Ultrastructure and Cytochemistry of Mature Oat (*Avena sativa L.*) Endosperm. The Aleurone Layer and Starchy Endosperm

D. B. BECHTEL and Y. POMERANZ, U.S. Department of Agriculture,[1] Science and Education Administration, Agricultural Research, U.S. Grain Marketing Research Laboratory, Manhattan, KS 66502

ABSTRACT

Cereal Chem. 58(1):61-69

The endosperm of five oat cultivars was studied with light and electron microscopy, cytochemistry, and enzymatic digestions. The aleurone was typically composed of a single layer of cells with an occasional area that was two cells thick. Aleurone grains, lipid bodies, plastids, mitochondria, endoplasmic reticulum, and a centrally located nucleus were common in the aleurone cells. Only the aleurone cells contained aleurone grains, which possessed a protein-carbohydrate body that contained inclusions. Cells in the subaleurone starchy endosperm contained numerous protein bodies and a few small starch granules, whereas cells in the central starchy endosperm were composed mostly of starch with protein bodies interspersed. The protein bodies ranged in size from 0.3–5 μm in diameter and in shape from round to angular to irregular masses. All protein bodies shared a common characteristic: they contained rounded electron-lucent inclusions embedded in a matrix. The protein bodies, particularly those from the subaleurone, were associated with densely stained regions containing ribosomes and endoplasmic reticulum. Simple and compound starch grains occurred in both regions of the starchy endosperm.

Oat protein has several unique features that separate it from proteins of other cerals (Frey 1977). First, oat protein, as demonstrated in rat feeding trials, has a high biological value. Second, the biological value does not diminish as the protein content increases, because the ratios of avenin to total protein remain unchanged. The amounts of the major typical protein fractions (albumin, globulin, prolamine, and glutelin) differ substantially from those of other cereals, the globulins being highest and the prolamines lowest (Pomeranz 1975). Third, the protein percentage of oat groats is the highest among cereal grains.

Although much information exists on chemical composition of the oat endosperm, little is known about the ultrastructure of the caryopsis. Pomeranz and Sachs (1972) used scanning electron microscopy to describe the oat kernel. Protein bodies from developing oat endosperm were briefly described by Sraon (1972). Because of the paucity of available information, we used a combined ultrastructural-cytochemical approach to investigate the mature oat endosperm structure.

MATERIALS AND METHODS

Oat Samples

Five oat (*Avena sativa* L.) cultivars—Froker, Holden, Lang, Stout, and Wright—were used for this study. All were grown on oat drill plots at Madison, WI, during 1976 and had a moisture content of about 12%. Test weights ranged from 38.1 to 41.4 lb/bu (average, 39.6 lb/bu), kernel weights from 27.7 to 32.6 mg (average 29.9 mg), and groat weights from 20.6 to 24.9 mg (average 22.1 mg).

Light and Transmission Electron Microscopy

Oat kernels were prepared for light and transmission electron microscopy by cutting the groat into small pieces with a sharp razor blade. Samples were fixed in 4% paraformaldehyde (w/v) and 4% glutaraldehyde (v/v) in 0.5*M* sodium dibasic and potassium monobasic phosphate buffer (Lillie 1954) for 1 hr at 21°C and 16 hr at 4°C. Samples were washed four times in buffer for a total of 2 hr and postfixed in phosphate-buffered 1% osmium tetroxide for 2 hr at 21°C. The oat pieces were then washed three times for 30 min in water, dehydrated in acetone, and infiltrated with and embedded in a low viscosity epoxy resin (Spurr 1969).

Plastic sections (1-μm thick) cut with glass knives were stained for light microscopy with 0.135 g of basic fuchsin and 0.365 g of toluidine blue O in 100 ml of 25% ethanol. Thin sections (70–80 nm thick) were cut with a diamond knife, stained with lead citrate (Reynolds 1963), and viewed in a Philips EM 201 electron microscope at 60 kV.

Enzymatic Digestions

All enzymes were purchased from Sigma Chemical Company (St. Louis, MO). Porcine α-amylase (No. A6255), twice crystallized, and treated with diisopropyl phosphorofluoridate was used at a concentration of 5 mg/ml in 20m*M* phosphate buffer at pH 7.0. Crystallized pepsin (No. 7012) from porcine stomach was used at 3 mg/ml in 0.1*N* HCl. Proteases V (No. P5005) and VI (No. P5130), isolated from *Streptomyces griseus*, were used at a

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

TABLE I
Enzymatic Digestions[a] of Thin Sections of Oat Aleurone Cells

Enzyme or Treatment	Thin Section Number	H_2O_2	Aleurone Grains		
			Matrix	Globoids	Protein-Carbohydrate Body Matrix[b]
Control: no treatment	1–2	No	–	–	–
Control: deosmicated H_2O_2	3–4	Yes	–	–	+++
Pepsin	5–6	Yes	++	++++	++++
Control	7–8	Yes	–	++++	++++
Protease V	9–10	Yes	+	++++	++++
Protease VI	11–12	Yes	+++	+++	++++
Control	13–14	Yes	–	–	++++
Trypsin	15–16	Yes	++++	+++	++++
Control	17–18	Yes	+	+++	++++
α-Amylase	19–20	Yes	+	++++	++++
Control	21–22	Yes	–	++++	++++
Control: no treatment	23–24	No	–	–	–
Control: deosmicated H_2O_2	25–26	Yes	–	–	+++

[a] – = Not digested or not substantially different from no treatment material, + = less than 25% digested, ++ = less than 50% but more that 25% digested, +++ = less than 75% but more than 50% digested, ++++ = 75–100% digested.
[b] Includes the electron-lucent, electron-dense, and electron-transparent inclusions which were not digested by any of the treatments.

TABLE II
Azocoll Assay for Protease Activity Determination

Incubation Time	Absorbance at 580 nm			
	α-Amylase	α-Amylase Control	Protease VI	Protease VI Control
10 min	0.005	0	0.56	0
30 min	0.036	0.005	...	...
90 min	0.045	0.010	...	...
150 min	0.059	0.013	...	...
24 hr	0.250	0.024	...	...

concentration of 5 mg/ml in distilled water at pH 7.4. Trypsin (No. T8253) type III, twice crystallized from porcine pancreas, was used at 3 mg/ml in 0.05*M* Tris buffer at pH 8.0 with 5m*M* $CaCl_2$ added.

Randomly chosen sections were broken from oat section ribbons and picked up on Formvar-coated gold grids. Osmium tetroxide was removed from the sections by floating the grids on a droplet of freshly prepared 3% hydrogen peroxide (diluted from concentrated 30% H_2O_2) for 20 min at 21°C (Knight and Lewis 1977). Grids were washed briefly in double-distilled water.

For enzymatic digestion, grids with deosmicated sections were floated on the enzyme solutions and incubated for 24 hr at 37°C. Precautions were taken against evaporation. For controls we used deosmicated sections treated in the same manner but without the enzyme and nontreated sections. All sections were first observed without poststaining but later were poststained in lead citrate for 5 min before being photographed. In addition, sections treated with protease V or α-amylase contained precipitated material on the entire grid after incubation. These grids were checked in the microscope and then treated with 3 mg/ml of pepsin in 0.1*N* HCl for 10 min at 21°C to remove the precipitated material. This pepsin treatment, however, had no visible effect on the sectioned oat material. Grid treatments are listed in Table I.

Protease Assay

Protease activity was determined by the azocoll assay (Jackson and Matsueda 1970). The test was conducted for the recommended time of 10 min and for a time course study lasting 24 hr (Table II).

Carbohydrate Cytochemistry

Carbohydrates were localized by use of a modification of the periodic acid-thiocarbohydrazide-silver protein technique (Knight and Lewis 1977). Oat groats of the cultivar Holden were cut into small pieces with a razor blade and fixed in 4% glutaraldehyde (v/v) in 0.05*M* phosphate buffer at pH 7.0 for 3 hr at 21°C and then for 18 hr at 4°C. The samples were washed with buffer ten times (20 min for each wash), dehydrated in a graded acetone series, and infiltrated and embedded in Spurr resin. Sections (60–70 nm thick) were cut with a diamond knife and picked up on Formvar-coated gold grids. For some sections, grids were floated on 1% aqueous periodic acid for 25 min at 21°C (Fig. 1). The grids were washed in running double-distilled water for 30 sec and on a droplet of water for 10 min. Oxidized grids were divided into three groups. The first group was taken through the entire staining procedure. The second group was first treated with a saturated solution of dimedone (5,5-dimethyl-1,3-cyclohexanedione, Polysciences, Inc., Warrington, PA) in 1% acetic acid for 1 hr at 60°C to block free aldehyde groups and then stained. The third group was treated with 2% mercaptoethanol in 0.05*M* tris buffer at pH 8.0 with 5m*M* $CaCl_2$ added for 90 min at 21°C. The grids were washed in double-distilled water for 10 min and then floated on a drop of alkylation solution, prepared by dissolving 1.86 g of iodoacetate and 1.235 g of boric acid in 100 ml of 50% *n*-propanol, for 4 hr at 21°C. The sections were rinsed in 20% *n*-propanol and then stained. Unoxidized sections were divided into two groups. The first group was treated with dimedone, washed in water, oxidized with periodic acid, and then stained. The other was stained without oxidation in periodic acid.

The staining procedure consisted of floating grids on a drop of freshly prepared 0.2% thiocarbohydrazide (Polysciences, Inc.) in 20% acetic acid for 45 min at 21°C. Grids were washed with running 10% acetic acid for 30 sec, then successively in two 15-min washes of 10%, a 5-min wash of 5%, and a 5-min wash of 1% acetic acid, and in two 5-min washes of double-distilled water. The grids were then floated on a freshly prepared solution of silver protein (Polysciences, Inc.). The silver protein solution was prepared in reduced light. Silver protein (0.5 g) was sprinkled onto the surface of 50 ml of double-distilled water and the mixture was kept undisturbed in the dark for 15 min. The resulting solution was then gently mixed, placed in a light-tight bottle, and kept at 4°C for 24 hr. The grids were stained for 30 min at 21°C in the dark. Grids were then washed for 60 sec in running double-distilled water and were viewed and photographed without poststaining. The best staining was obtained with staining solutions less than 48 hr old.

RESULTS

Aleurone Cells

Aleurone cells of all five oat cultivars appeared to be similar. These cells, each with a centrally located nucleus (Fig. 2), formed a single layer around the starchy endosperm, with an occasional area that was two cells thick (not shown). The major cytoplasmic components of the aleurone cells were lipid droplets and aleurone grains, a type of protein body (Fig. 3), which contained globoids and protein-carbohydrate bodies (described for barley by Jacobsen et al 1971) embedded in the proteinaceous matrix of the grain. The protein-carbohydrate body was made up of an electron-dense matrix with an electron-lucent inclusion embedded in it. This inclusion contained one or more electron-dense structures and a

transparent area (Fig. 3). Interspersed among the lipid droplets and aleurone grains (Fig. 4) were mitochondria, plastids containing osmiophilic globules and phytoferritin, and a rough endoplasmic reticulum (not shown). Mitochondria typically were smaller (0.5 μm) in diameter than plastids (1.0 μm). In addition, the mitochondria lacked phytoferritin and contained dispersed osmiophilic globules rather than globules grouped together (Fig. 4).

Enzymatic Digestions

Enzymatic digestion of thin sections of oat aleurone cells showed differential hydrolysis of the aleurone grain contents (Table I). The proteinaceous matrix was partially removed by pepsin (Fig. 5), protease V and VI, and α-amylase (not shown). Trypsin was the most effective enzyme in hydrolysis of the matrix, but some material was removed by the trypsin control (Figs. 6 and 7). Globoids were removed partially or completely (in comparison to standard fixed material) by every treatment except distilled water (the control for protease V and VI) and hydrogen peroxide (used for deosmication). Interpretation of the hydrolysis of the protein-carbohydrate body is difficult because either the deosmication process substantially removed the electron-dense matrix material or the osmium-positive staining was lost. The inclusions within this matrix were not affected by any of the treatments.

Carbohydrate Localization

Carbohydrate localization could not be conducted because thin sections of aleurone cells could not be obtained from the glutaraldehyde-fixed material. Material fixed with osmium tetroxide and deosmicated tissue could not be used because these procedures complicate (and possibly interfere with) interpretation of controls (Knight and Lewis 1977).

Starchy Endosperm

The starchy endosperm of all five cultivars generally appeared to be similar. The subaleurone starchy endosperm contained numerous protein bodies and a few small starch granules, whereas the central starchy endosperm was composed largely of starch with protein bodies interspersed. Two types of starch granules were observed: simple starch granules and compound granules composed of several to many granula (Fig. 8). Protein bodies were predominantly of one basic morphological type but were highly variable in both size and shape, ranging from 0.3 to 5 μm in diameter and from round to angular to irregular in shape. The protein bodies shared a common characteristic; they had a matrix of medium electron density and electron-lucent inclusions (Fig. 9). In addition, two cultivars, Stout and Lang, had lamellarlike material present in some of the electron-lucent inclusions (Fig. 10). The protein bodies, particularly those in the subaleurone region, were associated with densely stained areas containing ribosomes, rough endoplasmic reticulum (RER) (Figs. 11 and 12), and occasional dense structures, which could be another type of protein body (Fig. 12). Membranes of RER were typically difficult to visualize because of the large number of ribosomes present and the compacted nature of the endosperm. RER was identified by the parallel arrays of ribosomes (Fig. 11).

Enzymatic Digestions

Enzymatic digestions differentially extracted the various protein and starch components in deosmicated thin sections (Table III). For example, pepsin removed much of both the matrix and inclusion but left an electron-dense boundary around the remainder of the inclusion (Figs. 13 and 14). Similarly, proteases V and VI also distinguished between the matrix protein and the inclusions. With these enzymes, however, the matrix was more digested than were the inclusions (Figs. 15–17). Protease V digestion also revealed the matrix as a conglomerate of small rounded structures (Fig. 15). Trypsin hydrolyzed the matrix and the inclusions equally well (Figs. 18 and 19).

The α-amylase preparation not only digested the starch but also partially removed the matrix of the protein bodies (Figs. 20–22). To determine whether the α-amylase preparation possessed protease activity, we performed the azocoll assay (Jackson and Matsueda 1970). The assay, performed according to the protocol, revealed very little protease activity for the α-amylase in comparison to that for protease VI (Table II). Therefore, we used the periodic acid-thiocarbohydrazide-silver protein method for carbohydrate localization.

Carbohydrate Localization

Results of the carbohydrate localization are summarized in Table IV. The experimental treatment gave positive results for the protein bodies, ribosomal complexes, and starch granules (Fig. 23). Unoxidized controls gave positive results for only the protein bodies and ribosomal complexes (Table IV). Treatment of sections with dimedone, both before and after oxidation, and treatment

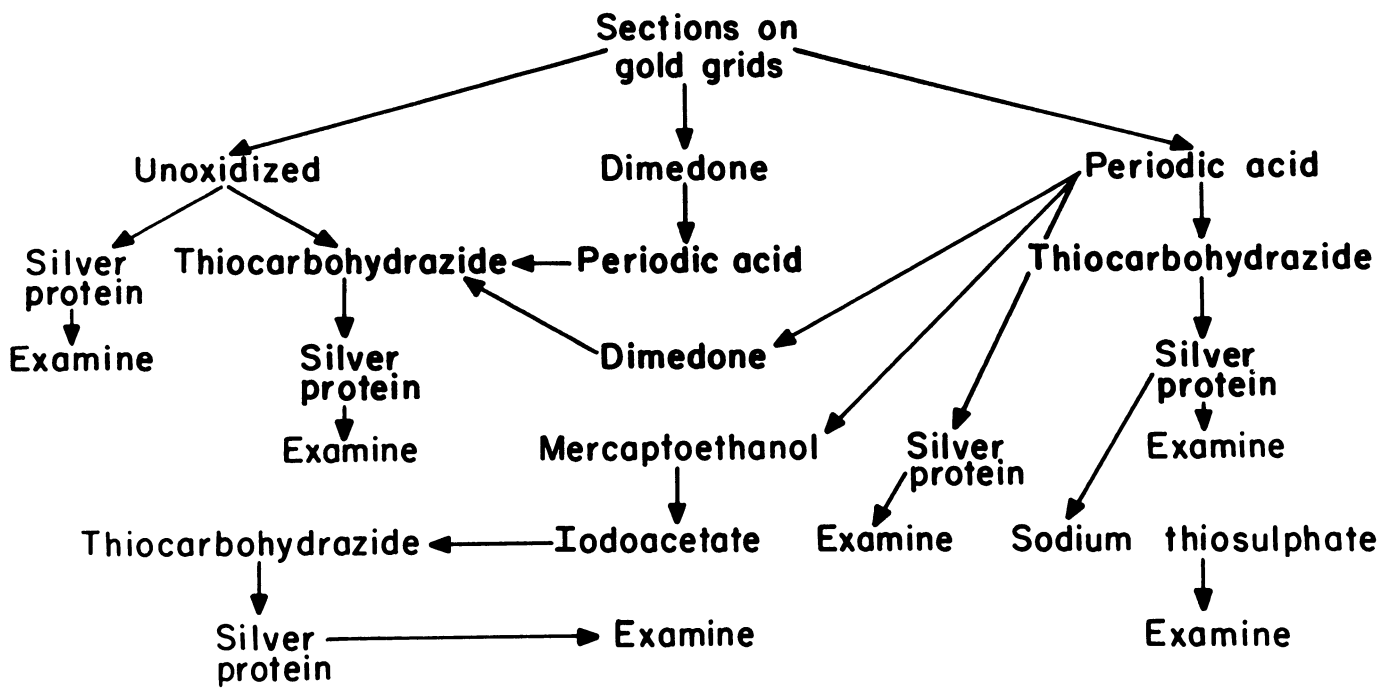


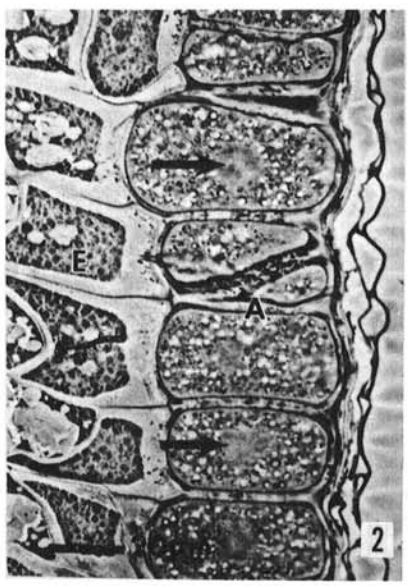
Fig. 1. Flow diagram of periodic acid-thiocarbohydrazide-silver protein procedure.

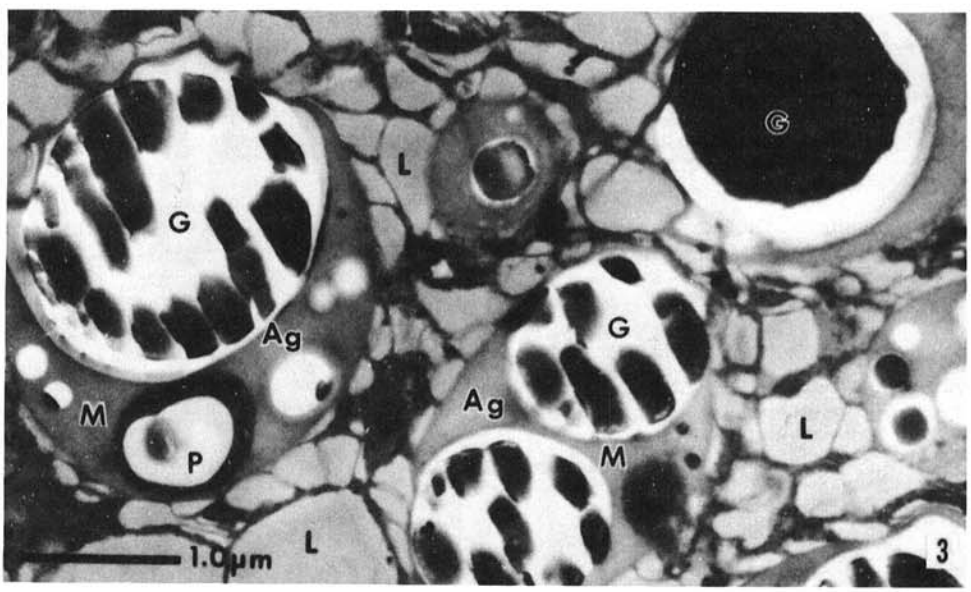
with mercaptoethanol and iodoacetate yielded positive localization for only the starch. Omitting the thiocarbohydrazide from the staining procedure gave some nonspecific staining of the ribosomal complexes, whereas omitting oxidation and thiocarbohydrazide resulted in no staining (Table IV).

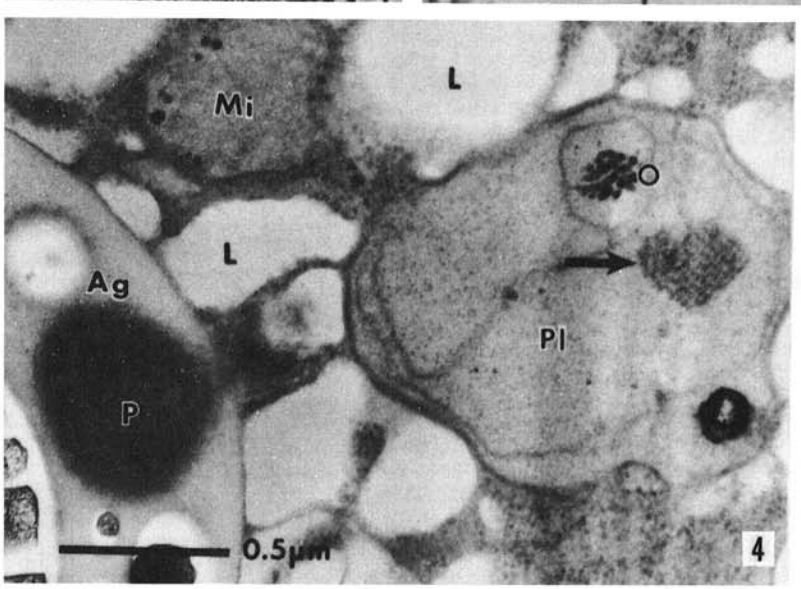
After the lack of stainable carbohydrate in the protein bodies was determined, another azocoll assay was conducted for a time-course study. This time the assay was run for 24 hr instead of the recommended 10 min. The α-amylase preparation clearly possessed some protease activity, even though the twice-crystallized α-amylase had been treated with diisopropyl phosphorofluoridate (Table II).

DISCUSSION

Mature oat aleurone cells were similar to mature aleurone cells of other cereals such as rice, *Oryza sativa,* (Bechtel and Pomeranz 1977) and barley, *Hordeum vulgare,* (Bechtel and Pomeranz 1978b, Buttrose 1971, Jones 1969). The effects of aqueous fixatives on seeds with low moisture content have been discussed previously (Bechtel and Pomeranz 1977). No major ultrastructural changes were induced by the fixatives. Recently, Bradbury et al (1980) found that the microscopic appearance of rice kernels soaked in water for three days was similar to that of dry kernels immediately fixed. Some changes, however, are likely to take place. The exact nature of the changes that occur in dry seeds when wetted by fixatives needs to be investigated. Freeze-fracture techniques probably will help determine the nature of those changes. Partial digestion of the matrix in aleurone grains by proteases indicated a proteinaceous matrix (Table I). Globoids were removed easily by all treatments except the deosmication and the control for proteases V and VI. Any treatment that contained an enzyme or buffer solution removed the globoids. Globoids of oats appeared structurally similar to those of other cereals. Globoids isolated from rice aleurone grains are composed predominantly of potassium and magnesium salts of phytic acid (Ogawa et al 1975), and we find little reason to believe that the globoids of oats are chemically much different. The protein-carbohydrate body of oats was different in structure from that described by Jacobsen et al (1971) for barley in that the oat body contained inclusions that were







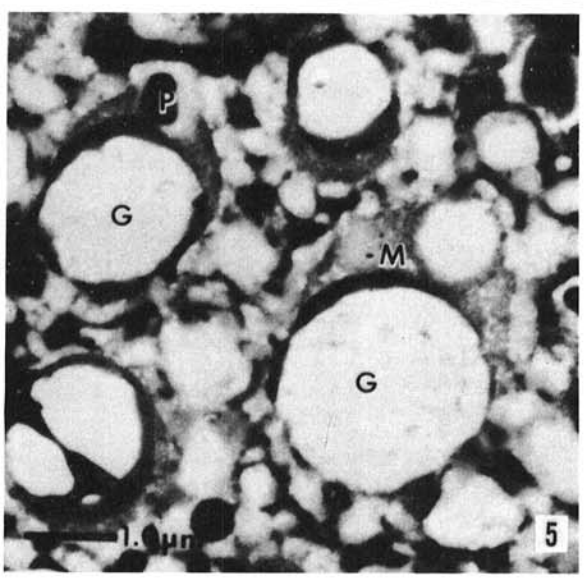
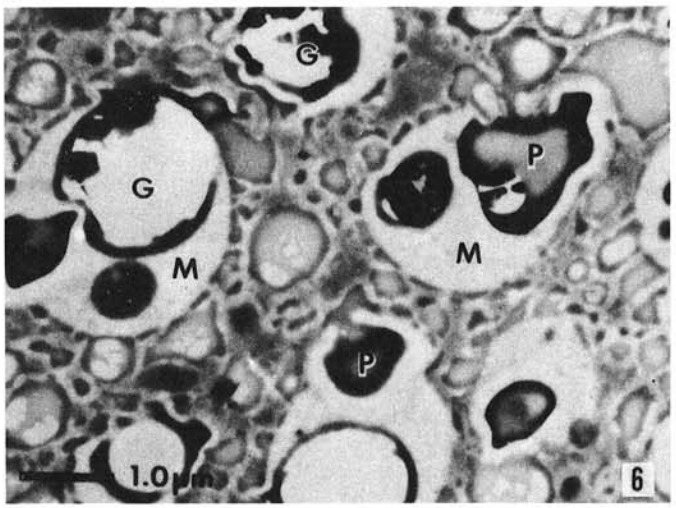
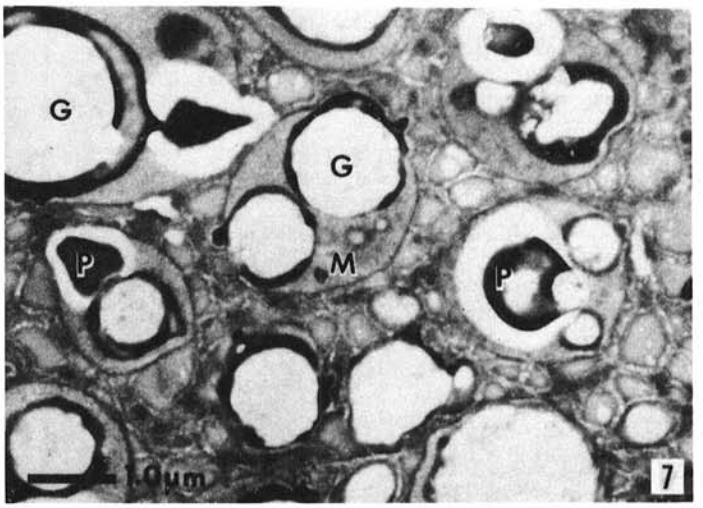
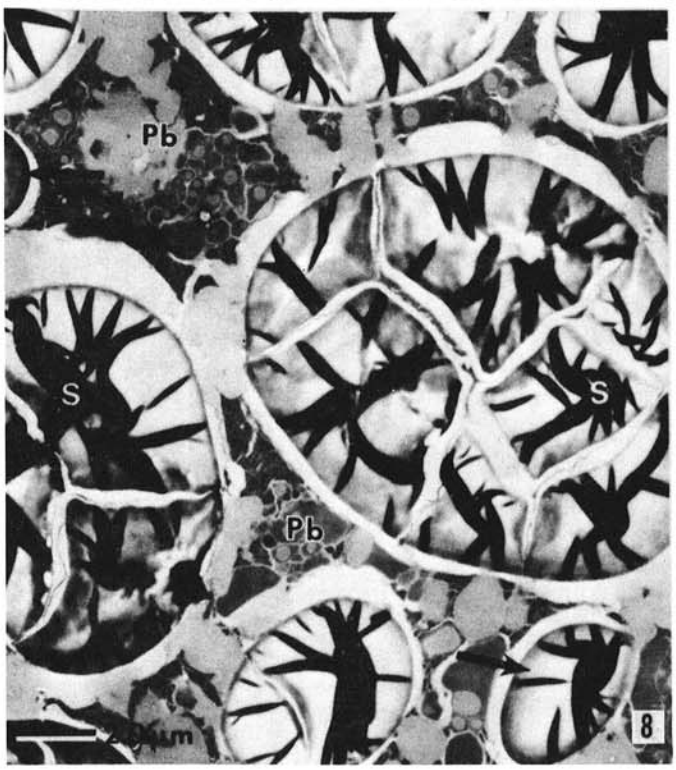
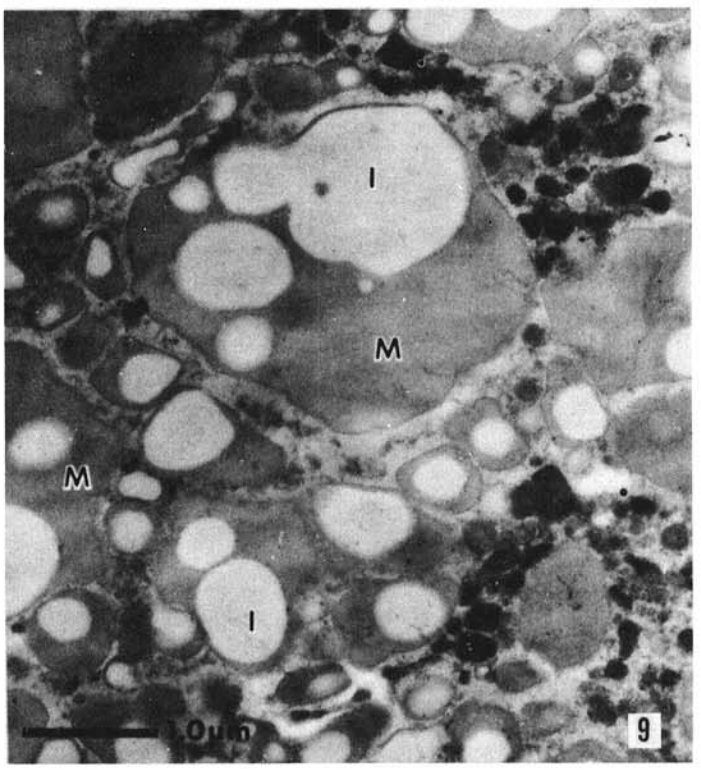


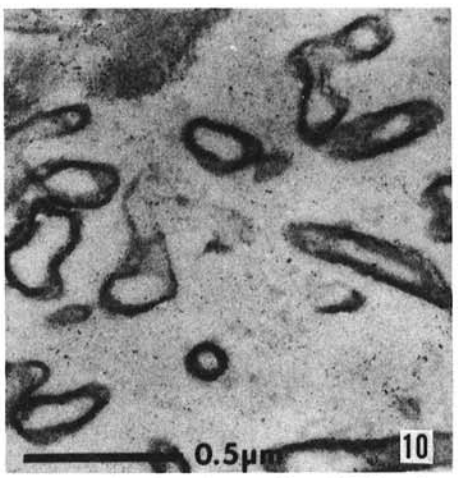
Fig. 2. Light microscopy of aleurone layer (**A**) and subaleurone (**E**) of oat cultivar Lang. Note central nuclei (arrows). **Fig. 3.** Transmission electron microscopy of oat cultivar Froker aleurone cell showing lipid droplets (**L**) and aleurone grains (**Ag**) containing globoids (**G**) and a protein-carbohydrate body (**P**) embedded in matrix protein (**M**). **Fig. 4.** High magnification of oat cultivar Froker aleurone cell showing lipid droplets (**L**) mitochondrion (**Mi**), and plastid (**Pl**) with osmiophilic globules (**O**) and phytoferritin (arrow). Portion of aleurone grain (**Ag**) contains oblique section through a protein-carbohydrate body (**P**). **Fig. 5.** Pepsin-digested oat cultivar Holden aleurone cell with removed globoids (**G**) and partially digested matrix (**M**) and protein-carbohydrate body (**P**).

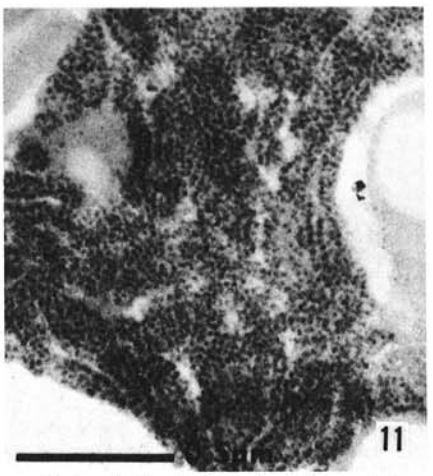












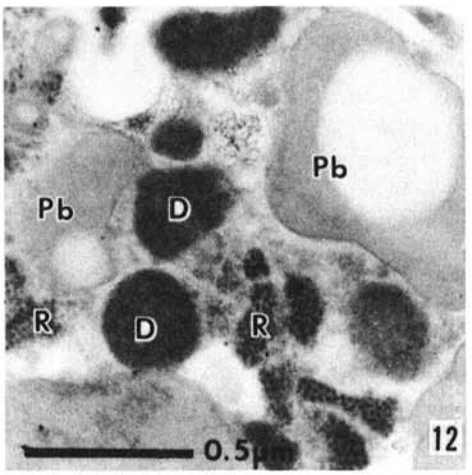
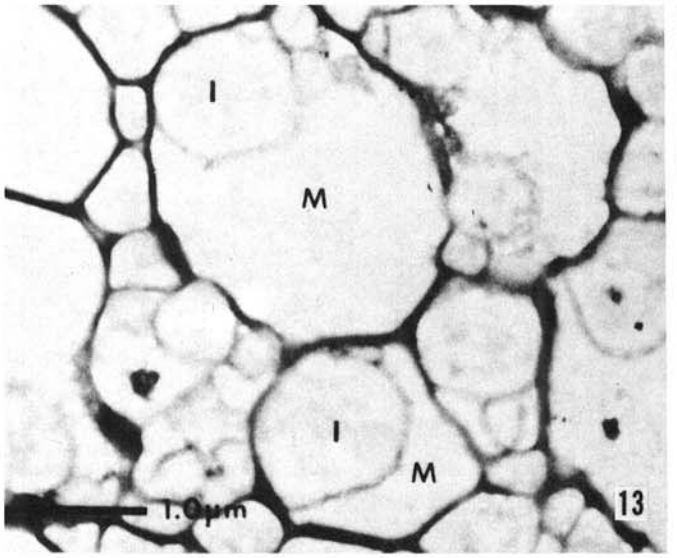
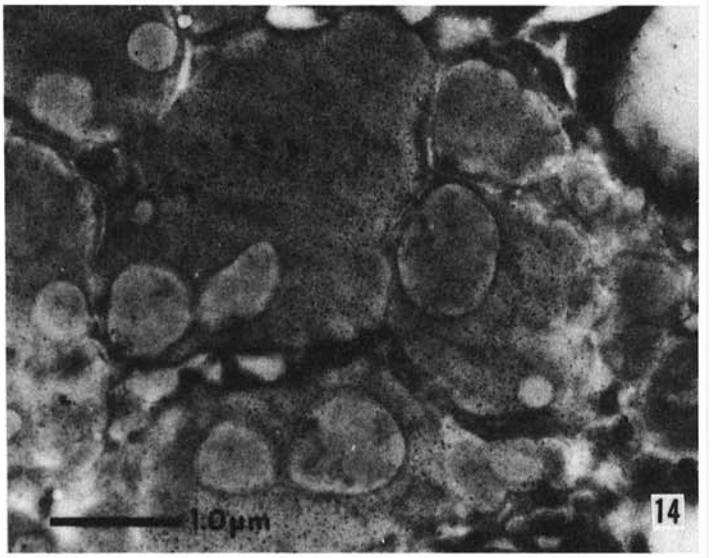
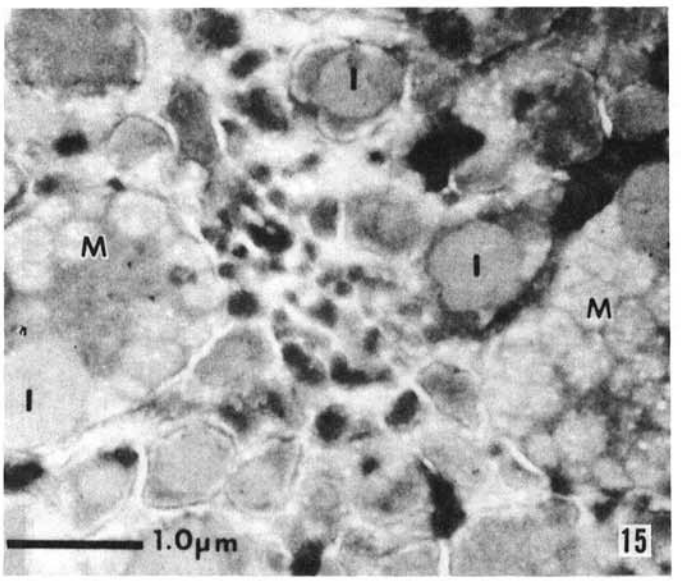
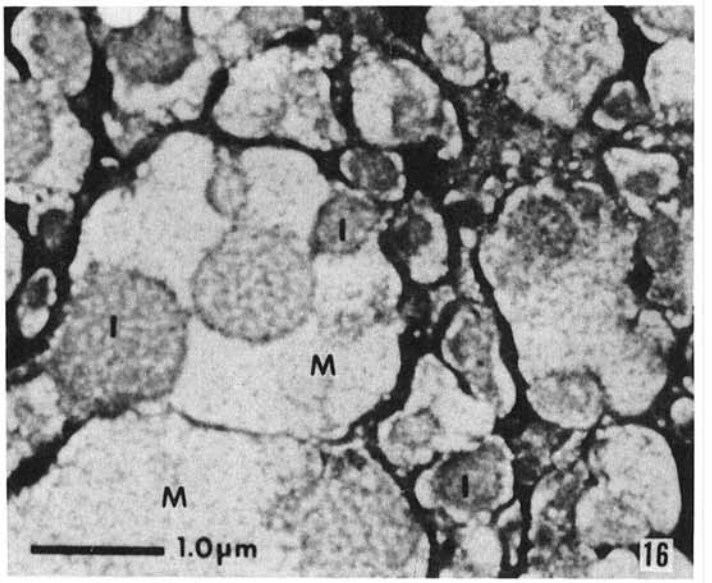


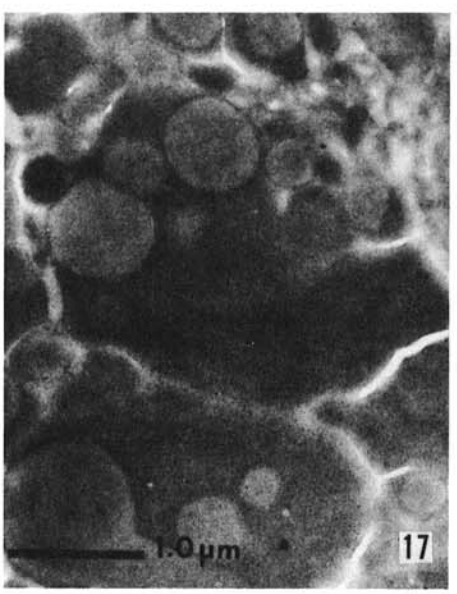
Fig. 6. Trypsin-digested oat cultivar Holden aleurone cell showing lack of matrix (**M**), partially removed globoid (**G**), and intact inclusion of protein-carbohydrate body (**P**). **Fig. 7.** Control of trypsin digestion. Note partial removal of matrix (**M**), protein-carbohydrate body (**P**), and globoid (**G**). **Fig. 8.** Oat cultivar Wright starchy endosperm showing compound (**S**) and simple starch granules (arrows) with protein bodies (**Pb**) between starch granules. **Fig. 9.** Protein bodies of oat cultivar Holden starchy endosperm showing matrix (**M**) and inclusions (**I**). **Fig. 10.** Lamellar material in protein body inclusion of oat cultivar Stout. **Fig. 11.** Ribosomes and rough endoplasmic reticulum of oat cultivar Wright. **Fig. 12.** Dense structure (**D**) in oat cultivar Holden located between protein bodies (**Pb**) and ribosomal complexes (**R**).

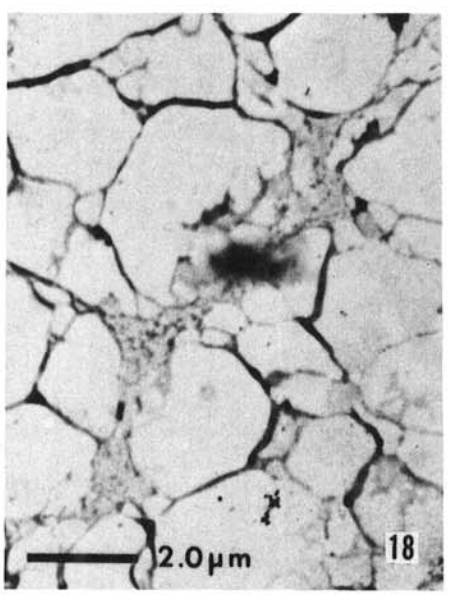












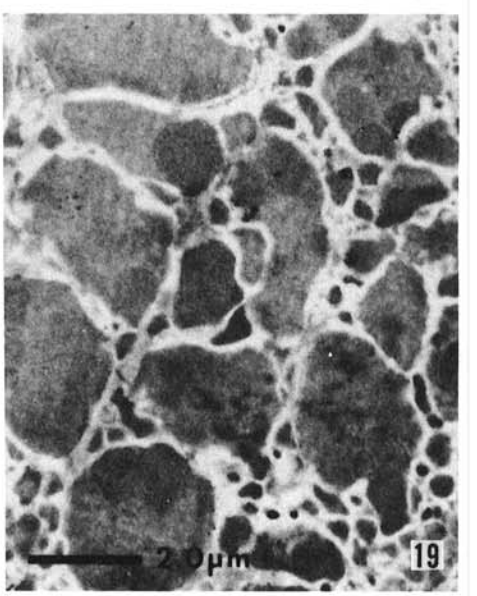
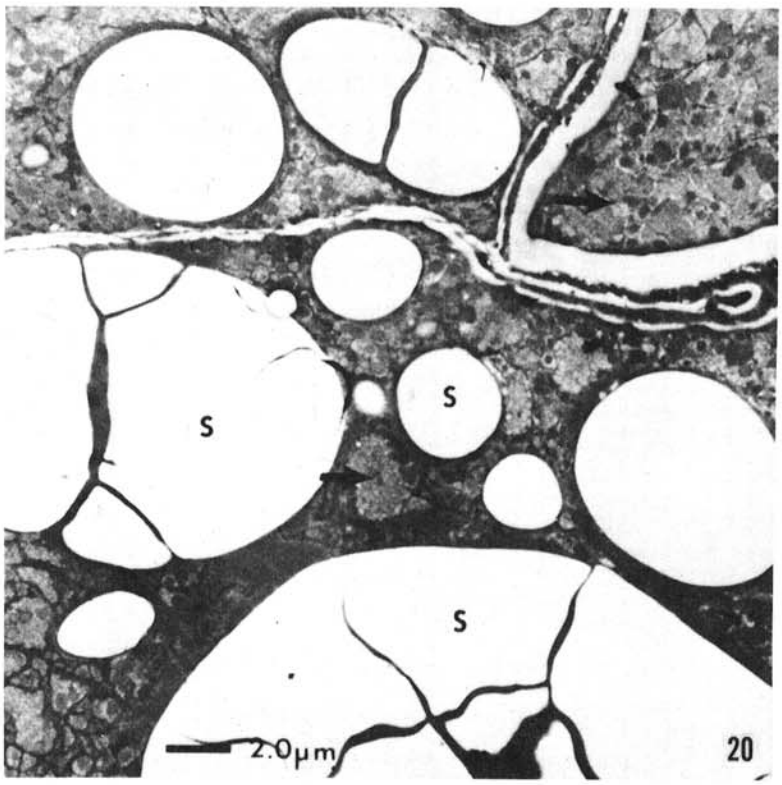
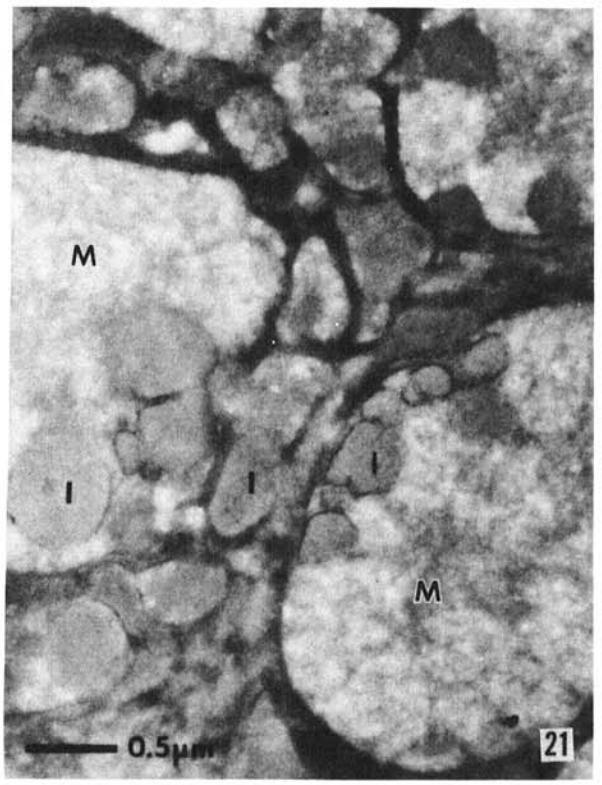


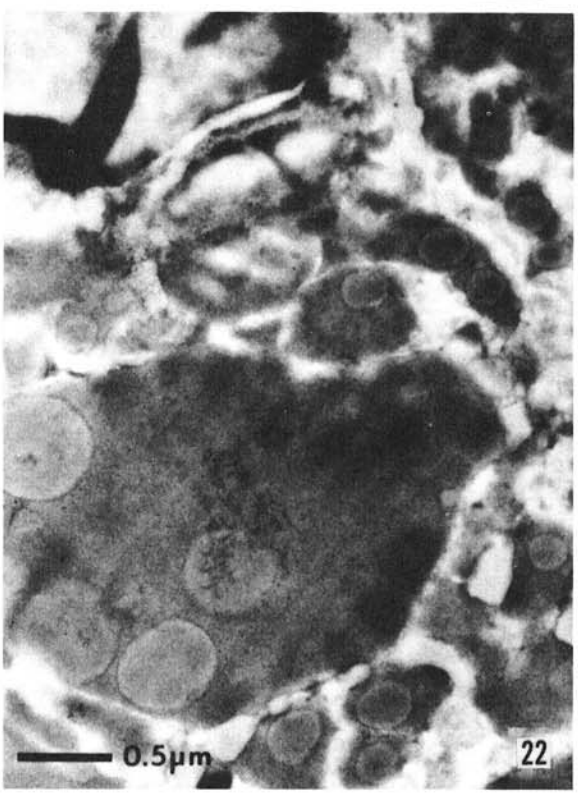
Fig. 13. Pepsin digestion of oat cultivar Holden starchy endosperm showing digested matrix (**M**) and partially removed inclusion (**I**). **Fig. 14.** Pepsin control exhibiting no protein body digestion. **Fig. 15.** Protease V digestion of endosperm showing matrix (**M**) digested more than inclusions (**I**). **Fig. 16.** Protease VI digestion of endosperm showing removal of most of matrix (**M**) but some inclusion protein (**I**) left. **Fig. 17.** Control for protease V and VI, showing undigested protein bodies. **Fig. 18.** Trypsin digestion of oat cultivar Holden starchy endosperm, showing total digestion of protein bodies. **Fig. 19.** Trypsin control, revealing partial removal of protein bodies.

resistant to all enzyme and control treatments. Most of the matrix portion of the protein-carbohydrate body, however, was partially removed by hydrogen peroxide (deosmication) and therefore, the determination of its composition by enzyme degradation was prohibited. Carbohydrate localization could not be conducted on aleurone cells because the aleurone cells of glutaraldehyde-fixed tissue could not be thin-sectioned. Tissue treated with osmium tetroxide could not be used because osmium tetroxide causes nonspecific staining (Jacobsen et al 1971).

The starch of the mature starchy endosperm of oats differed from that of other cereals studied to date. The starch was of two types: single granules and compound granules composed of two to many







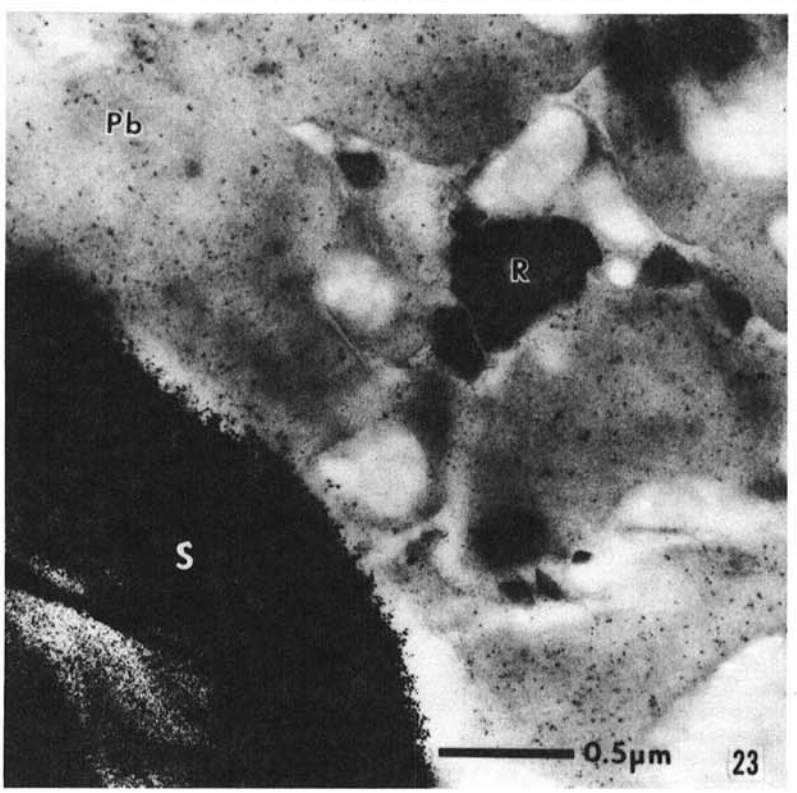


Fig. 20. α-Amylase-digested oat cultivar Holden starchy endosperm showing removal of starch granules (**S**) and partial removal of protein bodies (arrows). **Fig. 21.** High magnification of α-amylase-digested protein bodies. Note partial removal of matrix protein (**M**) but not of the inclusions (**I**). **Fig. 22.** Control for α-amylase digestion, showing intact protein bodies. **Fig. 23.** Carbohydrate localization of an experimental section, showing positive reaction product on **S**, protein bodies (**Pb**), and ribosomal complexes (**R**).

individual granula. All starch granules were compound in rice starchy endosperm (Bechtel and Pomeranz 1978a) and were single in the starchy endosperm of barley (Bechtel and Pomeranz 1979), wheat (*Triticum aestivum*, MacMasters et al 1971), corn (*Zea mays,* Wolf et al 1952), rye (*Secale cereale,* Lorenz et al 1978), and sorghum (*Sorghum bicolor,* Hoseney et al 1974).

The protein bodies of oats also differed from those in other grasses. Concentric-ringed protein bodies found in rice (Bechtel and Pomeranz 1978a, Mitsuda et al 1969), proso millet (*Panicum miliaceum,* Jones et al 1970), finger millet (*Eleusine coracana,* Adams and Liebenberg 1975), corn (Adams et al 1976, Khoo and Wolf 1970), sorghum (Adams and Novellie 1975, Seckinger and Wolf 1973), pearl millet (*Pennisetum typhoideum,* Adams et al 1976), and yellow foxtail (*Setaria lutescens*, Rost 1971) were not observed in oats. Oats also lacked the matrix type of storage protein typically found in wheat (Adams et al 1976), barley (Bechtel and Pomeranz 1979), rye (Lorenz et al 1978, Muszynski et al 1976), and triticale (Lorenz et al 1978). Instead, the oat protein bodies from the starchy endosperm were composed of a matrix in which electron-lucent inclusions were embedded. These two components were apparently of different composition, based upon differential removal by proteolytic enzymes (Table III). In addition, the protein bodies were partially digested by α-amylase preparation. Although this at first suggested the presence of carbohydrate in the protein body, two other tests proved this hypothesis incorrect. First, a 24-hr time-course azocoll test for protease activity showed that the α-amylase preparation possessed protease activity. Second, protein bodies failed to stain positively for carbohydrate at the transmission electron microscopy level.

Carbohydrate localization by the periodic acid-thiocarbohydrazide-silver protein procedure is very specific, especially if the proper controls are conducted. Although the protein bodies of the experimental treatment stained positively for carbohydrate, the staining was nonspecific because of fixative aldehydes in the protein bodies (shown in unoxidized sections and dimedone-blocked sections). Staining of ribosomal complexes was attributed to 1) the presence of aldehydes after fixation, 2) interference from proteins (shown by reducing and alkylating sulfhydryl groups and disulfide bonds), and 3) nonspecific binding of the silver protein to the complexes (shown by positive staining without the presence of thiocarbohydrazide).

The difference in structure of proteinaceous components between oats and other cereal grains is consistent with compositional differences and may explain, in part, the nutritional superiority of oat proteins (Frey 1977). The absence of numerous types of protein bodies and the matrix type of storage protein in oats should not be construed, however, to indicate complete uniformity in structure-composition of oat protein bodies. Differential digestion by several enzymes points to a microheterogeneity that probably can be traced to differences in composition.

TABLE III
Enzyme Digestion[a] of Starchy Endosperm of Oat Cultivar Holden

Enzyme or Treatment	Protein Body		Starch Granule
	Matrix	Inclusion	
No treatment	−	−	−
Deosmication	−	−	−
Pepsin	++++	+++	−
Control	−	−	−
Protease V	++	+	++++
Protease VI	++++	++ to ++++	−
Control	−	−	−
Trypsin	++++	++++	−
Control	−	−	−
α-Amylase	++	+	++++
Control	−	−	−

[a]− = Not digested or not substantially different from untreated material, + = less than 25% digested, ++ = less than 50% but more than 25% digested, +++ = less than 75% but more than 50% digested, ++++ = 75−100% digested.

TABLE IV
Carbohydrate Localization[a] Using the Periodic Acid-Thiocarbohydrazide-Silver Protein Method

Treatment	Protein Body	Ribosome Complexes	Starch
Experimental	+	++	+++
Unoxidized	+	+	−
Dimedone-oxidized	−	−	+++
Oxidized-dimedone	−	−	++
Mercaptoethanol-iodoacetate	−	+	++
No thiocarbohydrazide	−	+	−
Unoxidized and no thiocarbohydrazide	−	−	−

[a]− = No silver grains detected, + = some silver grains present, ++ = large numbers of silver grains present, +++ = silver grains completely cover material.

LITERATURE CITED

ADAMS, C. A., and LIEBENBERG, N. v.d.W. 1975. Ultrastructure of storage protein in cereals. Proc. Electron Microsc. Soc. S. Afr. 5:39.

ADAMS, C. A., and NOVELLIE, L. 1975. Composition and structure of protein bodies and spherosomes isolated from ungerminated seeds of Sorghum bicolor (Linn.) Moench. Plant Physiol. 55:1.

ADAMS, C. A., NOVELLIE, L., and LIEBENBERG, N. v.d.W. 1976. Biochemical properties and ultrastructure of protein bodies isolated from selected cereals. Cereal Chem. 53:1.

BECHTEL, D. B., and POMERANZ, Y. 1977. Ultrastructure of the mature ungerminated rice (Oryza sativa) caryopsis. The caryopsis coat and the aleurone cell. Am. J. Bot. 64:966.

BECHTEL, D. B., and POMERANZ, Y. 1978a. Ultrastructure of the mature ungerminated rice (Oryza sativa) caryopsis. The starchy endosperm. Am. J. Bot. 65:684.

BECHTEL, D. B., and POMERANZ, Y. 1978b. Ultrastructure of the mature ungerminated barley caryopsis. Cereal Foods World 23:480 (Abstr.).

BECHTEL, D. B., and POMERANZ, Y. 1979. Endosperm ultrastructure of barley isogenic lines. Cereal Chem. 56:446.

BRADBURY, J. H., COLLINS, J. G., and PYLIOTIS, N. A. 1980. Methods of separation of the major histological components of rice and characterization of their proteins by amino acid analysis. Cereal Chem. 57:133.

BUTTROSE, M. S. 1971. Ultrastructure of barley aleurone cells as shown by freeze-etching. Planta 96:13.

FREY, K. J. 1977. Protein of oats. Z. Pflanzenzuecht. 78:185.

HOSENEY, R. C., DAVIS, A. B., and HARBERS, L. H. 1974. Pericarp and endosperm structure of sorghum grain shown by scanning electron microscopy. Cereal Chem. 51:552.

JACKSON, R. L., and MATSUEDA, G. R. 1970. Myxobacter AL-1 protease. Page 591 in: Perlmann, G. E., and Lorand, L., eds. Methods in Enzymology, Vol. 14, Proteolytic Enzymes. Academic Press: New York.

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.

JONES, R. L. 1969. The fine structure of barley aleurone cells. Planta 85:359.

JONES, R. W., BECKWITH, A. C., KHOO, U., and INGLETT, G. E. 1970. Protein composition of proso millet. J. Agric. Food Chem. 18:37.

KHOO, U., and WOLF, M. J. 1970. Origin and development of protein granules in maize endosperm. Am. J. Bot. 57:1042.

KNIGHT, D. P., and LEWIS, P. R. 1977. General cytochemical methods. Page 77 in: Glauert, A. M., ed. Staining Methods for Sectioned Material. North-Holland Publishing Co.: Amsterdam.

LILLIE, R. D. 1954. Histopathologic technique and practical histochemistry. The Blakiston Co.: New York.

LORENZ, K., YETTER, M., and SAUNDERS, R. M. 1978. Scanning electron microscopy of maturing triticale, wheat, and rye. Cereal Chem. 55:66.

MacMASTERS, M. H., HINTON, J. J. C., and BRADBURY, D. 1971. Microscopic structure and composition of the wheat kernel. Page 51 in: Pomeranz, Y., ed. Wheat Chemistry and Technology. Am. Assoc. Cereal Chem.: St. Paul, MN.

MITSUDA, H., MURAKAMI, K., KUSANO, T., and YASUMOTO, K. 1969. Fine structure of protein bodies isolated from rice endosperm. Arch. Biochem. Biophys. 130:678.

MUSZYNSKI, S., KOCON, J., DARLEWSKA, M., and GROMADKA, M. 1976. The ultrastructure of endosperm in rye (Secale cereale) as

revealed by SEM. Bull. Acad. Pol. Sci. Ser. Sci. Biol. 24(8):449.
OGAWA, M., TANAKA, K., and KASI, Z. 1975. Isolation of high phytin containing particles from rice grains using an aqueous polymer two phase system. Agric. Biol. Chem. 39:695.
POMERANZ, Y. 1975. Proteins and amino acids of barley, oats and buckwheat. Page 13 in: Friedman, M., ed. Protein Nutritional Quality of Foods and Feeds, Part 2. Marcel Dekker, Inc.: New York.
POMERANZ, Y., and SACHS, I. B. 1972. Scanning electron microscopy of the oat kernel. Cereal Chem. 49:11.
REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208.
ROST, T. L. 1971. Fine structure of endosperm protein bodies in Setaria lutescens (Graminae). Protoplasma 73:475.
SECKINGER, H. L., and WOLF, M. J. 1973. Sorghum protein ultrastructure as it relates to composition. Cereal Chem. 50:455.
SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31.
SRAON, H. S. 1972. An electron microscopic study of protein bodies of developing oat seed. Proc. S.D. Acad. Sci. 51:69.
WOLF, M. J., BUZAN, C. L., MacMASTERS, M. M., and RIST, C. E. 1952. Microscopic structure of the endosperm of dent corn. Cereal Chem. 29:349.

[Received April 1, 1980. Accepted July 22, 1980]