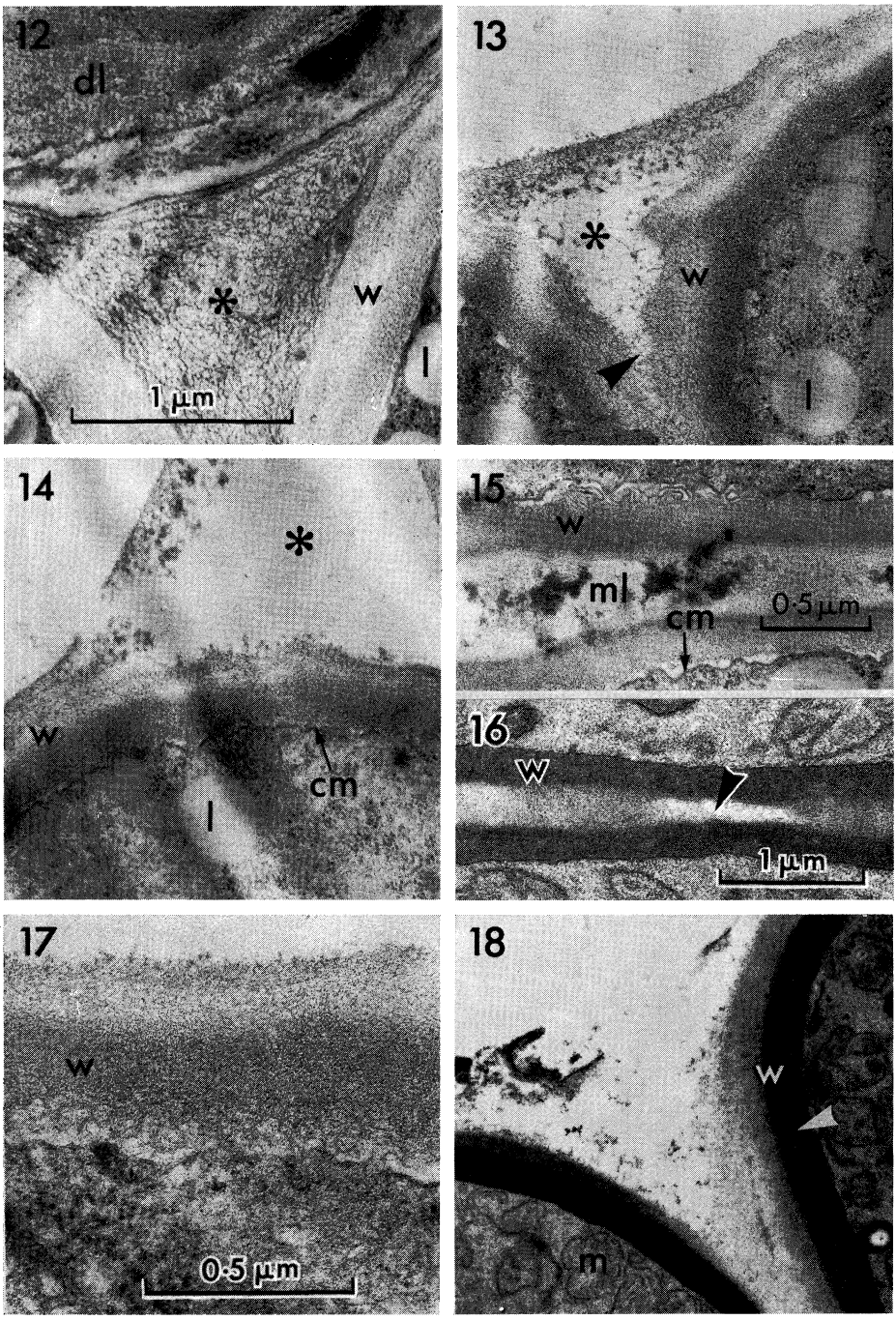
Fig. 14.—24 hr after soaking. Most of the fibrillar material has been broken down (asterisk) and the inner half of the wall next to the cell membrane (*cm*) is more electron-dense than the outer half.

Fig. 15.—Part of the walls of two adjacent epithelial cells at the 10-mm coleoptile stage. Some of the wall material in the middle lamella region (*ml*) is breaking down. *cm*, cell membrane; *w*, wall of epithelial cell. Glutaraldehyde–OsO_4.

Fig. 16.—Portion of the walls between two neighbouring epithelial cells at the 30-mm coleoptile stage. In the wall (*w*) between the cells there is an electron-dense zone adjacent to the cell membrane, while in the region of the middle lamella the wall material is of lower contrast and appears to be breaking down (arrowhead). $KMnO_4$ fixation.

Fig. 17.—The cell wall (*w*) at the tip of an epithelial cell, 20-mm coleoptile stage. The inner wall material is more electron-dense than the outer material and close to the cell membrane it shows a more open, fibrillar texture. Glutaraldehyde–OsO_4.

Fig. 18.—The tips of two adjacent epithelial cells in a 30-mm coleoptile seedling. Note the very high electron contrast of the inner wall material (arrowhead; cf. Fig. 23). *m*, mitochondrion; *w*, cell wall. $KMnO_4$ fixation. Magnification as for Figure 16.



Figs. 12–14.—Electron micrographs at the same magnification of the tips of two adjacent epithelial cells where they are in contact with the depleted layer of the endosperm, illustrating the changes that take place in the cell walls between 6 hr and 24 hr after soaking. Asterisk, material between the walls of adjacent epithelial cells; *dl*, depleted layer of the endosperm; *l*, lipid body; *w*, wall of epithelial cell. Glutaraldehyde–OsO_4.

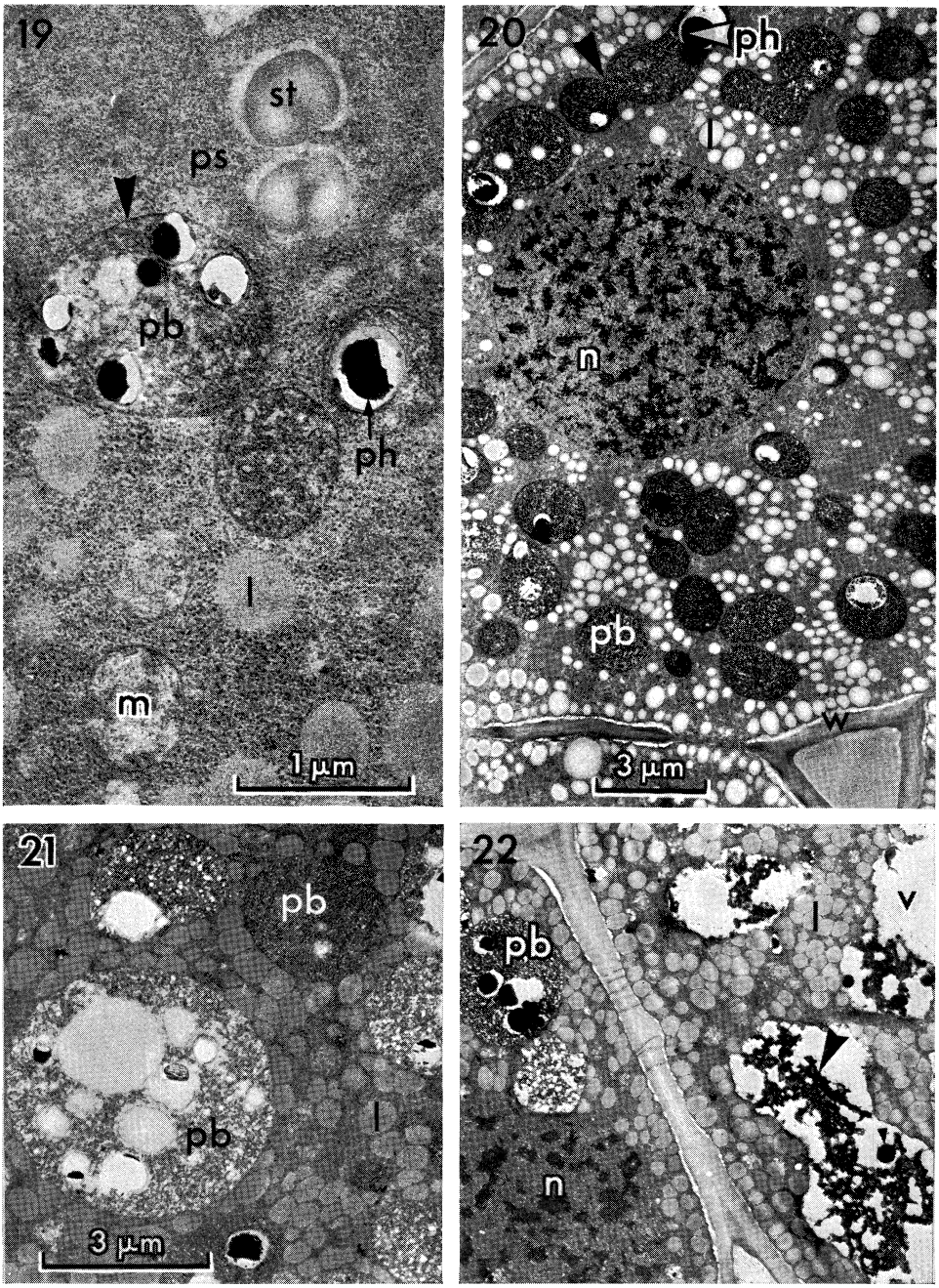


Fig. 19.—Portion of the cytoplasm in an epithelial cell, 6 hr after soaking. The protein matrix of the protein bodies (*pb*) is granular in appearance, due to its partial hydrolysis and a membrane is visible around the protein bodies (arrowhead). Two starch granules (*st*) are present in a plastid (*ps*), free cytoplasmic ribosomes are abundant, and the cristae of mitochondria (*m*) are indistinct and poorly developed. *ph*, phytate deposit in protein body; *l*, lipid body. Glutaraldehyde–OsO_4.

droplets often appear in these plastids. The plastids in the parenchyma cells still contain large starch grains when the coleoptile is 40 mm long. However, small fragments of starch are present also, implying that some of the large granules are being broken down (Fig. 31).

(v) *Dictyosomes.*—By 24 hr many dictyosomes are seen, and consist of four or five individual cisternae, with numerous associated vesicles (Fig. 23). Later in germination they appear to become less abundant (Fig. 30).

(vi) *Protein Bodies.*—6 hr after soaking, hydrolysis of the protein matrix is well-advanced in many of the protein bodies in the epithelial cells, and the matrix appears granular or fibrillar (Fig. 19). In the parenchyma, breakdown of the large protein bodies is well-advanced in some cells, while in others it has not commenced. In an individual cell the degree of hydrolysis is fairly uniform throughout its complement of protein bodies. Some of the partly hydrolysed bodies are beginning to fuse (Figs. 20, 21). Hydrolysis of the protein bodies is rapid in the epithelial cells; by 24 hr most of the reserve protein has been utilized and only a few fibrous remnants are left in the small vacuoles, which are coalescing. In the parenchyma the rate of protein breakdown is variable, even in adjacent cells (Fig. 22), and after 24–30 hr some of these cells have a few large vacuoles, while others still retain almost half of their protein reserves. A few phytate deposits are found in the vacuoles (Fig. 24).

The fusion of protein body vacuoles is more difficult to trace after $KMnO_4$ fixation, but regions limited by a single membrane and often stellate or crenate in outline are interpreted as fusing vacuoles (Fig. 28). Occasionally areas that were occupied by phytate inclusions can be identified within these bodies (Fig. 28). In the epithelium, electron-dense areas of irregular outline that often resemble or appear to be associated with cisternae of ER may represent fusion of partly hydrolysed protein bodies (Fig. 27).

Most of the protein and phytin reserves in the parenchyma cells have been utilized by the time the coleoptile is 20 mm long. The remaining small vacuoles fuse to form one or a few very large vacuoles in each cell. The coalescence of small vacuoles often produces an extensive network of canalicular vacuoles before a large single vacuole develops (Fig. 25).

(vii) *Lipid Bodies.*—Lipid bodies are abundant in both epithelium and parenchyma and show no detectable change in appearance or distribution 12 hr after soaking (Figs. 13, 19, 22), but by 24 hr they are less common near the cell membrane at

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Fig. 20.—View of a parenchyma cell, 6 hr after soaking. The protein matrix of the protein bodies (*pb*) is being hydrolysed and appears granular. Some adjacent protein bodies have fused (arrowhead). Note the uniformity in the degree of protein breakdown throughout the cell. The chromatin is dispersed throughout the nucleus (*n*) in small clumps. *l*, lipid body; *ph*, phytate inclusion of protein body; *w*, cell wall. Glutaraldehyde–OsO_4.

Fig. 21.—Portion of the cytoplasm in a parenchyma cell, 6 hr after soaking, showing details of the structure of the protein bodies (*pb*) during their hydrolysis. *l*, lipid body. Glutaraldehyde–OsO_4.

Fig. 22.—Two neighbouring parenchyma cells 12 hr after soaking. Hydrolysis of protein bodies is more advanced in the cell on the right, in which the fusion of protein bodies during their breakdown has produced large vacuoles (*v*), containing irregular masses of partly hydrolysed protein (large arrowhead) and some phytate deposits (small arrowhead). *l*, lipid bodies; *n*, nucleus; *pb*, protein body. Glutaraldehyde–OsO_4. Magnification as in Figure 20.

the tips of the epithelial cells (Figs. 14, 23). In the later stages of germination there appears to be a gradual decline in the number of lipid bodies present. Only a few are seen near the cell wall and most lie scattered within the cytoplasm. However, the utilization of these bodies is slow and many still remain when the coleoptile is 40 mm long.

(viii) *Epithelial Cell Walls.*—At 6 hr the walls at the tips of the epithelial cells are similar to those in dry grains, except that the material between the tips has a more open, fibrillar texture (Fig. 12). Between 6 and 24 hr after soaking, the material between the tips of the epithelial cells breaks down and the region of the middle lamella between adjacent cells becomes looser and more open in structure. The inner half of the wall adjacent to the cell membrane becomes more electron-dense than the outer half. Most of the material of the depleted layer has disappeared by 24 hr leaving just a few wall remnants (Figs. 13, 14; cf. Fig. 3). Permanganate fixation produces a slightly different image of the wall structure at 24 hr; the middle lamella is not apparent, but the inner one-third of the wall immediately adjacent to the cell membrane is considerably more electron-dense than the outer two-thirds. This dense zone is found at the tips and in the walls between neighbouring epithelial cells (Fig. 23). As the epithelial cells elongate during germination, they separate from each other along their lateral walls. This separation is achieved by dissolution of the wall material in the region of the middle lamella, the more electron-dense inner layer of the wall remaining intact (Figs. 15, 16). Although plasmodesmata are present in these walls, they do not appear to be sites of preferential hydrolysis. In the later stages of germination there is a loosening of the wall structure within the inner, dense region of the wall and next to the cell membrane the wall has an open, fibrillar appearance (Fig. 17). Permanganate fixation reveals a further increase in the electron contrast of the inner wall material (Fig. 18).

(ix) *Microbodies.*—Microbodies are present in the scutellum of 10–40 mm coleoptile seedlings. They could not be identified with certainty in the earlier stages of germination, because their appearance resembles that of partly hydrolysed protein bodies (compare Figs. 19 and 26). The presence of phytate inclusions facilitates identification of the protein bodies.

IV. Discussion

The changes in the organelles of the scutellar cells can be correlated with the increase in metabolic activity that accompanies germination. Soon after seeds are imbibed, their respiratory activity increases several fold and the protein- and carbohydrate-synthesizing systems become active (Abdul-Baki 1969). Early in imbibition the ribosomes of the wheat embryo are converted from a non-functional to a functional form (i.e. to polysomes), indicating that protein synthesis is in process early in germination (Marcus 1969). Holdgate and Goodwin (1965) found a comparatively rapid increase in the RNA content of the rye scutellum during the first few hours of imbibition and *de novo* synthesis of RNA was shown to occur.

By 6 hr the chromatin of the nucleus has dispersed into very small aggregates, in marked contrast to its aggregated form in the dry state; this change may allow the onset of intense nuclear activity, since condensed chromatin is believed to be relatively inactive in *m*-RNA synthesis (Prescott 1961; Littau *et al.* 1964; Karasaki

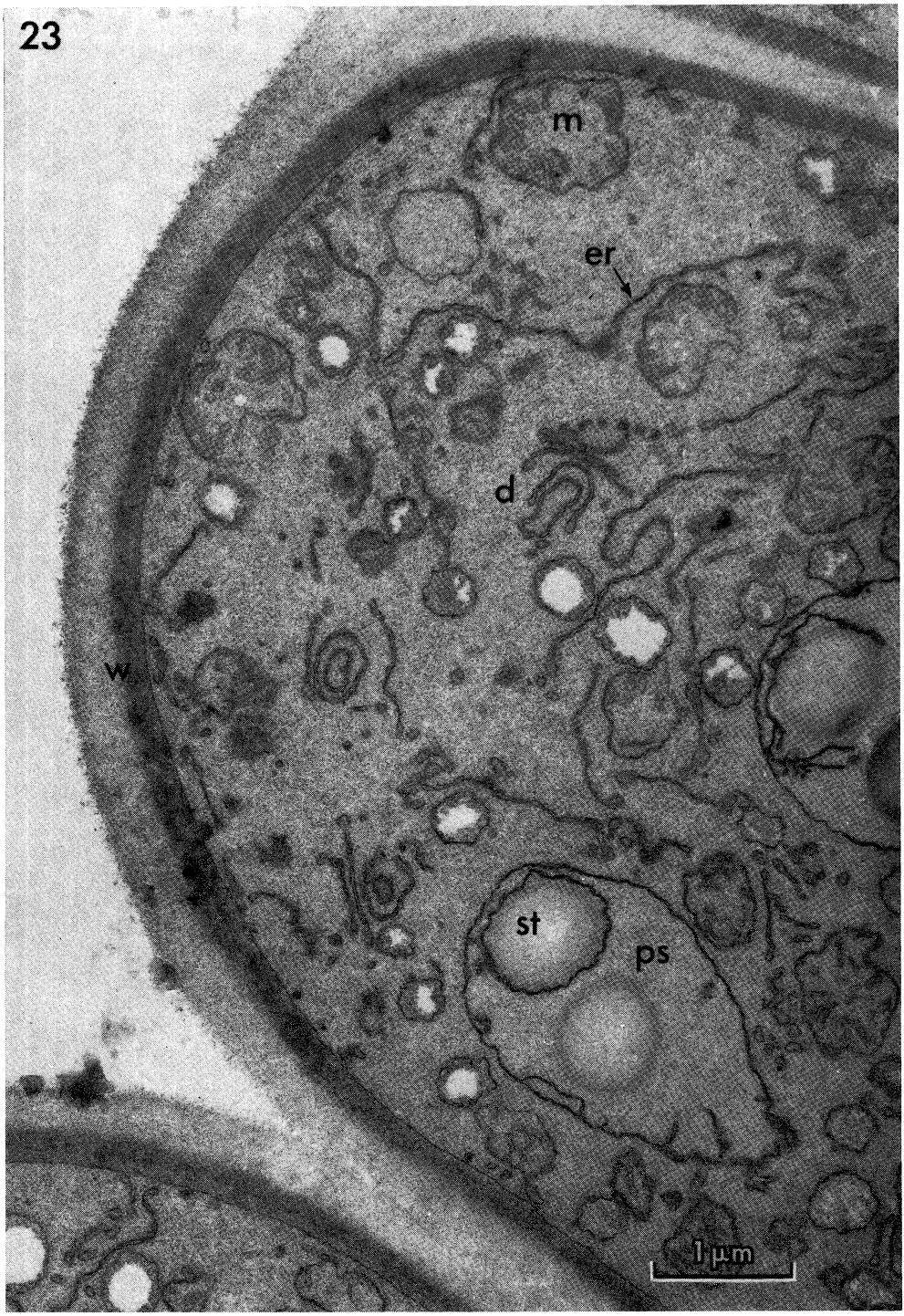

Fig. 23.—The tip of an epithelial cell fixed in $KMnO_4$, 24 hr after soaking. Note the increased electron contrast of the inner region of the cell wall (*w*), the long strands of ER (*er*) and the numerous cristae in the mitochondria (*m*). The dictyosomes (*d*) are well developed and there are fewer lipid bodies (*l*) near the cell wall. *st*, starch grain; *ps*, plastid.

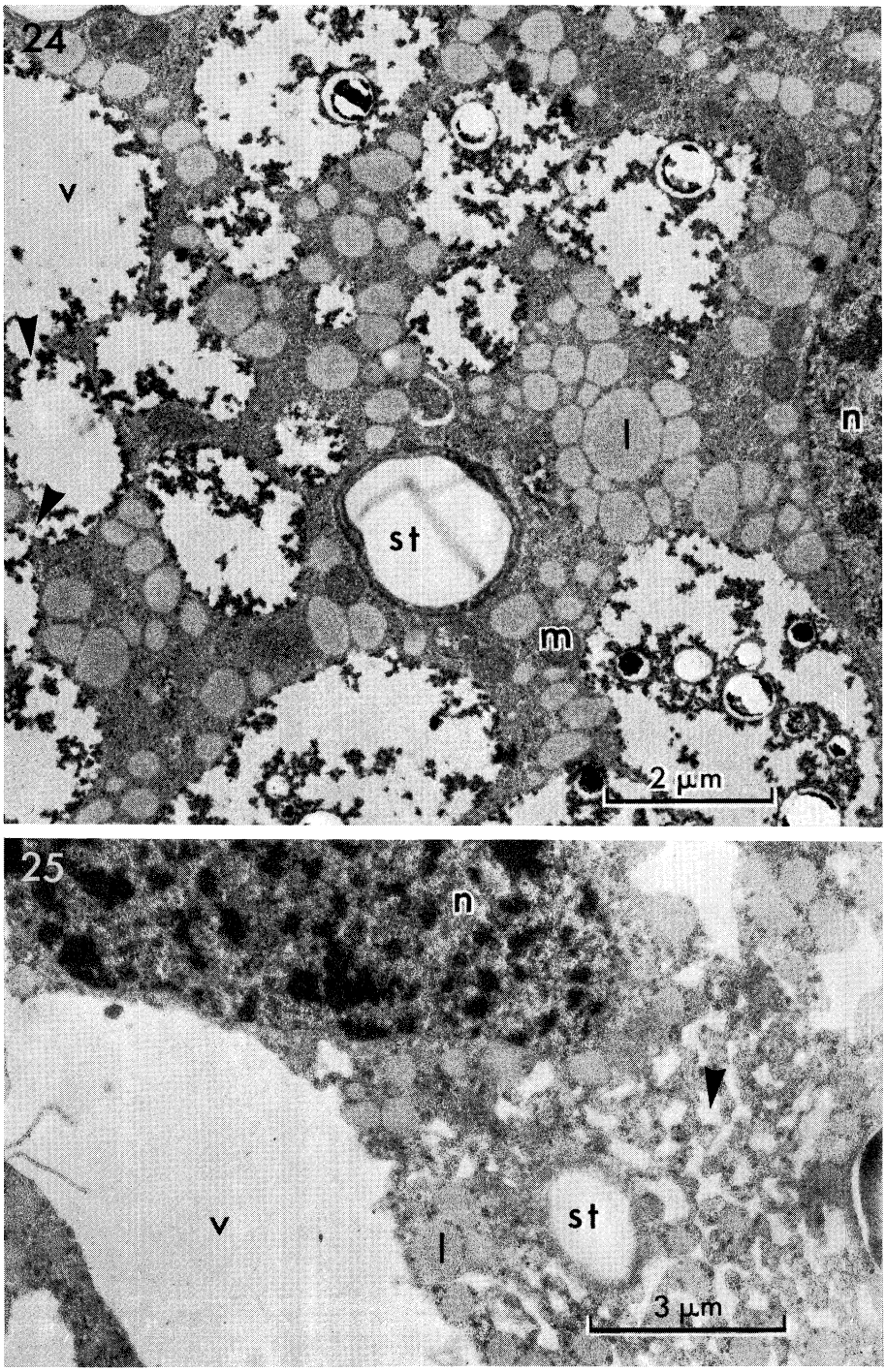

Fig. 24.—View of a parenchyma cell 30 hr after soaking. Hydrolysis of protein bodies is almost complete and only a few phytate deposits and remnants of protein lie in the vacuoles (*v*), which are in the process of fusing (arrowheads). A starch grain (*st*) is present in a plastid. The stroma in both plastids and mitochondria (*m*) is electron-dense. *l*,lipid body; *n*, nucleus. Glutaraldehyde–OsO_4.

1965; John and Lewis 1969). Other studies of germination have also revealed a similar development of the ER, mitochondria, and dictyosomes (e.g. Nieuwdorp and Buys 1964; Nougarède and Pilet 1964; Srivastava and Paulson 1968; Yoo 1970). An increase in the electron density of the mitochondrial stroma was noted by Briarty, Coult, and Boulter (1970) in bean cotyledons towards the end of germination and in *Phaseolus* by Opik (1966), who interpreted this as a degenerative change.

Although dictyosomes are well-represented in the epithelium, and during germination numerous vesicles are associated with them, there was no evidence of dictyosome vesicles in contact with the cell membrane. During germination the epithelial cells elongate to about twice their original length, without any apparent decrease in wall thickness. Clearly, a considerable amount of new material must be added to the walls and cell membrane of these cells. It has been suggested that dictyosome vesicles contribute materials to the cell wall during its synthesis and membranes to the cell membrane (Mollenhauer and Morré 1966); if dictyosomes perform this function it is surprising that there is no evidence of it in the epithelial cells.

It is not possible to define chemically the nature of the changes that take place in the walls of the epithelial cells during germination from the evidence presented here. In the course of mobilization of endosperm reserves, the walls of the endosperm cells are broken down, while the scutellar cell walls remain intact. The zone of densely staining material that forms in the walls of the epithelial cells at the time when hydrolysis of endosperm walls and reserves is in progress may represent the structural basis for the resistance of the scutellar walls. It will be of interest to determine if this layer is rich in the ferulic acid–carbohydrate complex that appears to be responsible for the resistance of the aleurone cell wall to digestion in wheat (Fulcher, O'Brien, and Lee 1972). As the epithelial cells elongate, the adjacent cells separate along the middle lamella, where none of this electron-dense material is found. By the time the coleoptile is 40 mm long in light-grown seedlings, photosynthesis would be beginning to supply the growing plant with metabolites, and dependence upon the endosperm would be greatly reduced. Consequently, a continued synthesis of resistant wall-material would be less important by this stage and the loosening in the structure of the inner wall material may be due to a reduction in the rate of synthesis of the protective wall material. Nieuwdorp and Buys (1964) also reported that the epithelial cell walls in the barley scutellum increase in electron density during germination, but after 30 days at 11°C the electron density declined to a level similar to that at the start of germination.

In the cotyledons of germinating pea, the formation of ER coincided with a disappearance of fat from the cytoplasm, while fat accumulation coincided with a disappearance of membranes as pea cotyledons became mature (Bain and Mercer 1966*a*, 1966*b*). In the present study no association was seen between the lipid bodies and any cytoplasmic organelles, and there was little evidence of lipid breakdown. The only morphological evidence of lipid utilization may be a gradual decrease in size of

Fig. 25.—Part of a parenchyma cell, 20-mm coleoptile stage. After hydrolysis of the protein bodies an extensive network of small vacuoles often develops (arrowheads) before a larger vacuole (*v*) forms. *l*, lipid body; *n*, nucleus; *st*, starch grain. Glutaraldehyde–OsO_4.

the droplets. Abundant lipid reserves still remain in the scutellar cells after 5 days germination. This is in agreement with Toole's (1924) observation that in maize, fat utilization is limited, and the amount of fat decreases slowly. Tavener and Laidman (1968), using wheat germinated in the dark at 24°C, found that although triglyceride breakdown had commenced in the embryo axis within 24 hr, breakdown in the scutellum did not start until 48 hr after imbibition. They also found that scutellar lipase activity remained constant at about 5 units per 90 grains for 2 days, and then increased to about 60 units per 90 grains after 6 days of germination. Edelman, Shibko, and Keys (1959) found relatively high soluble-sugar concentrations in the scutellum of grains soaked for 3 hr, so the early growth of the axis may be supported by soluble reserves until the reserves in the protein bodies, lipid bodies, and endosperm are mobilized. Ingle, Beevers, and Hageman (1964) suggest that, in maize, the fat is transformed to sugar or serves as a respiratory substrate, and Oaks and Beevers (1964) have shown that the tricarboxylic acid and glyoxylate cycles are active in this tissue. Other ultrastructural studies have revealed that lipid bodies move away from the cell wall in the course of germination (Nieuwdorp and Buys 1964; Srivastava and Paulson 1968; Yoo 1970). Nieuwdorp and Buys (1964) suggest that the lipid bodies are dissolved and their products taken up by the ER, but this is not the kind of speculation that can be tested by an examination of electron micrographs.

Microbodies have been found in ultrastructural studies of many plant tissues (e.g. Frederick *et al.* 1968) and glyoxysomes, which are bodies similar in appearance, have been isolated from homogenates of fat-storing tissues and shown to contain the enzymes necessary for the operation of the glyoxylate cycle (e.g. Longo and Longo 1970). Longo and Longo (1970) found that in the scutella of germinating maize seeds the specific activities of isocitratase, malate synthetase, and catalase associated with the glyoxysomes rise sharply until the fourth day of germination and then decline again. Recently it has been established that the microbodies observed *in situ* are identical to the isolated glyoxysomes (Gruber *et al.* 1970). These observations indicate that in the scutellum microbodies may function in the synthesis of sugars from the stored lipids of the lipid bodies.

Fig. 27.—Portion of the cytoplasm in an epithelial cell, 24 hr after soaking. The dark irregular areas (arrowheads) limited by a single membrane may represent the fusion of protein bodies following partial hydrolysis. ER (*er*) appears to be closely associated with these dark areas in some places. *n*, nucleus; *ps*, plastid; *st*, starch grain. $KMnO_4$ fixation.

Fig. 28.—Part of a parenchyma cell, 24 hr after soaking. The stellate region (*sr*), bounded by a single membrane (arrowhead) and containing a circular area where a phytate inclusion was located (*ph*), is probably a vacuole resulting from partial hydrolysis of a protein body. *l*, lipid body. $KMnO_4$ fixation.

Fig. 29.—Detail of a plastid (*ps*) in an epithelial cell at the 20-mm coleoptile stage. Note the concentric rings of internal membranes (arrowhead) and the electron-dense stroma. Glutaraldehyde–OsO_4.

Fig. 30.—Portion of the cytoplasm in an epithelial cell, 30-mm coleoptile stage. The membranes of the plastid (*ps*) are clear, but other cytoplasmic membranes are indistinct. *d*, dictyosome; *m*, mitochondrion. $KMnO_4$ fixation.

Fig. 31.—Part of a plastid in a parenchyma cell, 30-mm coleoptile stage. Small fragments of starch (arrowheads) are seen close to the larger starch grains (*st*) (cf. Fig. 5). $KMnO_4$ fixation.

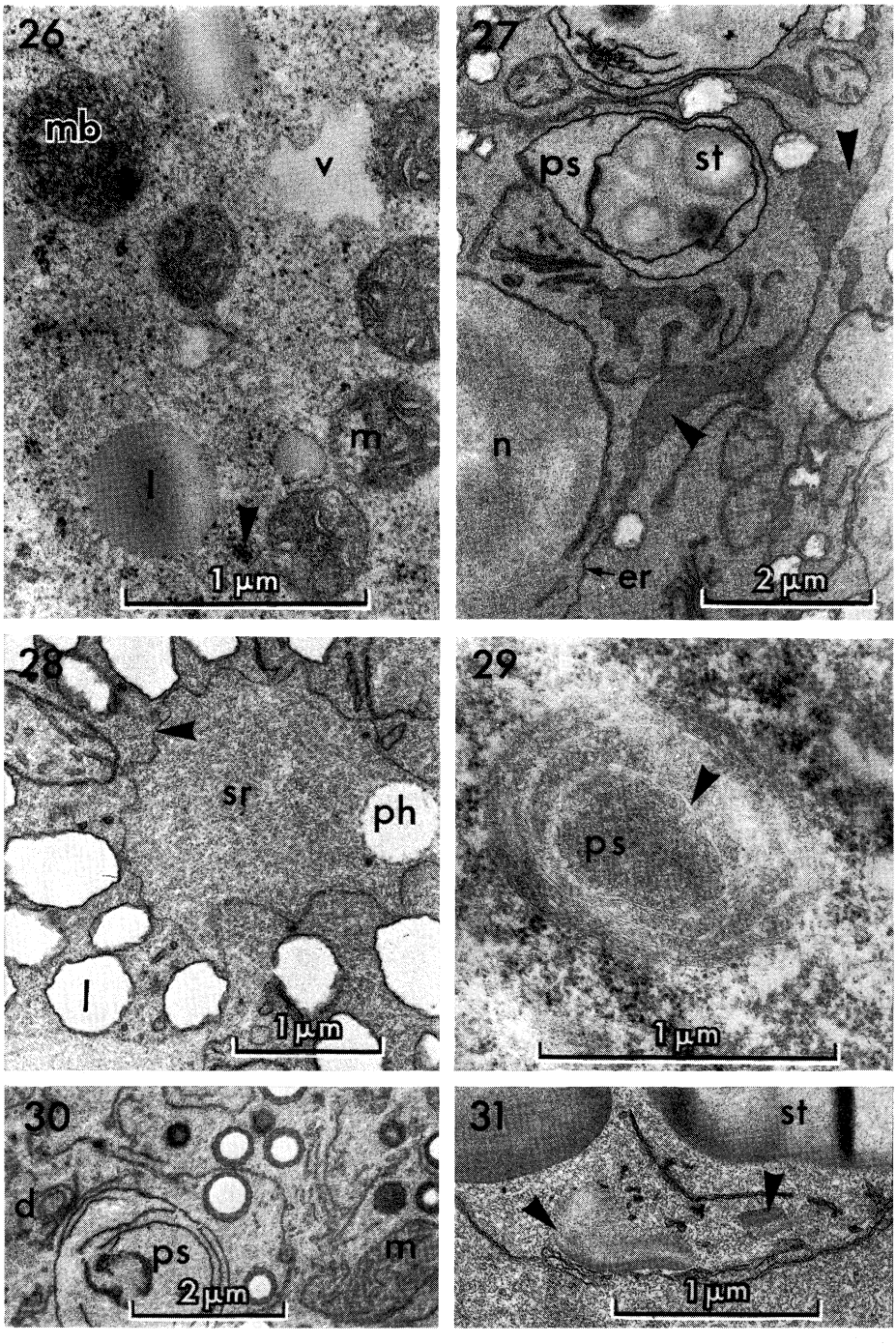

Fig. 26.—40-mm coleoptile, portion of the cytoplasm in a parenchyma cell. The mitochondria (*m*) have an electron-dense stroma with well-formed cristae. There appear to be fewer ribosomes and many are in short rows or small groups (arrowhead). *l*, lipid body; *mb*, microbody; *v*, vacuole. Glutaraldehyde–OsO_4.

It is not clear why starch should be synthesized and stored temporarily in the plastids of the scutellum during germination. It is unlikely that the starch is synthesized from the glucose absorbed from the endosperm, since starch appears in the scutellum before significant mobilization of endosperm reserves has occurred, and starch granules are deposited in the scutellum if embryos are grown in isolation on water (MacLeod and Palmer 1966). These authors note that lipid can be converted to starch during the germination of oil-containing seeds, which suggests that the starch that appears in the plastids of the scutellum is derived ultimately from the lipid bodies.

Seeds in the early stages of germination are completely independent of exogenous sources of nitrogen, and reserve proteins are broken down rapidly to supply amino acids to the growing axis. Thus the protein bodies of the scutellum constitute an important supply of nitrogen for the seedling until mobilization of endosperm protein reserves becomes significant. The breakdown and coalescence of protein bodies during germination and the resulting formation of large vacuoles have been described in a range of tissues (e.g. Poux 1963; Nougarède and Pilet 1964; Srivastava and Paulson 1968; Briarty, Coult, and Boulter 1970). The protein matrix is hydrolysed uniformly throughout the protein body and it seems likely that proteolysis is not initiated by proteases supplied from outside the membrane of the body. Rather, the evidence suggests that enzymes incorporated into the bodies during their formation are activated on hydration.

In wheat the phytin phosphorus constitutes 70–75% of the total phosphorus of the grain (Peers 1953) and this represents an important reserve of inorganic phosphorus that is made available to the plant through the action of phytases. The phytase activity is high in the wheat scutellum and Peers (1953) reports an increase of 6·5-fold in phytase on germination, while there is a sharp decrease in phytic acid between 24 and 120 hr (Albaum and Umbreit 1943). These observations are in agreement with the onset of ATP-requiring metabolic activities with germination. Poux (1963), using the Gomori's lead procedure, concluded that phosphates are found only within "globoids" (corresponding to the regions occupied by phytate deposits in this study) in protein bodies of germinating wheat. Acid phosphatase activity appeared both intensively around globoids and often within the protein matrix. She identifies an internal membrane limiting the globoid and, although a similar image was obtained occasionally in the present study (e.g. Fig. 21), we are uncertain about the interpretation of this image. Jones and Price (1970) also identify a limiting envelope around the phytin globoids in aleurone grains of barley aleurone cells and their evidence shows that this envelope is not a "typical membrane".

Finally, we wish to emphasize that we have encountered no cytological evidence that sheds any light on the processes of nutrient absorption or secretion of diastase, processes that are known to be functions of the scutellum. Of the many possible explanations for this fact, we favour the view that the uptake of soluble nutrients from the free space of plant cells and the secretion of modest levels of hydrolases are *not* accompanied by the gross specialization of ultrastructure that characterizes many animal cells engaged in similar activities (see Fawcett 1966; Porter and Bonneville 1964). We think it is likely that the structural changes that accompany these processes in the scutellum may not be evident except at the molecular level of resolu-

tion and that such changes are not detected by standard transmission microscopy of the tissue because the technique is not adequate for the task.

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