THE FINE STRUCTURE OF THE WHEAT SCUTELLUM BEFORE GERMINATION

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Abstract

The structure of the cells of the scutellar epithelium and parenchyma is described and illustrated by light and electron microscopy of air-dry grains and compared with that seen in grains soaked for 3 hr. In the air-dry state, nuclear chromatin is strongly aggregated, mitochondria and plastids appear to be intact, endoplasmic reticulum is present but not abundant, and dictyosomes cannot be readily identified. The ground substance contains an abundance of free ribosomes which appear to coat protein bodies, lipid droplets, and mitochondria. In material soaked only for 3 hr, endoplasmic reticulum and dictyosomes are apparent, the nuclear chromatin has dispersed, and some mobilization of storage protein appears to have begun in the scutellar epithelium. No differences in fine structure of other organelles or in the cell walls could be detected.

I. Introduction

In the dry seed the development of the embryo has been arrested temporarily until conditions are favorable for germination and growth of the embryo can resume. Dry embryos are characterized by a low water content and a very low metabolic activity. However, the potential for active metabolism is retained and on imbibition enzymes are activated (Bonner and Varner 1965; Abdul-Baki 1969), the respiration rate rises rapidly, and the breakdown of reserve materials is initiated (Mayer and Poljakoff-Mayber 1963).

Ultrastructural studies of seed development have been made on aleurone (Buttrose 1963*a*), endosperm (Graham *et al.* 1962; Jennings, Morton, and Palk 1963; Buttrose 1963*b*) and scutellar cells of wheat (Hrsel, Wolfova, and Mohelska 1961), cotyledons of pea (Bain and Mercer 1966*a*) and bean (Briarty, Coult, and Boulter 1969), and embryonic leaves of *Tropaeolum* (Nougarède 1963*a*). The changes during germination have been followed in a wide range of tissues, for example pea (Bain and Mercer 1966*b*) and bean (Opik 1966; Briarty, Coult, and Boulter 1970) cotyledons, pea embryo radicles (Yoo 1970), *Lactuca* embryos (Srivastava and Paulson 1968),

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embryonic leaves of *Tropaeolum* (Nougarède 1963*a*), and scutella of wheat (Nougarède and Pilet 1964) and barley (Nieuwdorp and Buys 1964).

When considering the changes that accompany maturation or follow imbibition, it is important to establish the structure of the mature, dry seed as a point of reference. Perner (1965) obtained useful information by fixing dry dormant pea radicles in OsO_4 vapour for several months, whereas Yatsu (1965) and Paulson and Srivastava (1968) used aqueous fixatives for dry embryos to avoid long fixation times. However, most studies of the changes in germinating embryos have involved soaking for various periods before fixation, because earlier techniques of fixation and embedding yielded unsatisfactory results with dry embryos (e.g. Nieuwdorp 1963; Nougarède and Pilet 1964).

The extent to which the structure of naturally dehydrated tissues is altered during aqueous fixation is unknown. Some hydration and swelling of cellular components must occur during aqueous fixation, altering structure to some extent from that which pertained in the air-dry state. Our preliminary attempts to use non-aqueous fixation procedures on wheat scutella were marred by poor infiltration and imperfect sections. However, the images that were obtained with these methods lead us to believe that while there must be structural changes induced, perhaps at the molecular level, by aqueous fixation, gross structural changes were not induced by the methods used in this study. Accordingly, all of the observations and illustrations are based upon specimens treated with aqueous fixatives. Although the results are superior to those achieved with earlier methods of specimen preparation, it is important that the structure of the air-dry state revealed in these micrographs be interpreted with caution.

In the mature grain, the embryo, which lies partly embedded in the endosperm at the base of the grain, is appressed to the endosperm by the scutellum (see Swift and O'Brien 1970, Fig. 1*a*). Specialized epithelial cells cover the surface of the scutellum adjacent to the endosperm and the remainder of the organ consists of storage parenchyma and vascular tissue.

The vascular system of the scutellum and its differentiation after germination have been described in earlier papers (Swift and O'Brien 1970, 1971). In this paper, the structure of the parenchyma cells and the epithelial cells is described in the air-dry state and in grains soaked for 3 hr in water.

II. Materials and Methods

Dry wheat grains (*Triticum aestivum* L. cv. Heron) and grains soaked for 3 hr in distilled water at 24°C were cut transversely through the embryo-containing region into slices about 1 mm thick. For light microscopy, the slices were fixed in 10% acrolein for 4 days at 0°C (0·025M phosphate buffer, pH 7·0). The tissues were then dehydrated and embedded in glycol methacrylate (Feder and O'Brien 1968), after prolonged periods (4 weeks or more) of cold infiltration (Kajtar, O'Brien, and Cohen 1970), and the scutellum was sectioned transversely at 1–2 μm. For electron microscopy, some of the slices were fixed in phosphate-buffered 6% glutaraldehyde for 3 hr, rinsed in buffer, and post-fixed in 2% OsO_4 for 10 min (longer times in OsO_4 gave inferior results). Other slices were exposed to 2% $KMnO_4$ for 1 hr. All fixations for electron microscopy were at room temperature. The tissues from both treatments were dehydrated in alcohol and embedded in a low viscosity epoxy resin (Spurr 1969). Sections were doubly stained with uranyl acetate and lead citrate and examined in an Hitachi HU 11E electron microscope at 75 kV.

III. Results

(a) *Light Microscopy*

Scutella from air-dry seeds and seeds soaked for 3 hr differed only in the structure of their chromatin aggregates when viewed with the light microscope. In the air-dry state, the chromatin aggregates are larger and more densely clumped (cf. Figs. 1 and 2). In both cases, the chromatin is well stained by toluidine blue but is almost unstained by anionic dyes (Fig. 3). On the other hand, the nucleoli (often 2–3 per nucleus, especially in the epithelial cells) take up both cationic and anionic dyes (Figs. 1–3).

Protein bodies are abundant in the epithelium and parenchyma of the scutellum and are the sites of protein and phytin storage. The protein matrix of these bodies stains strongly with anionic dyes such as fast green or acid fuchsin (Figs. 1 and 3) and is almost unstained by toluidine blue O (Fig. 2). Circular, unstained regions within the matrix in sections stained with the periodic acid–Schiff's reaction (PAS) before treatment with an anionic dye represent the positions from which the phytate deposits have been dissolved by the periodic acid treatment (Fig. 1). When stained with toluidine blue only, the phytate deposits stain metachromatically (Fig. 2). In the epithelial cells the protein bodies are usually about 1 μm in diameter, but in the parenchyma cells they may be up to 5 μm or more.

The ground substance of the cytoplasm also stains strongly and metachromatically with toluidine blue O. Epithelial cells show a higher level of cytoplasmic metachromasy than the cells of the parenchyma.

Lipid is present in abundance as small droplets in the cytoplasm but it is not retained by the techniques used for light microscopy unless the tissue is post-fixed in OsO_4 (see Fig. 4). After OsO_4 fixation, the lipid may be coloured by Sudan IV or Sudan black B but it cannot be resolved as discrete droplets in the light microscope, even in sections that are only 1 μm thick.

Starch and watery vacuoles are absent from all scutellar cells in air-dry grains.

(b) *Electron Microscopy*

(i) *Air-dry Scutella* (Figs. 4, 6, 8–10)

(1) *Membranes.*—In specimens fixed with glutaraldehyde–OsO_4, the electron contrast of the membranes varies considerably in different preparations. In most cases, the cell membrane and the membranes of plastids and mitochondria can be resolved clearly, even at low magnification (Figs. 4 and 6). The membranes of plastids stand out quite clearly in thin sections at high magnification (Figs. 9 and 10). The membranes of the nuclear envelope usually appear as shown in Figure 6, though images such as that shown in Figure 6, inset, are seen occasionally. The membrane that bounds the protein bodies can usually be demonstrated easily in thin sections (Figs. 9 and 10), but occasionally, these membranes are not revealed (Fig. 8).

These facts make it very difficult to assess the status of the endoplasmic reticulum (ER) and dictyosomes in these air-dry cells. Figures 8 and 9 show two different views of what may be rough ER present as stacks of short cisternae that tend to run between lipid droplets. Similar stacks of membrane-like material are not

seen in cells treated with $KMnO_4$ although cisternal elements of ER can be detected (Fig. 8, inset). No structure resembling a dictyosome can be identified in any of our micrographs.

(2) *Ribosomes.*—The strong affinity of these cells for cationic dyes is explained by the wealth of cytoplasmic ribosomes. Though polyribosomal aggregates appear to be absent, many ribosomes are present as sheets (rows in the thin sections) which partly encircle lipid droplets, protein bodies, and mitochondria (see especially Fig. 8).

(3) *Nucleus.*—The clumped dense aggregates of chromatin are the most marked feature of the nucleus (Fig. 6). Even though the nuclear envelope is often indistinct, the boundary between the nucleoplasm and cytoplasm is quite apparent. It is a remarkable fact that most of the dense chromatin aggregates are not in contact with the nuclear envelope (Fig. 6 and inset).

(4) *Mitochrondria and Plastids.*—Mitochondria are small ($0 \cdot 5$–$1 \cdot 0$ μm in diameter) and their appearance in sections suggests that they are mostly short rods and spheres. Cristae are poorly developed and the stroma appears clumped and lacks osmiophilic globules. Mitochondria are numerous in both the epithelium and the parenchyma. They appear to lie at random in the parenchyma cells whereas they are found mainly near the tips and bases of epithelial cells.

Plastids are less numerous, larger, and more irregular in shape than the mitochondria. They have a few internal membranes and some osmiophilic globules, but lack starch grains (Figs. 9, 10).

(5) *Lipid Bodies.*—Lipid droplets, $0 \cdot 5$–1 μm in diameter, are very numerous in the cytoplasm of epithelial and parenchyma cells. They form an almost uninterrupted line around the cell adjacent to the cell membrane and are dispersed liberally throughout the cytoplasm. Often they form a ring around the larger protein bodies (Figs. 4 and 6). In some cases, but not in all, the surface of the lipid droplets is bounded by a thin electron-dense line (cf. Figs. 6, 9, and 10 with Fig. 8).

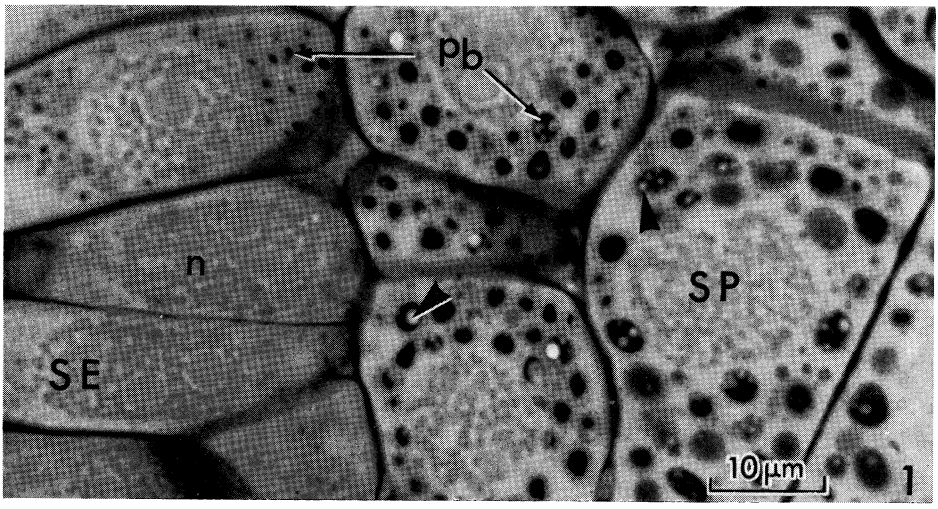
(6) *Protein Bodies.*—The protein bodies of the epithelial cells consist of a homogeneous, electron-dense protein matrix, in which one or a few large phytate deposits are embedded (Fig. 4). Often one very large phytate inclusion occupies almost the entire body and there is only a narrow peripheral band of proteinaceous material. In the dry seeds the protein bodies are almost spherical. The electron density of the protein matrix may vary slightly within the one cell.

Figs. 1–3.—Light micrographs of transverse sections of the scutellum. *DL*, depleted layer of endosperm; *SE*, cell of the scutellar epithelium; *SP*, cell of the scutellar parenchyma; *n*, nucleus; *pb*, protein body.

Fig. 1.—Scutellum of a dry embryo. Note the numerous small protein bodies in the epithelium and the large protein bodies in the parenchyma. Some of the protein bodies in the parenchyma contain unstained regions where phytate deposits have been lost from the section (arrowheads). The nuclear chromatin is clumped into dense aggregates. PAS–fast green FCF–toluidine blue O.

Fig. 2.—Scutellum 3 hr after soaking. Phytate deposits are the darkly stained bodies present in the epithelium and parenchyma (large arrowheads). The cytoplasmic ground substance stains metachromatically and pale or unstained regions within the cytoplasm represent areas occupied by protein bodies (small arrowheads). The chromatin appears granular and more dispersed than in Figure 1. Toluidine blue O.

Fig. 3.—Scutellum 3 hr after soaking. There is no evidence of protein body breakdown in the epithelium or parenchyma. PAS–fast green FCF.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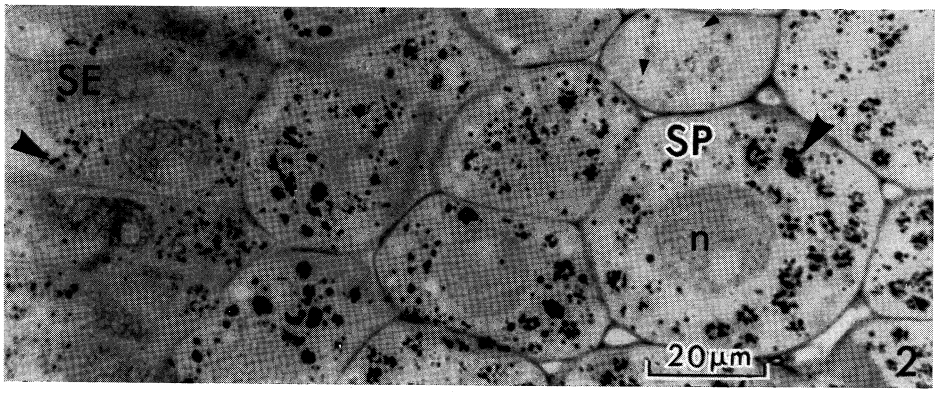
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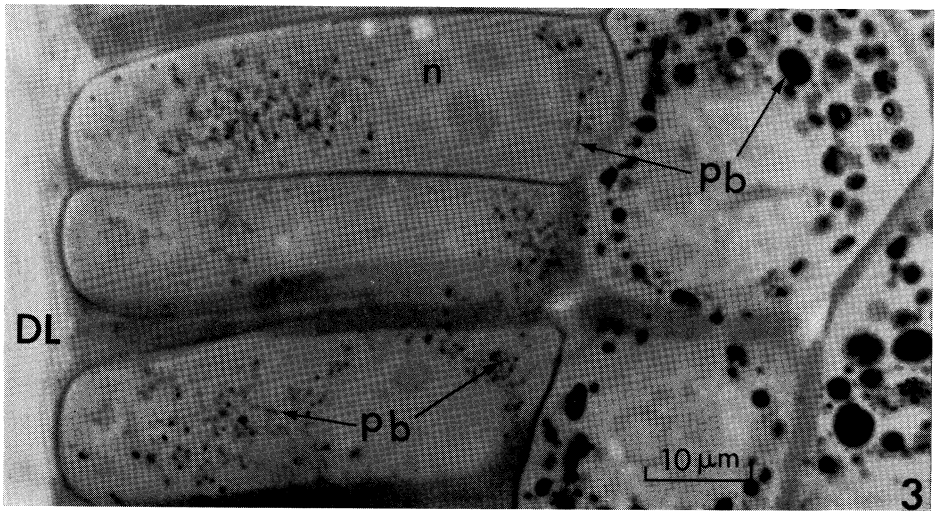
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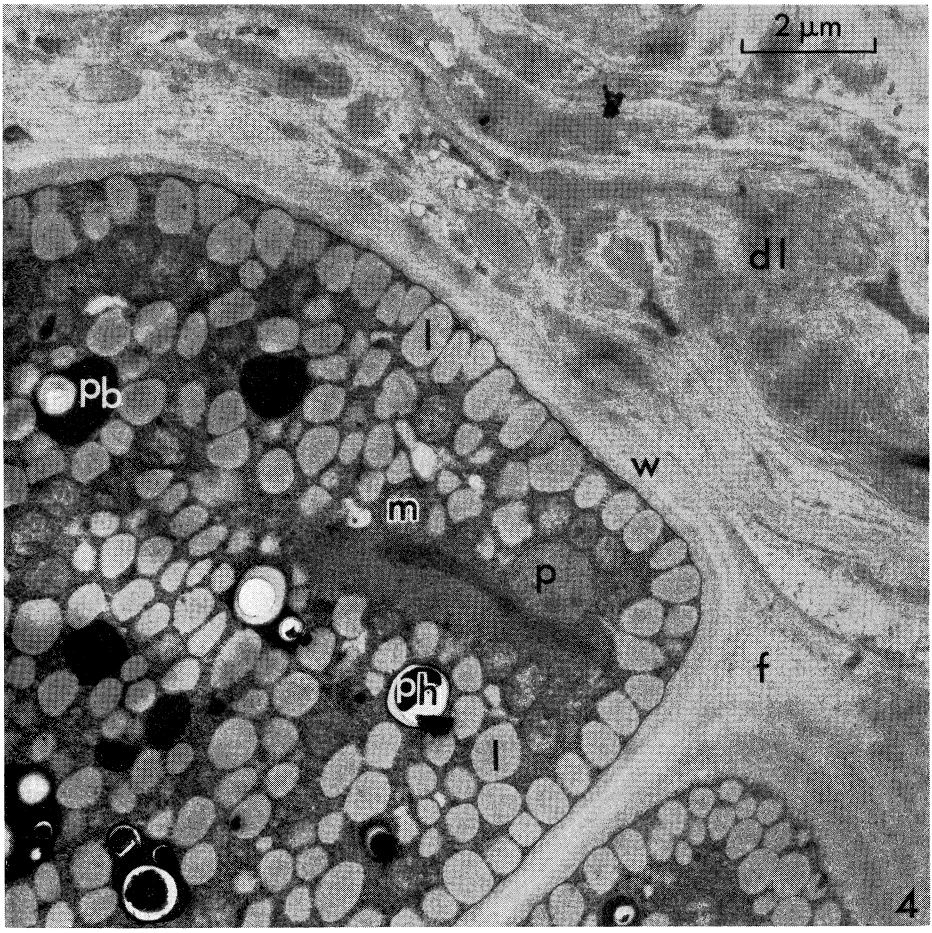


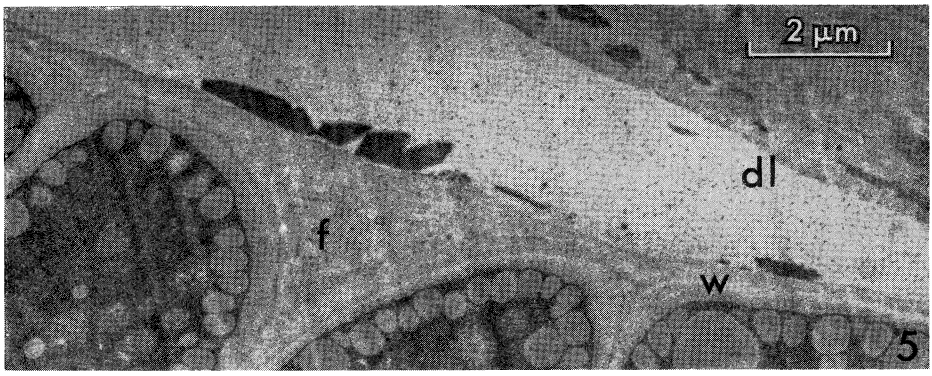
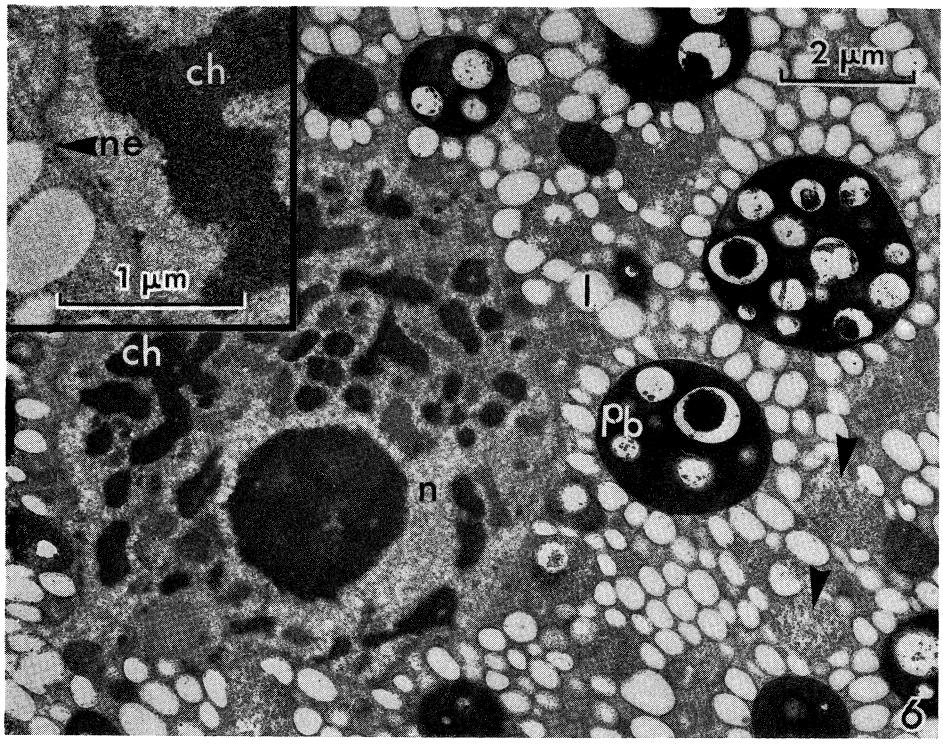


Fig. 4.—Electron micrograph of the apical ends of two adjacent epithelial cells and part of the depleted layer of the endosperm (*dl*) in a dry grain. The electron-dense cytoplasm contains mitochondria (*m*), plastids (*p*), numerous lipid bodies (*l*), and protein bodies (*pb*) with phytate inclusions (*ph*). Note the layer of lipid bodies next to the cell membrane. *f*, fibrous material between tips of epithelial cells; *w*, cell wall. Glutaraldehyde–OsO_4 fixation.

Fig. 5.—A view of the tips of the epithelial cells and part of the depleted layer (*dl*) after 3 hr soaking. *f*, amorphous material between the tips of the epithelial cells; *w*, epithelial cell wall. Glutaraldehyde–OsO_4.



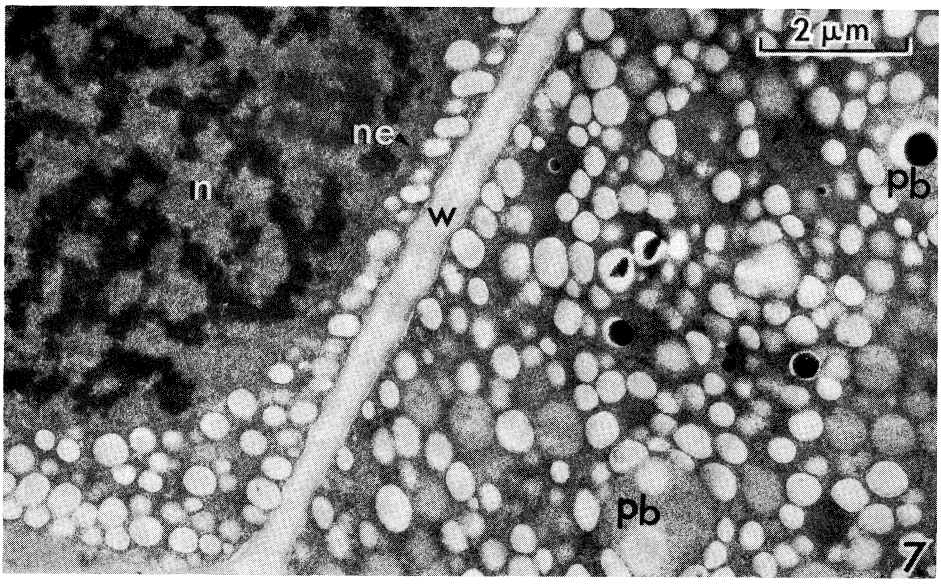
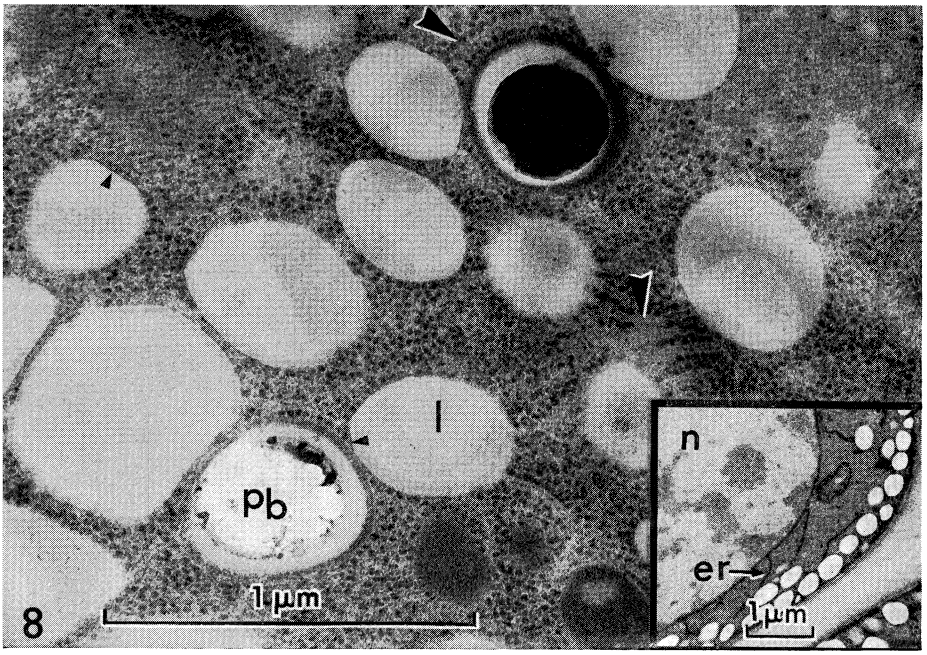
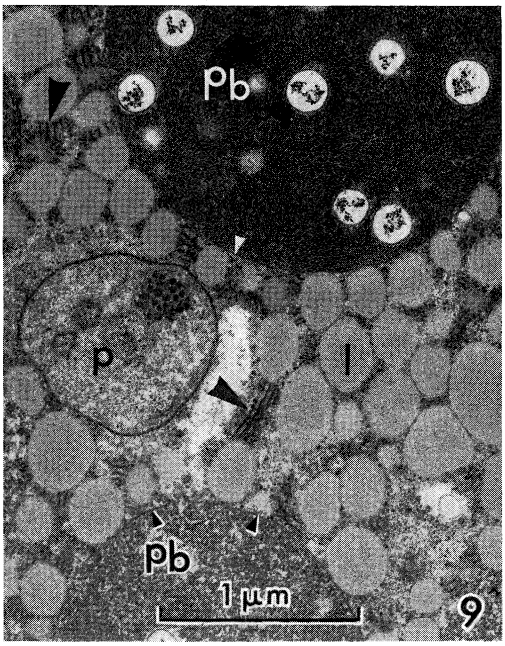


Fig. 6.—Dry scutellar parenchyma cell, showing the aggregated form of the chromatin (*ch*) in the nucleus (*n*), and the very large protein bodies (*pb*), some with numerous phytate inclusions. The protein matrix of some small protein bodies appears granular (arrowheads). The lipid droplets (*l*) form a layer around the protein bodies. *Inset:* an example of a more clearly defined nuclear envelope (*ne*), with chromatin (*ch*) withdrawn from it. Both glutaraldehyde–OsO_4.

Fig. 7.—The mid-region of two adjacent epithelial cells 3 hr after soaking. The chromatin of the nucleus (*n*) has dispersed into smaller aggregates some of which are in contact with the nuclear envelope (*ne*) (cf. Fig. 6). The protein matrix of some of the protein bodies (*pb*) is granular. *w*, cell wall. Glutaraldehyde–OsO_4.





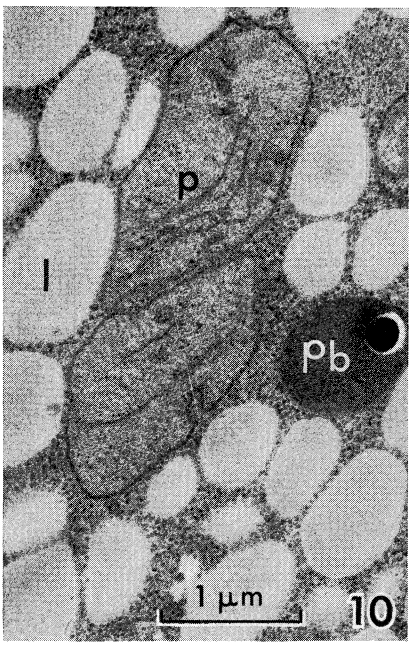


Fig. 8.—Portion of the cytoplasm of a dry epithelial cell showing the distribution of ribosomes. Rows of ribosomes (large arrowheads) lie between some lipid bodies (*l*) and around the protein bodies (*pb*). Many lipid bodies are partly surrounded by a sheet of ribosomes. An electron-dense lamella can be seen at the surface of some lipid bodies (small arrows). Glutaraldehyde–OsO_4. *Inset:* endoplasmic reticulum (*er*) in a dry epithelial cell; *n*, nucleus; $KMnO_4$ fixation.

In the parenchyma cells the protein bodies show a wider variation in size. Some are very large and contain many phytate deposits; these large bodies are usually almost spherical. In some of the smaller protein bodies the matrix is granular and less electron-dense (Fig. 6). A single membrane encloses the protein bodies (Fig. 9). The region of low electron contrast that often surrounds the phytate inclusions is probably the embedding medium (Fig. 4).

(7) *Cell Walls.*—Plasmodesmata are found between all scutellar cells. However, they do not occur at the tips of the epithelium where these cells are in contact with the depleted layer of the endosperm.

The cell walls at the tips of the epithelium consist of at least two layers, the inner of which is somewhat more electron-dense. Finely fibrous material lies between these walls at their tips (Fig. 4). The depleted layer of the endosperm consists of numerous layers of collapsed cell walls within which are a variety of structures that may be cytoplasmic remnants (Fig. 4).

(ii) *Scutella after Soaking for 3 hr* (Figs. 5, 7, 11–13)

Significant changes were detected in only three features of fine structure. The most obvious is the partial dispersal of the chromatin aggregates and the re-establishment of numerous regions of contact between the chromatin and nuclear envelope (Fig. 7). Secondly, in specimens treated with $KMnO_4$, profiles of ER (many of which lie close to the cell membrane) and structures that are probably small dictyosomes are apparent (Figs. 11 and 13). Thirdly, protein bodies with an amoeboid shape are more common in the epithelial cells (Figs. 12 and 13).

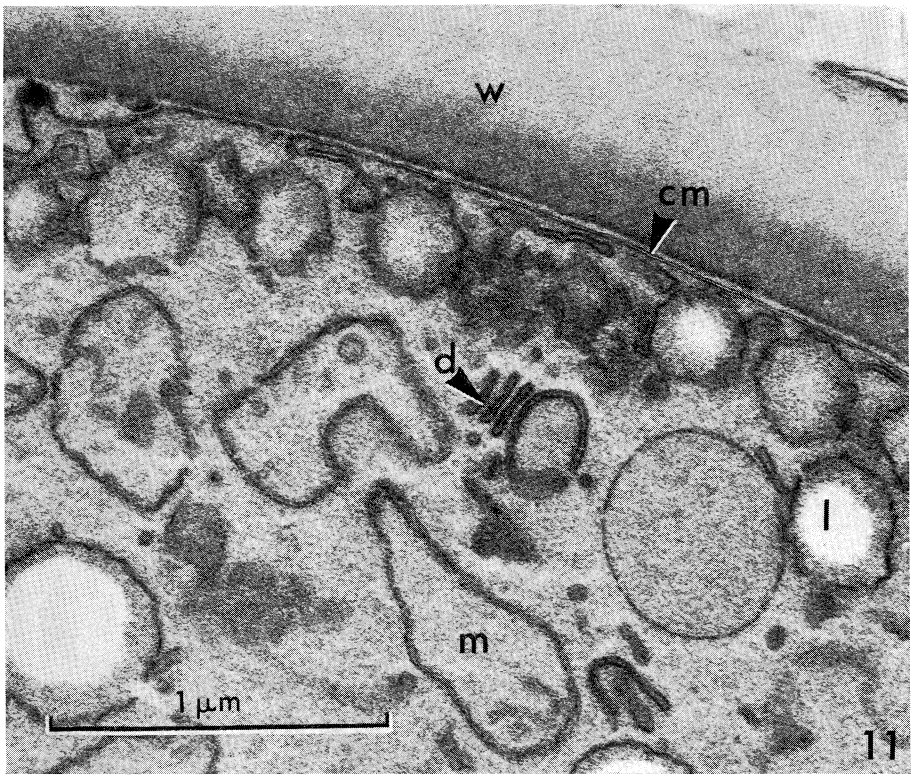
Several points, all of which could have been illustrated equally well in preparations of air-dry seeds, deserve further comment. Firstly, specimens treated with $KMnO_4$ and in which the cell membrane is resolved very clearly show a definite electron-dense layer at the surface of lipid droplets (Fig. 11). Secondly, the greater electron density of the inner layer of the epithelial cell wall is demonstrated very clearly by $KMnO_4$ treatment. Finally, it should be noted that the structure of the depleted layer of the endosperm varies considerably in different positions on the scutellar surface (cf. Figs. 4 and 5).

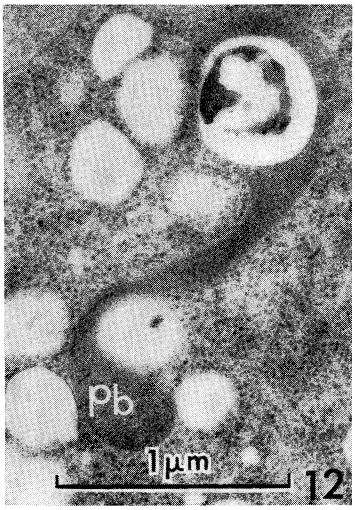
IV. Discussion

The structure of cells whose metabolism has been depressed to low levels by natural desiccation accompanying maturation is of considerable interest. It is, after all, the structure of a special kind of "living state", one whose metabolism can be returned to normal simply by rehydration at an appropriate temperature. The cells of the scutellar epithelium and the aleurone layer of cereal grains are especially

Fig. 9.—Portion of the cytoplasm in a dry parenchyma cell. Note the membrane-like structures (large arrowheads) that extend from one lipid body (*l*) to another. The plastid (*p*) contains osmiophilic droplets and a membrane can be distinguished around the protein bodies (*pb*) at the positions indicated by the small arrowheads. Glutaraldehyde–OsO_4.

Fig. 10.—Plastids and a protein body (*pb*) in a dry epithelial cell. Some internal membranes are present in the plastids. *l*, lipid body. Glutaraldehyde–OsO_4.





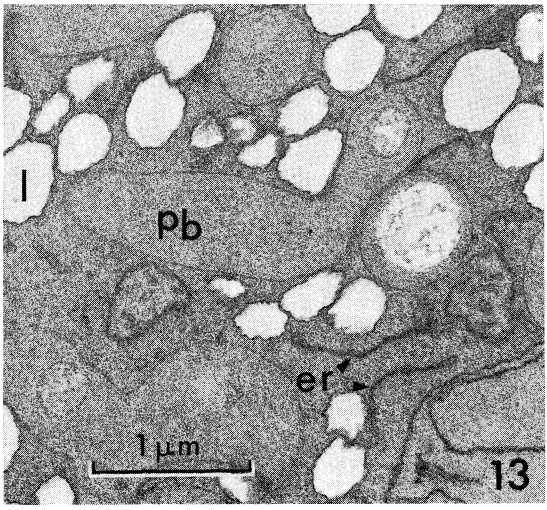


Fig. 11.—Portion of the cytoplasm and cell wall (*w*) at the tip of an epithelial cell 3 hr after soaking. A dictyosome (*d*) is present, but shows little structural differentiation. The mitochondria (*m*) have few cristae and lipid bodies (*l*) lie close to the cell membrane (*cm*). The inner wall material near the cell membrane is more electron-dense than the outer wall material. $KMnO_4$ fixation.

Fig. 12.—An amoeboid protein body (*pb*) in an epithelial cell after 3 hr soaking. Glutaraldehyde–OsO_4.

interesting in this connection since they lie so closely adjacent to the cells of the endosperm which are dead. In the past it has been difficult to obtain good images of the structure of any of the tissues of mature cereal grains whose high lipid content, dense cytoplasm, and resistance to infiltration led to poor preparations. The methods used in this work demonstrate that all of the organelles and membrane systems of these dry cells are now amenable to detailed study and that it should be possible to contrast the structure of maturing and mature endosperm cells with that of the embryo.

A number of other investigators have reported that in dry embryos, membranes are difficult to resolve, mitochondria are small with ill-defined cristae, free ribosomes are abundant, and the ER is sparse (e.g. Nieuwdorp 1963; Paulson and Srivastava 1968; Yoo 1970). We can confirm most of these observations in the wheat scutellum but the clarity with which membranes can be seen depends very much upon the time of exposure to OsO_4. Though one still encounters some cells in which membranes lack electron contrast, specimens treated with OsO_4 for only 10 min are usually quite satisfactory and the electron density of the ground substance is very much reduced. We urge all who are trying to study dry, lipid-rich tissues after aldehyde fixation to test very short exposures to OsO_4.

Dictyosomes could not be identified in the dry scutellum but structures that resemble them are present in tissues soaked for 3 hr. This suggests, but does not prove, that hydrophobic bonds may be important in stabilizing the membrane structure of dictyosomes. Dictyosomes were present in the scutellar cells of developing wheat embryos (Hrsel, Wolfova, and Mohelska 1961), and Bain and Mercer (1966*a*) suggest that dictyosomes are disorganized during seed maturation. Since 3 hr imbibition seems to be rather little time to manufacture membrane subunits *de novo*, perhaps dictyosome membranes are self-assembled during hydration from the dispersed subunits of dictyosomes that existed prior to maturation.

The most obvious difference between the structure of the dry seeds and those soaked for 3 hr is the appearance and distribution of the nuclear chromatin. Chromatin aggregates in hydrated cells are known to be regions rich in DNA–histone complexes (John and Lewis 1969) and such aggregates are known to be relatively inactive in RNA synthesis (Karasaki 1965; John and Lewis 1969). It has been demonstrated (Comings and Kakefuda 1968) that DNA replication commences in the chromatin that is closely appressed to the nuclear envelope, and in hydrated cells chromatin aggregates do not normally dissociate from the nuclear envelope until the aggregates have formed late-prophase chromosomes during mitosis. Clearly, the structure of these nuclei and their metabolic activity during dehydration and rehydration provide ample opportunity for future study, especially since Nir, Klein, and Poljakoff-Mayber (1969) have shown that nuclear chromatin aggregates during desiccation under experimental conditions.

The structure of the surface of lipid droplets continues to be controversial. Many workers identify what they believe to be a membrane around lipid droplets

Fig. 13.—Part of the cytoplasm of an epithelial cell 3 hr after soaking. showing amoeboid and spherical protein bodies (*pb*), short membranes of endoplasmic reticulum (*er*), and lipid bodies (*l*). $KMnO_4$ fixation.

(e.g. Nieuwdorp 1963, barley scutellum, $KMnO_4$ or OsO_4 fixation; Nougarède and Pilet 1964, wheat scutellum, OsO_4 fixation; Yoo 1970, pea-embryo radicle, glutaraldehyde–OsO_4 fixation; Buttrose 1970, freeze-etched wheat aleurone) while others deny its existence (O'Brien 1967, oat coleoptile; Paulson and Srivastava 1968, lettuce embryos, both glutaraldehyde–OsO_4 fixation). The observations presented here sometimes reveal a thin, electron-dense lamella at the surface of the lipid droplets, both in specimens fixed in glutaraldehyde–OsO_4 or $KMnO_4$ (Figs. 8, 10, 11, and 13). We incline towards Dixon's view (1970) that such lipid droplets may contain not a bimolecular leaflet of lipid and protein at their surface but a monomolecular layer of phospholipid, perhaps with some protein adsorbed. Though almost nothing is known about the reactions of $KMnO_4$ with phospholipids and proteins, it is known that OsO_4 will react with the polar end of some phospholipids (Stoeckenius 1959). The ability to demonstrate an electron-dense lamella at the surface of lipid droplets might well depend upon the amount and type of phospholipid present in the cell.

The fine structure of protein bodies has been described for a wide range of tissues and there is considerable variation in the appearance and composition of the reserve materials they contain (Altschul *et al.* 1966). The terms "globoid", "crystal" and "crystalloid" have been used to describe the various inclusions found embedded in the protein matrix, but there is a lack of consistency in the use of these terms by different workers, so they have not been used here. In this study two forms of reserve material are identified: a homogeneous, electron-dense protein matrix, and phytate deposits, which are insoluble salts of phytic acid. The protein bodies in aleurone cells contain an additional inclusion (Jones 1969) that was not seen in the scutellar bodies.

Many workers have established the presence of a membrane around the protein bodies of mature seeds (e.g. Nougarède and Pilet 1964; Paleg and Hyde 1964; Briarty, Coult, and Boulter 1970) and studies of immature seeds indicate that protein bodies are formed by secretion of proteins into vacuoles (Guillermond 1941; Graham *et al.* 1962; Nougarède 1963*a*, 1963*b*; Buttrose 1963*a*; Briarty, Coult, and Boulter 1969; Bain and Mercer 1966*a*). In the present study the protein matrix of some of the small protein bodies was granular and appeared to have been partly broken down. These structures may represent vacuoles that were not completely filled with protein at the time of maturation of the grain, or it is possible that some of the reserves stored in the protein bodies had been used already by the developing embryo before maturation. The proteins in the completely dry protein bodies may be in the crystalline state, as Perner (1965) found in pea roots fixed in OsO_4 vapour.

We suggest that hydrolysis of the protein bodies in the epithelium is initiated very rapidly after hydration, even within 3 hr. Nougarède and Pilet (1964) state that imbibition is very rapid in the epithelium and much slower and irregular in the parenchyma cells. After 4 hr soaking they found that breakdown of the protein bodies in the epithelial cells had commenced.

V. Acknowledgements

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