

Development of Oat Aleurone Cells and Their Protein Bodies¹

DAVID M. PETERSON,² ROY H. SAIGO,³ and JON HOLY³

ABSTRACT

Cereal Chem. 62(5):366-371

The development of oat (*Avena sativa* L.) aleurone cells was studied by electron microscopy of tissues from various stages of development. Aleurone protein bodies were formed in vacuoles by sequential deposition of phytin and protein. Light micrographs of sections stained for protein and electron micrographs of sections digested with pepsin confirmed the proteinaceous nature of the protein bodies. A densely stained region within the protein component of the protein body appeared analogous to the niacin-containing inclusion (protein-carbohydrate body) described in

barley, wheat, and oats. Starch grains were present in aleurone cells during early stages of development but disappeared with maturation. Lipid bodies increased in number in aleurone cells up to 10 days after anthesis. No evidence for endoplasmic reticulum origin of lipid bodies was seen. Rough endoplasmic reticulum was present but was not as abundant in the aleurone as in the starchy endosperm. Free ribosomes were numerous in the cytoplasm of younger specimens. Dictyosomes were infrequent.

During early development of cereal endosperm, cambium-like cells in a peripheral layer adjacent to the inner nucellar epidermis divide tangentially to form starchy endosperm and subaleurone cells (Evers 1970). Later, this outer layer of cells ceases mitotic activity and develops thick walls and other characteristics of mature aleurone cells. Despite their similar origin, aleurone cells become morphologically distinct from those of the subaleurone and starchy endosperm; at maturity they lack starch grains and have more abundant lipid bodies and a different type of protein body than starchy endosperm cells. Aleurone protein bodies are markedly different from those of the starchy endosperm (Bechtel and Pomeranz 1981), although their protein composition is similar (Donhowe and Peterson 1983). The primary differences between protein bodies of the two tissues are presence, in the aleurone protein bodies, of a phytin globoid surrounded by a protein matrix and, within the protein matrix, electron dense inclusions termed protein-carbohydrate bodies (Jacobsen et al 1971) or protein crystalloids (Lott 1980). Other workers have referred to these inclusions as Type I and Type II, respectively (Fulcher et al 1981).

Development of the oat aleurone layer has not been studied at the ultrastructural level, although such studies have been conducted with wheat (Buttrose 1963, Morrison et al 1975) and maize (Kyle and Styles 1977). Previously, we published light micrographs showing aleurone layer development in oats (Saigo et al 1983).

In this work our aim was to document aleurone cell development in oats by electron microscopy of tissues fixed at different stages of maturity beginning shortly after anthesis. Particular attention was given to formation of protein bodies. Comparisons are made with oat starchy endosperm development (Saigo et al 1983) and with aleurone development in wheat (Morrison et al 1975).

MATERIALS AND METHODS

Culture of oats (*Avena sativa* L. cv. Goodland), harvest of the kernels, preparation of tissues, and electron microscopy have been described previously (Saigo et al 1983). The caryopses examined weighed 11, 15, 18, 25, and 35 mg (fresh weight), which corresponds to about four to 16 days after anthesis (Saigo et al 1983).

¹Research supported by the United States Department of Agriculture, Agricultural Research Service, the School of Arts and Sciences, University of Wisconsin-Eau Claire and the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

²Cereal Crops Research Unit, USDA/ARS and Department of Agronomy, University of Wisconsin-Madison 53706.

³Department of Biology, University of Wisconsin-Eau Claire 54701. Present address of R. H. Saigo: College of Natural Sciences, University of Northern Iowa, Cedar Falls 50614. Present address of J. Holy: Department of Anatomy, University of Wisconsin-Madison 53706.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. American Association of Cereal Chemists, Inc., 1985.

Protein Digestion

After primary fixation in 4% paraformaldehyde and 2.5% glutaraldehyde (0.1M cacodylate buffer, pH 6.8), specimens were incubated in 0.4% pepsin in 0.02N HCl at 40°C overnight. They were rinsed in distilled water, postfixed in 1% OsO₄ in 0.1M cacodylate buffer, and embedded in Spurr's low viscosity resin as before (Saigo et al 1983).

Light Microscopy

Thin (1 μm) sections of tissue digested in pepsin were stained with Ponceau 2R using the procedure of Gori (1978). Undigested identical specimens were treated similarly.

RESULTS

Aleurone Cell Development

At 11 mg (Fig. 1) the roughly rectangular peripheral endosperm (future aleurone) cells were surrounded by thin cell walls. The vacuoles in these cells varied from 1 to 8 μm in diameter. Most of the vacuoles appeared empty, but some contained a lightly distributed flocculent material, and a few had densely stained deposits that appeared to be phytin of the developing globoid inclusion. The cytoplasm contained numerous free ribosomes and some rough endoplasmic reticulum (RER). Mitochondria, amyloplasts (with starch grains), and lipid bodies were present, but dictyosomes were not observed.

At the 15-mg stage (Fig. 2) vacuoles were more numerous but smaller, and many of them contained small phytin deposits. Some vacuoles contained internal membranes. The abundance of free ribosomes and RER was similar to the 11-mg stage. Mitochondria and amyloplasts were present, and lipid bodies appeared to be more numerous, but dictyosomes were infrequent.

Vacuoles of aleurone cells of 18-mg caryopses (Fig. 3) were smaller than those of younger cells, and they were uniformly about 1 μm in diameter. These aleurone vacuoles had now accumulated considerable amounts of phytin forming the globoid inclusion. The number of lipid bodies had increased as compared to younger stages. A few large plastids containing starch grains were present.

By the 25-mg stage (Fig. 4), cell walls had thickened considerably. The developing protein bodies had increased in diameter as compared to younger caryopses. Globoid inclusions frequently appeared missing because shattering during sectioning (Lott 1980) left vacant globoid cavities. Flocculent material at the periphery of the aleurone vacuoles surrounding the globoid inclusion is believed to be proteinaceous. Lipid bodies were smaller than before, except those adjacent to the cell wall. Mitochondria were about as abundant as before, but starch-containing plastids were no longer present. The cytoplasm, now reduced in proportion to the vacuoles and lipid bodies, still contained both RER and free ribosomes. Dictyosomes were not observed.

At 35 mg (Fig. 5), the cells of the caryopsis appeared to be fully developed; further maturation consisted of dehydration of the

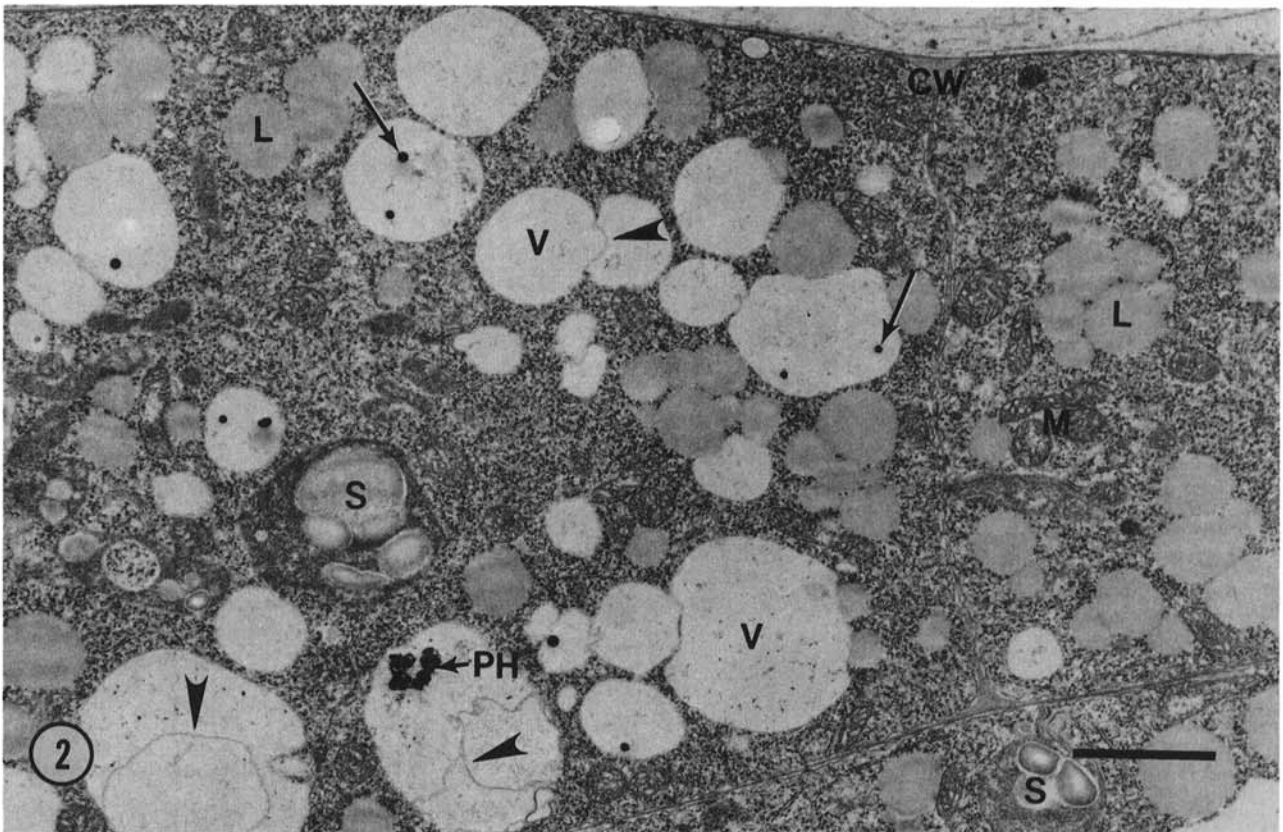
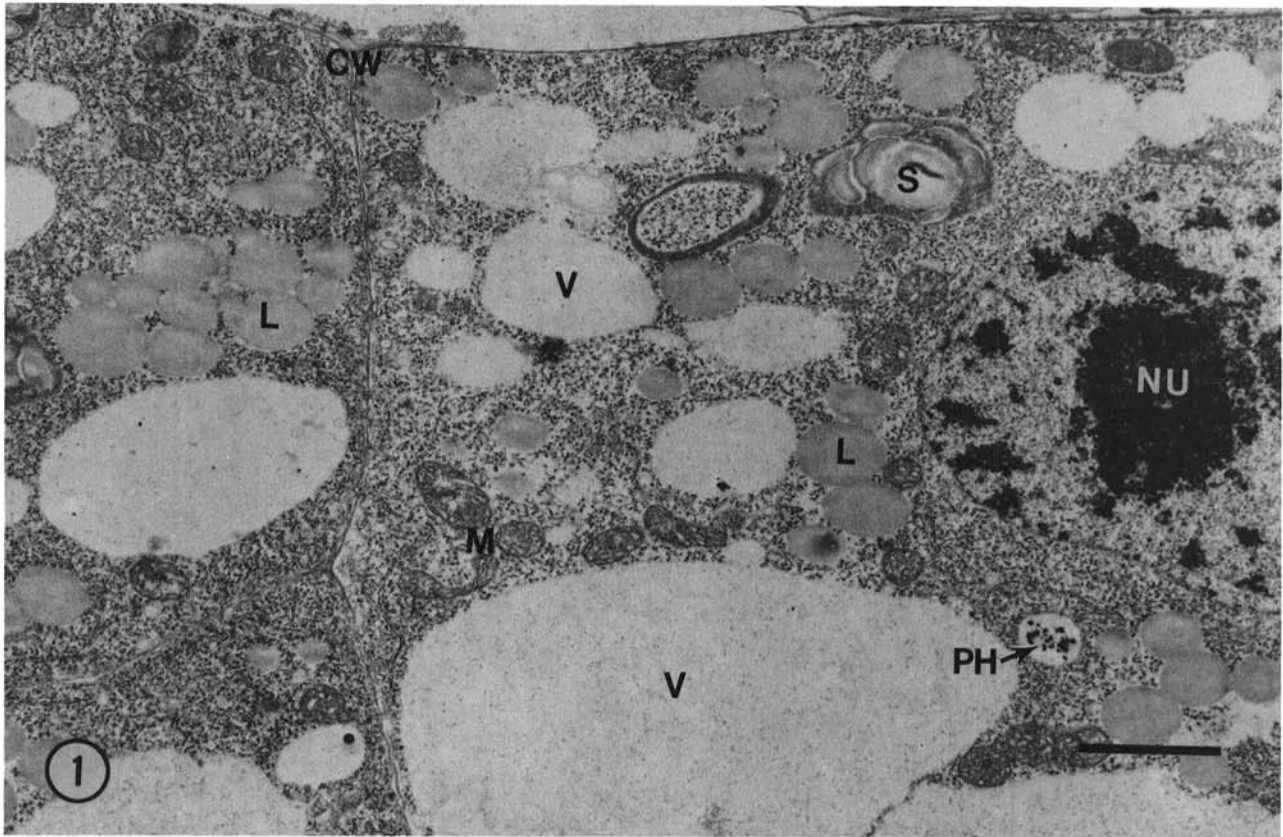


Fig. 1. Aleurone cells from an 11-mg caryopsis showing large vacuoles that appear empty. A small vacuole contains a dense deposit that appears to be phytin. Bar = 2 μ m. **Fig. 2.** Similar cells from a 15-mg caryopsis have smaller vacuoles, and phytin deposits (arrows) within are more numerous. Some vacuoles have internal membranes (arrowheads). Bar = 2 μ m. CW, cell wall; L, lipid body; M, mitochondrion; NU, nucleolus; PH, phytin; S, starch grain; and V, vacuole.

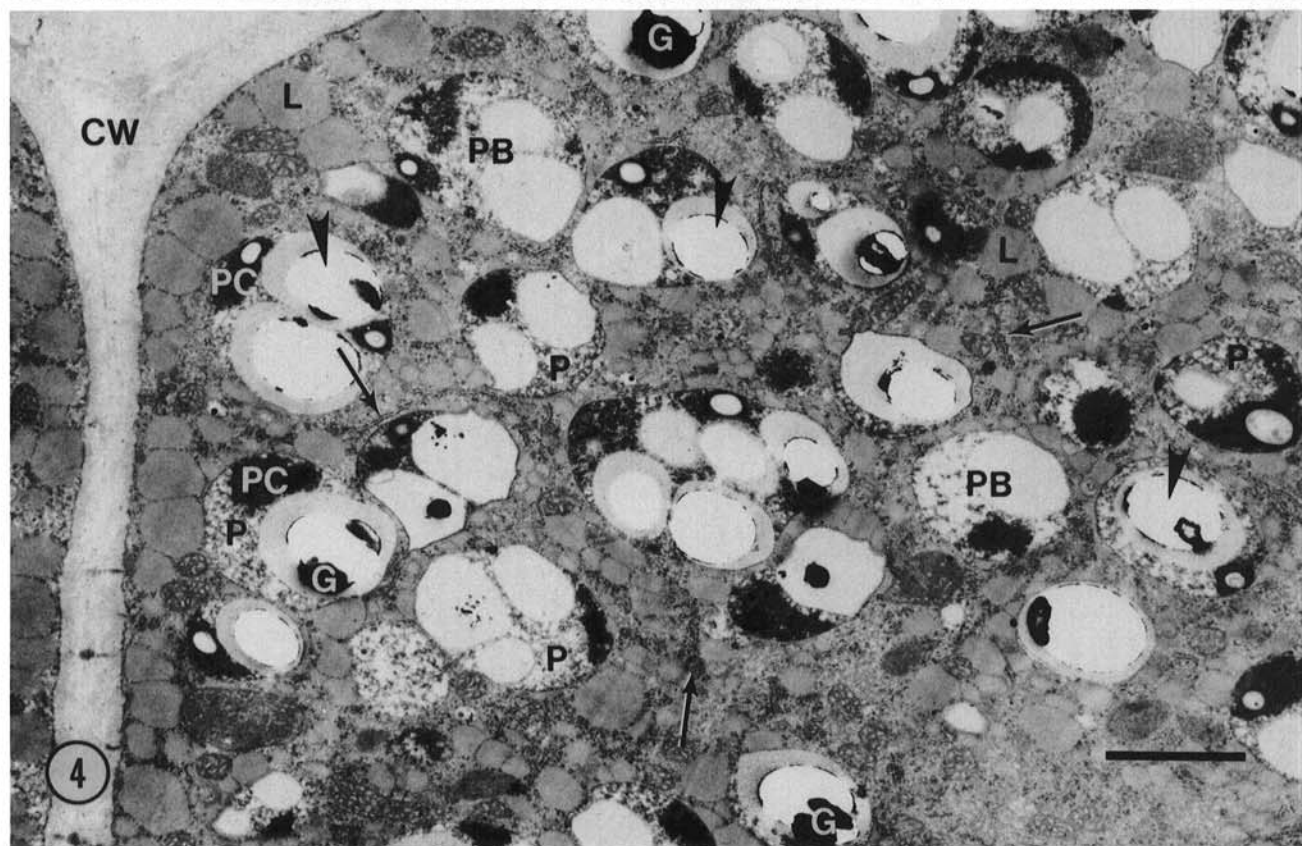
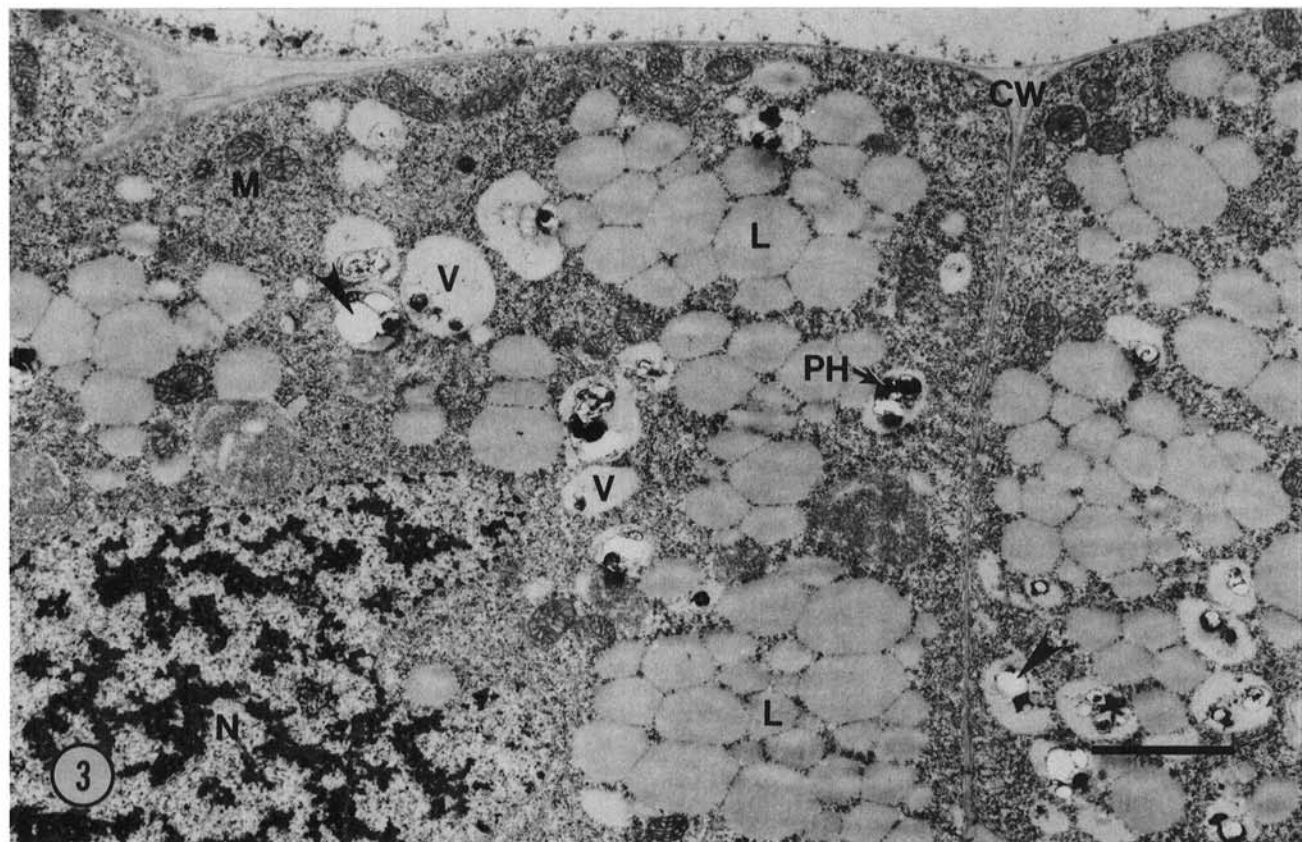


Fig. 3. Aleurone cells from an 18-mg caryopsis have more numerous lipid bodies. Vacuoles are smaller, and most contain phytin deposits (globoids). Sometimes the globoid inclusions have become torn from the section, leaving a hollow cavity (arrowheads). Bar = 2 μ m. **Fig. 4.** In aleurone cells at the 25-mg stage, vacuoles, now containing phytin and protein, are larger and entirely filled, except for the hollow cavities (arrowheads). The proteinaceous deposits contain dense areas that correspond to protein-carbohydrate bodies. As in younger stages, some rough endoplasmic reticulum is present (arrows). Cell walls have thickened considerably. Bar = 2 μ m. CW, cell wall; G, globoid; L, lipid body; M, mitochondrion; N, nucleus; P, protein; PB, protein body; PC, protein-carbohydrate body; PH, phytin; and V, vacuole.

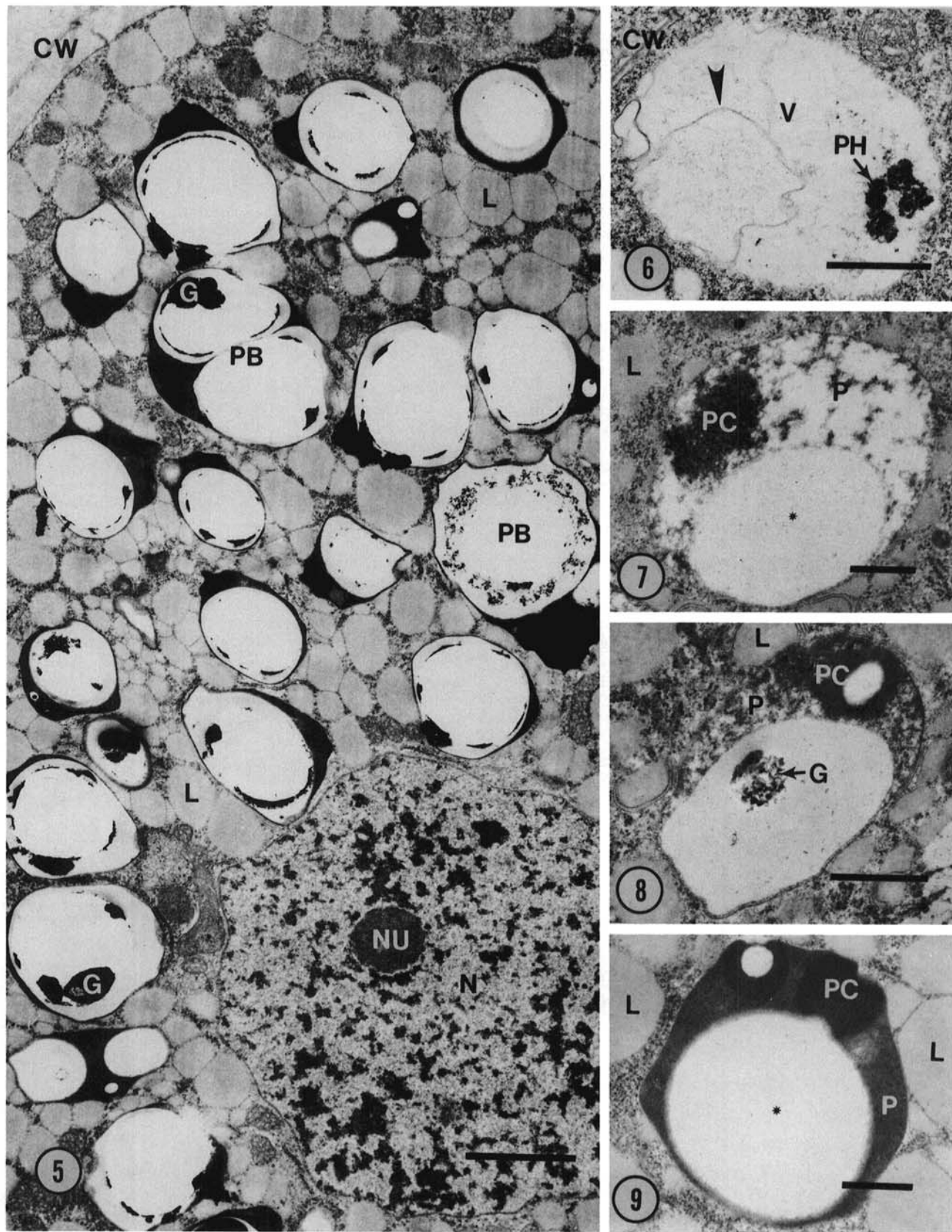


Fig. 5. Cells from a 35-mg caryopsis showing numerous large protein bodies and smaller lipid bodies. Most globoid inclusions have been lost from the section, leaving only the globoid cavities. Bar = 2 μ m. **Figs. 6-9.** Development of protein bodies from vacuoles. **6,** Enlargement from Fig. 1 showing vacuole from aleurone cell of an 11-mg caryopsis. Note phytin deposit (PH) and internal vacuolar membrane (arrowhead). Bar = 1 μ m. **7,** Enlargement from Fig. 4 showing vacuole from aleurone cell of a 25-mg caryopsis. Protein is deposited on the periphery of the globoid cavity (*). The dense area appears to be forming a protein-carbohydrate body (PC). Bar = 0.5 μ m. **8,** Protein body from a 25-mg caryopsis, containing more protein (P) than the previous one in Fig. 7. A portion of the globoid (G) is present in the globoid cavity. Bar = 0.5 μ m. **9,** Protein body from aleurone cell of 35-mg caryopsis, showing completely condensed protein (P) with protein-carbohydrate bodies (PC) surrounding a globoid cavity (*). Bar = 0.5 μ m. CW, cell wall; G, globoid; L, lipid body; N, nucleus; NU, nucleolus; PB, protein body; V, vacuole.

caryopsis. Aleurone cells at this stage were now arranged as a single layer of cells having thick cell walls. These aleurone cells contained primarily a nucleus, protein bodies, and lipid bodies, which together almost completely filled the cellular space. The remaining cytoplasm contained RER, free ribosomes, mitochondria, and a few plastids without starch.

Protein Body Development

Protein bodies were formed by progressive deposition of phytin and protein within the vacuoles, beginning with phytin (Figs. 6–9). Whereas the 11-mg stage vacuoles generally lacked electron-dense materials (Fig. 1), by 15 mg, vacuoles containing phytin deposits were frequently observed (Fig. 6). Typically the vacuoles at this stage were subdivided by intravacuolar membranes (Figs. 2 and 6). These membranes could serve to separate the phytin deposits from the protein, which first appeared at the 25-mg stage (Fig. 7). At this stage, the phytin-containing (globoid) cavity was roughly spherical. The phytin itself had either shattered during sectioning or occupied only a small portion of the cavity, thus causing its absence in the plane of this section. The protein component first appeared as flocculent material surrounding the globoid cavity. A denser region within the protein matrix was already apparent and might be the niacin-containing inclusion (protein-carbohydrate body) previously described (Jacobsen et al 1971, Fulcher et al 1981). With maturation, this inclusion condensed (Fig. 8) and sometimes exhibited a hollow center. Near maturity, protein bodies contained large globoid cavities (typically one but sometimes two) surrounded by a protein matrix in which one or more inclusions were embedded (Fig. 9).

The protein stain, Ponceau 2R, specifically stained the protein bodies of the aleurone layer and the starchy endosperm (Fig. 10). Predigestion of sections with pepsin completely eliminated the stainable substance (not shown). Electron micrographs of pepsin-digested sections showed the complete removal of aleurone protein bodies including globoids (Fig. 11). Dense, granular material that may or may not be proteinaceous remained in the cytoplasm, however.

Lipid Body Development

Lipid bodies were present in the earliest specimens examined (Fig. 1). They ranged in size from about 0.5 to 1.5 μm in diameter. No apparent connections to endoplasmic reticulum (ER) were observed. The number of lipid bodies increased considerably between the 15- and 18-mg stage, although the size range remained about the same (Figs. 2 and 3). In 25-mg caryopses, a reorientation

of lipid bodies adjacent to the newly thickened cell walls was apparent (Fig. 4). The average size of those remaining among the enlarging protein bodies was smaller than before and smaller than those at the periphery of the cells.

DISCUSSION

This is the first report on the development of the aleurone layer of oats at the ultrastructural level. Among the cereals, oats is unusually high in protein and lipid. An analysis of hand-dissected oat caryopsis fractions showed that the bran contained 18–32% protein (Youngs 1972) and 6–10% lipid (Youngs et al 1977), concentrations considerably higher than in the starchy endosperm.

Our studies show that aleurone protein bodies form by sequential and progressive deposition of phytin and protein into vacuoles. Phytin was deposited before any evidence of protein deposition was seen; it was observed as early as the 11-mg stage. Vacuoles at early stages were often subdivided by internal membranes (Fig. 6) indicating the existence of a mechanism for compartmentalization of phytin from later deposited protein. However, membranes were not observed surrounding the globoid of protein bodies at later stages. Protein first appeared as a flocculent material surrounding the globoid cavity (Fig. 7). During maturation, the vacuolar space surrounding the cavity was filled, except for one or more areas of greater density that also contained small cavities. These dense areas in the protein matrix appear equivalent to those described previously (Jacobson et al 1971, Fulcher et al 1981). These inclusions stained positively for protein and carbohydrate in barley (Jacobson et al 1971), but Fulcher et al (1981) could not detect the presence of protein. The later group found these inclusions to be a major site of niacin deposition in wheat, oats, and barley.

Also in wheat (Morrison et al 1975), phytin was deposited first into vacuoles. However, little was deposited until after cell walls had thickened, causing these workers to speculate that oxidation of *myo*-inositol to form cell wall polysaccharides suppressed phytin synthesis. In oats, most of the vacuoles contained phytin before cell walls thickened (Fig. 3). It was difficult to estimate the relative quantity of phytin at different stages because of the loss of the globoid inclusions from the sections, but judging from the number and size of the protein bodies and the size of the globoid cavities, we suggest that, as in wheat, the majority of phytin was synthesized during or after cell walls had thickened.

The stage where protein deposition commenced coincided with an apparent increase in RER (Figs. 3 and 4), although RER is never

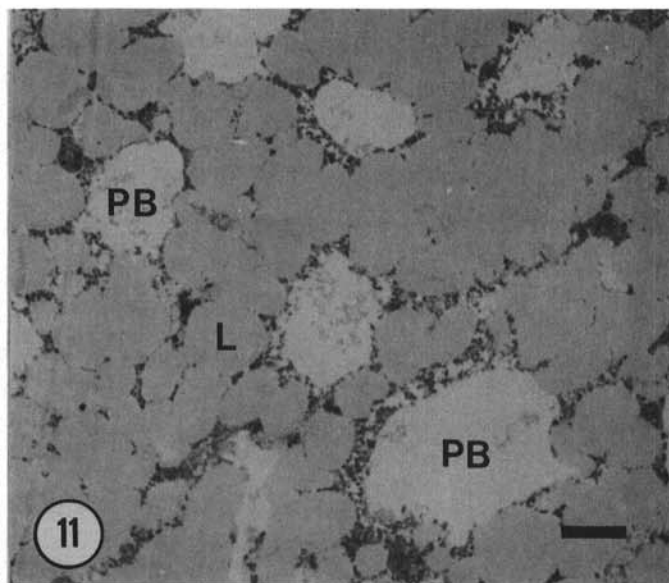
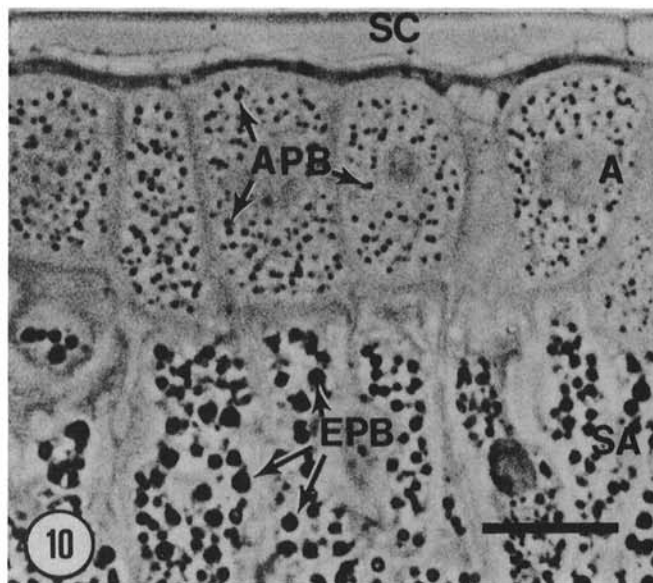


Fig. 10. Light micrograph of aleurone layer and subaleurone cells of starchy endosperm stained for protein with Ponceau 2R. Bar = 25 μm . Fig. 11. Electron micrograph of aleurone cell from tissue of a 35-mg caryopsis that had been predigested with pepsin. Bar = 1 μm . A, aleurone; APB, aleurone protein body; EPB, endosperm protein body; L, lipid body; PB, protein body; SA, subaleurone; SC, seed coat.

abundant in aleurone as compared to subaleurone cells (Saigo et al 1983). Free ribosomes remained abundant in the cytoplasm although cytoplasmic volume diminished considerably as protein bodies enlarged (Fig. 4). We do not know whether free or bound ribosomes are active in synthesis of the storage protein that is deposited in the protein bodies. Dictyosomes have been implicated in the transport of storage proteins into protein bodies in other species, particularly the cotyledons of dicots (Lott 1980). However, because of their scarcity, we suggest that they may not be an important part of the mechanism for protein deposition into protein bodies in oat aleurone. Neither did we observe any direct ER-vacuole connections as found in subaleurone cells (Saigo et al 1983). Thus, a mechanism whereby protein synthesized on the ribosomes is transported into the protein bodies was not evident. In wheat, RER was abundant during the period of protein deposition (Morrison et al 1975), whereas in corn, like oats, free ribosomes predominated during this period (Kyle and Styles 1977).

Histochemical staining with Ponceau 2R and digestion with pepsin confirmed the proteinaceous nature of the aleurone protein bodies. The loss of globoid inclusions in the pepsin-digested sections does not necessarily suggest that these inclusions contain protein, because Bechtel and Pomeranz (1981) found that globoid inclusions of mature oat aleurone protein bodies were lost by treatment with any other several enzymes or even with buffer.

We found starch in the aleurone layer of very young caryopses, but it did not persist through maturation. Similar results have been reported with wheat (Buttrose 1963, Morrison et al 1975). Aleurone cells apparently lose the capacity for starch synthesis early in development, whereas in the underlying starchy endosperm, starch becomes the primary storage reserve.

The formation of lipid bodies has not been addressed in cereal aleurone tissue, but several reports from oilseeds are available. In some species, lipid-containing vesicles were seen by electron microscopy to originate from the ER (Schwarzenbach 1971, Wanner and Theimer 1978). Other workers found no evidence for ER involvement, and they postulated a cytoplasmic origin for lipid bodies (Rest and Vaughn 1972, Bergfeld et al 1978). The former type have been called spherosomes, and the latter oleosomes (Bergfeld et al 1978).

In the oat aleurone cells, we found no evidence of ER connections to lipid bodies in the stages we investigated. These lipid bodies therefore appear to be of the oleosome type. While we cannot rule out an ER origin of lipid bodies in caryopses smaller than 11 mg, there was very little ER present in caryopses at this stage, so this would seem unlikely.

LITERATURE CITED

- BECHTEL, D. B., and POMERANZ, Y. 1981. Ultrastructure and cytochemistry of mature oat (*Avena sativa* L.) endosperm. The aleurone layer and starchy endosperm. *Cereal Chem.* 58:61.
- BERGFELD, R., HONG, Y.-N., KUHN, T., and SCHOPFER, T. 1978. Formation of oleosomes (storage lipid bodies) during embryogenesis and their breakdown during seedling development in cotyledons of *Sinapis alba* L. *Planta* 143:297.
- BUTTROSE, M. S. 1963. Ultrastructure of the developing aleurone cells of wheat grain. *Aust. J. Biol. Sci.* 16:768.
- DONHOWE, E. T., and PETERSON, D. M. 1983. Isolation and characterization of oat aleurone and starchy endosperm protein bodies. *Plant Physiol.* 71:519.
- EVERS, A. D. 1970. Development of the endosperm of wheat. *Ann. Bot.* 34:547.
- FULCHER, R. G. 1972. Observations on the aleurone layer with emphasis on wheat. Ph.D. dissertation. Monash University: Clayton, Victoria, Australia.
- FULCHER, R. G., O'BRIEN, T. P., and WONG, S. I. 1981. Microchemical detection of niacin, aromatic amine, and phytin reserves in cereal bran. *Cereal Chem.* 58:130.
- GORI, P. 1978. Ponceau 2R staining of proteins and periodic acid bleaching of osmicated subcellular structures on semithin sections of tissues processed for electron microscopy: a simplified procedure. *J. Microsc.* 114:111.
- JACOBSEN, J. V., KNOX, R. B., and PYLIOTIS, N. A. 1971. The structure and composition of aleurone grains in the barley aleurone layer. *Planta* 101:189.
- KYLE, D. J. and STYLES, E. D. 1977. Development of aleurone and subaleurone layers in maize. *Planta* 137:185.
- LOTT, J. N. A. 1980. Protein bodies. Pages 589-623 in: *The Biochemistry of Plants*. Vol. 1. The Plant Cell. N. E. Tolbert, ed. Academic Press: New York.
- MORRISON, I. N., KUO, J., and O'BRIEN, T. P. 1975. Histochemistry and fine structure of developing wheat aleurone cells. *Planta* 123:105.
- REST, J. A., and VAUGHN, J. G. 1972. The development of protein and oil bodies in the seed of *Sinapis alba* L. *Planta* 105:245.
- SAIGO, R. H., PETERSON, D. M., and HOLY, J. 1983. Development of protein bodies in oat starchy endosperm. *Can. J. Bot.* 61:1206.
- SCHWARZENBACH, A. M. 1971. Observations on spherosomal membranes. *Cytobiology* 4:145.
- WANNER, G., and THEIMER, R. R. 1978. Membranous appendices of spherosomes (oleosomes). Possible role in fat utilization in germinating oil seeds. *Planta* 140:163.
- YOUNGS, V. L. 1972. Protein distribution in the oat kernel. *Cereal Chem.* 49:407.
- YOUNGS, V. L., PUSKULCU, M., and SMITH, R. R. 1977. Oat lipids. I. Composition and distribution of lipid components in two oat cultivars. *Cereal Chem.* 54:803.

[Received January 9, 1985. Revision received August 16, 1985. Accepted August 19, 1985.]