Scanning Electron Microscopy of the Endosperm of Malted Barley[1]

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(Refer to pages 9-11 for Figures 1-5)

ABSTRACT

Changes in the aleurone layer and in the starchy endosperm of steeped, malted, and kilned barley were followed by scanning electron microscopy. The surface of aleurone cells in steeped barley was highly pitted. The walls of aleurone cells were progressively degraded during malting and kilning. Increase in diameter of aleurone grains during steeping was followed by further distortion of the spherical granules during kilning. Partial breakdown of cell-walls in the center of the starchy endosperm of malted barley was accompanied by extensive dissolution of the protein matrix and "freeing" of small starch granules that were previously embedded in that matrix; the effect on the appearance of the starch granules was small. In the central endosperm of kilned barley malt, the cell-wall dissolution was extensive and was accompanied by mechanical breakdown of the large starch granules.

The objectives of malting barley for brewing purposes are to modify grain into a product which can yield an aqueous extract containing: a) fermentable products, b) available substrate for yeast nutrition, and c) precursors for imparting the desirable organoleptic qualities to the beer (1).

The sum total of physical and chemical changes which take place during malting is termed "modification". According to MacLeod (2), the term modification describes "a rather nebulous but none the less real condition which has resulted from the transformation of endospermic constituents to give the best possible material for mashing." In practice, malting conditions are selected with the purpose of attaining optimum modification that will yield a maximum of extractable solids while minimizing malting losses and excessive degradation of the barley's high-molecular-weight components. Modification results in transformation of tough barley into friable malt. That transformation can be assessed by physical methods ranging from the simple biting test to tests employing elaborate self-recording mechanical devices. Among chemical indices, the increase in soluble proteins is probably the most important single parameter. Another index which has gained acceptance in evaluating modification is the difference in extract of fine- and coarse-ground malt. Known chemical and physical tests for evaluating modification are of limited value (2).

[1]Cooperative investigations between the Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the Agricultural Experiment Station, University of Wisconsin, Madison. The work of the Barley and Malt Laboratory is supported by an Industrial Research Grant from the Malting Barley Improvement Association.

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As early as 1890, Brown and Morris concluded on the basis of microscopic observations that the cell-walls of the barley endosperm gradually dissolve away during malting, leading to softening of the tissue (1). Subsequent work has shown that the dissolution of cell-wall material is incomplete, and is most evident near the scutellal epithelium and in the subaleurone starchy endosperm. The changes are accompanied by progressive decreases in viscosity of grain extracts, resulting from enzymatic degradation of proteins and, primarily, of polysaccharides other than starch. Dickson and Shands (3) have shown by microscopy that dissolution of endosperm cell-walls progresses from the scutellum to the distal end of the kernel. Disappearance of the cell-walls is followed closely by dissolution of the protein matrix embedding the starch granules.

This report deals with using scanning electron microscopy to follow changes taking place in the aleurone and starchy endosperm of barley from steeping, through malting and kilning.

MATERIALS AND METHODS

A six-row malting barley (*Hordeum vulgare* L.) of Dickson variety was used. The barley was steeped for 30 hr. at 16°C., malted for 5 days at 16°C., and kilned at a maximum temperature of 85°C. for 2 hr. The micro-malting equipment and conditions used were described elsewhere (4,5). Steeped and malted samples for use in electron scan microscopy were freeze-dried. Sections of freeze-dried and kilned material were prepared by slicing the grain with a razor blade.

The pictures shown in this report were from transversal sections of the middle portion of the grain. The samples were mounted on circular (9-mm. diam.) specimen holders with an adhesive, coated with graphite, and covered with a 200 to 300 A gold layer. A Cambridge Stereoscan electron microscope was used.

RESULTS AND DISCUSSION

A comparison of aleurone cell-walls with endosperm cell-walls of steeped grain (from a section above the crease) is shown in Fig. 1, a and b. A cross-section through the aleurone layer indicates that the cell-wall in steeped barley (Fig. 2, b) is almost twice as thick as in mature barley that has not been steeped (Fig. 2, a) (6). In addition, the cell-wall surface of steeped barley is highly pitted, whereas that of untreated barley has a finely wrinkled, yet intact appearance. Average diameter of aleurone grains increased from about 2 μ to 3 μ during steeping. A comparison of the aleurone layer in steeped, malted, and kilned barley (Fig. 2, b to d) indicates slight dissolution of the cell-wall after germination and almost complete breakdown following kilning. The latter is to be expected, as modification is known to occur during the early or drying stages of kilning (7). The findings are in agreement with those of Taitz and Jones (8), who reported recently that following treatment of isolated aleurone layers with gibberellic acid, digestion of the aleurone cell-walls containing a β-1,3-linked polymer occurs. The digestion is apparently the result of β-1-3-glucanase produced by aleurone cell-walls. Under the conditions described by Taitz and Jones (8), in the aleurone cells which lie adjacent to the starchy endosperm, cell-wall digestion occurred in the cell-wall regions closest to the endosperm cells.

A closer look at the aleurone grains of steeped barley is shown in Fig. 3, a

(continued on page 18)

Determining the Structure of the Barley Kernel by Scanning Electron Microscopy

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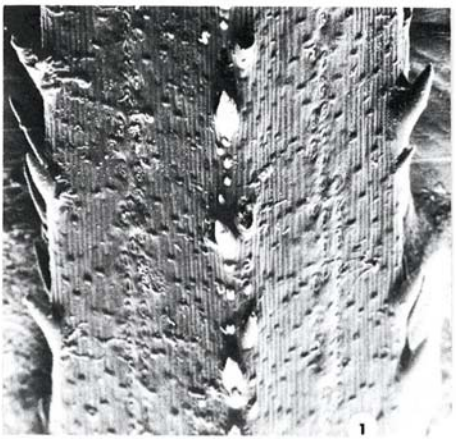


Fig. 1. Awn in barley (60X).

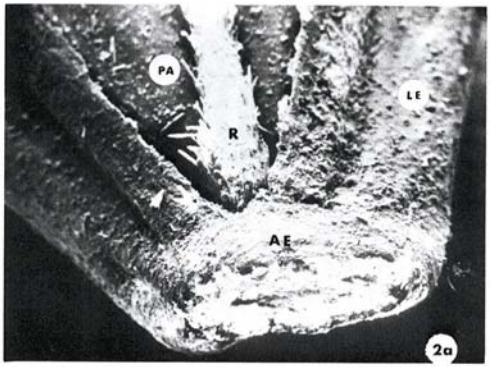
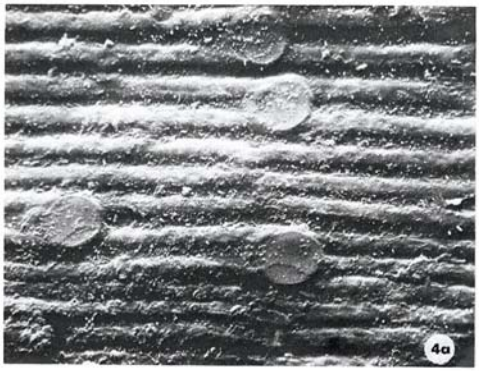


Fig. 2. Ventral view of barley. a) Basal end and rachilla (42X); AE = attachment end, R = rachilla, LE = lemma. PA = palea. b) Crease with rachilla (67X).



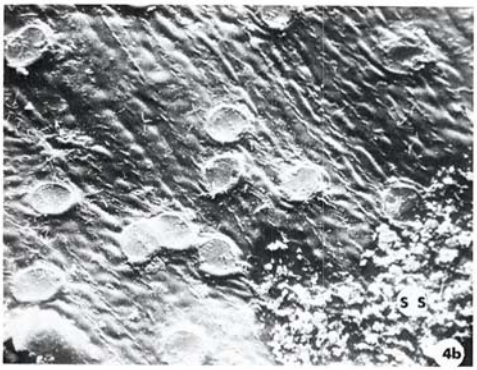
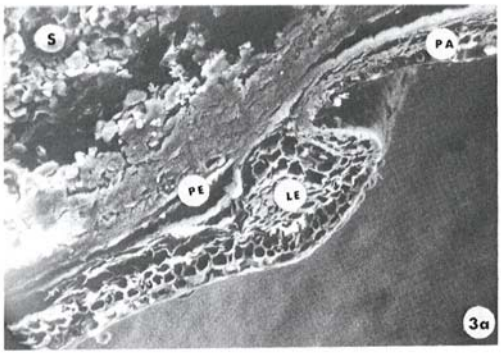


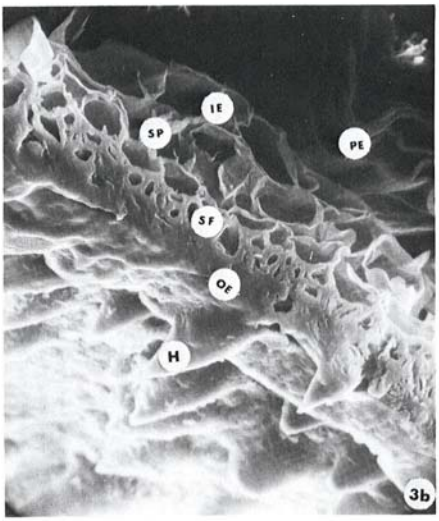
Fig. 4. Stomata-like cells and ridges on outer surface of barley. a) Immature barley (590X). b) Mature barley with silica-like scale (SS) at bottom right (430X).

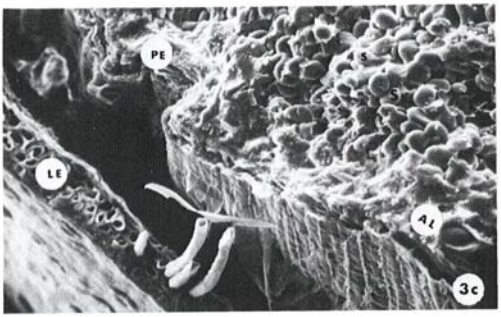
Fig. 5. Transverse section through pericarp and endosperm of barley (710X); PE multilayered, cord-like pericarp; AW = aleurone-cell walls, AG = aleurone grain, PM = protein matrix of subaleurone layer, S = starch granules, EW = cell walls of subaleurone layer, CL = compound middle lamellae.

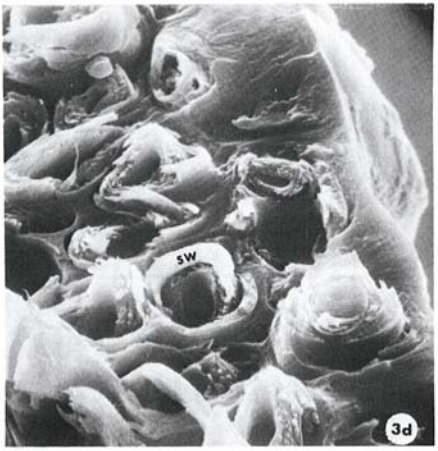
Fig. 6. High magnification (3,500X) of a single aleurone cell; ST = pitted cell-wall.

Fig. 7. Longitudinal section through central endosperm of barley. a) Central endosperm of immature barley (1,450X); S = starch granule. b) Mature barley (1,450X), S = starch granule with attached protein (bottom) or with indentations (top), EW = cell-wall.









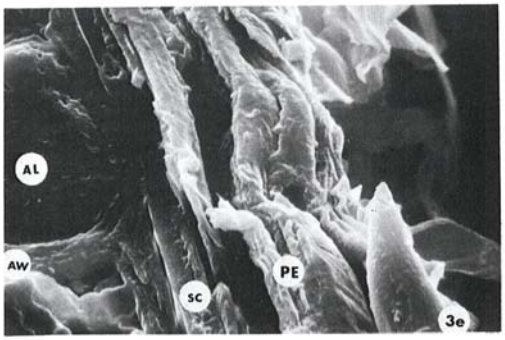
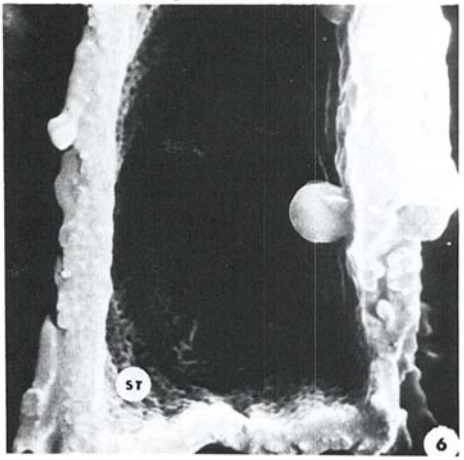
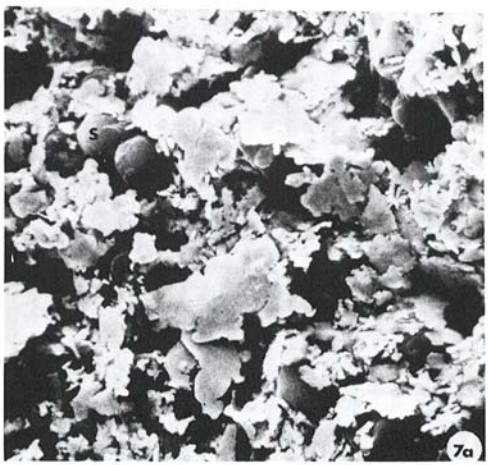
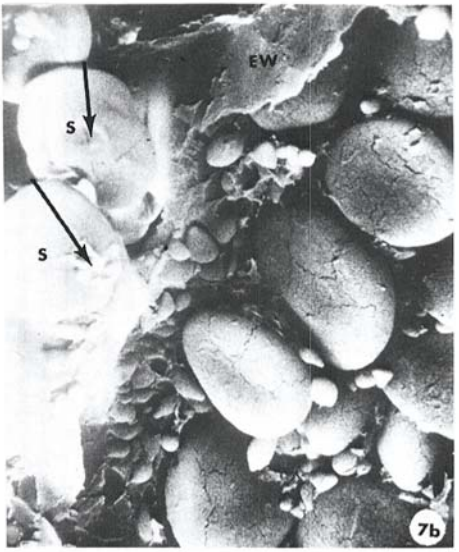


Fig. 3. Transverse sections through outer barley layers. a) Transverse section through the palea (PA), lemma (LE), pericarp (PE), and subaleurone layer, including starch granules (S), of steeped barley (165X). b) Section through lemma and pericarp (PE). Oblique view showing hair (H), outer epiderm (OE), sclarenchyma fibers (SF), spongy parenchyma (SP), and inner epiderm (IE) (410X). c) Section through the lemma (LE) with protruding tubes, pericarp (PE), aleurone (AL), and starch-containing (S) endosperm (170X). d) Section (top view) through the lemma; SW = secondary walls (1,570X). e) Section through pericarp (PE), seed coat (SC), aleurone cell-walls (AW), and aleurone cells (AL) of steeped barley (1,570X).







Scanning Electron Microscopy of the Endosperm of Malted Barley

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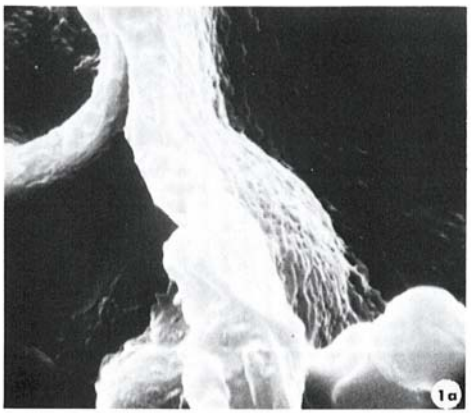


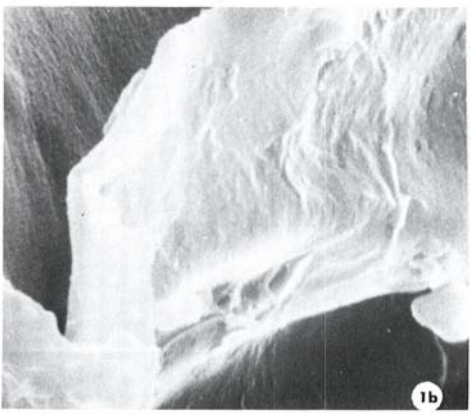
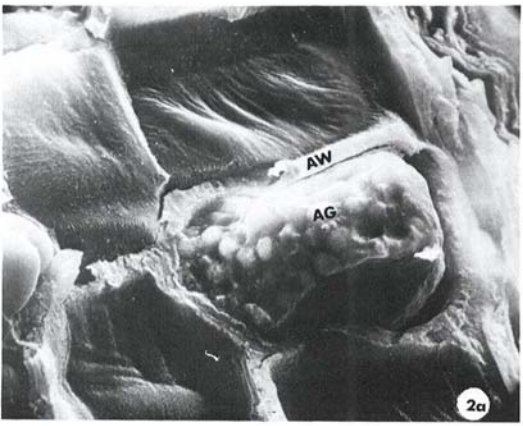
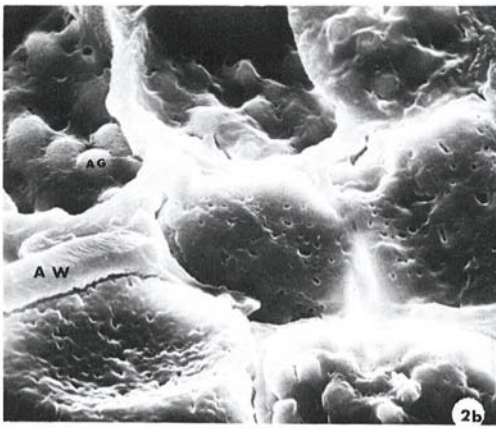
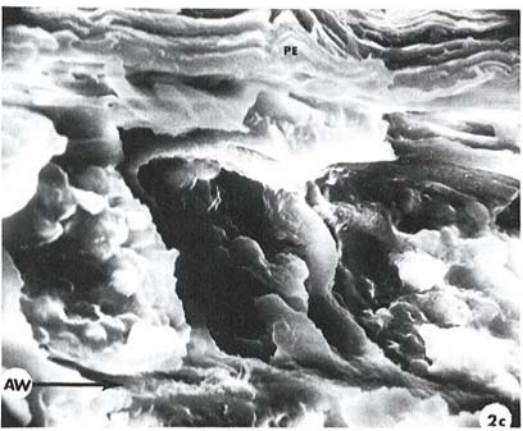


Fig. 1. Transverse sections of cell-wall material in steeped barley. a) Aleurone cell-wall (3,900X). b) endosperm cell-wall (3,500X).







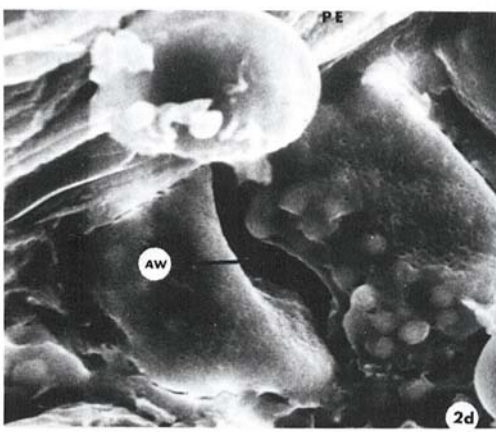


Fig. 2. Transverse sections through the aleurone layer. a) Unsteeped barley (1,350×); AW = cell-wall, AG = aleurone grain. b) Steeped barley (1,600×); AW = cell-wall, AG = aleurone grain. c) Malted and freeze-dried barley (1,700×); PE = pericarp, AW = slightly degraded aleurone cell-wall. d) Malted and kilned barley (1,650×); PE = pericarp, AW = extensively degraded aleurone cell-wall.

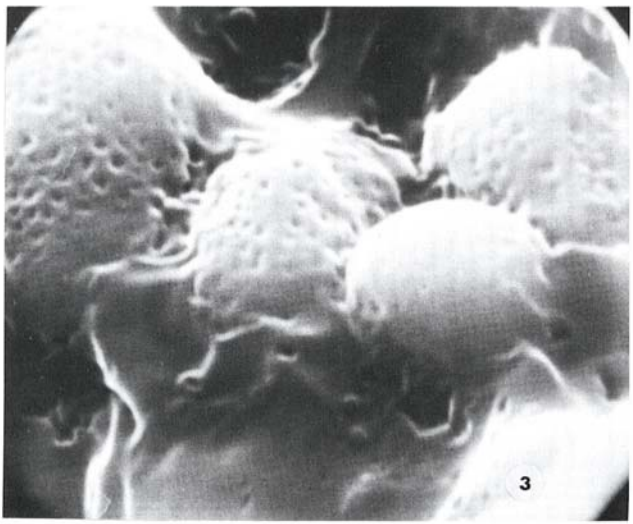
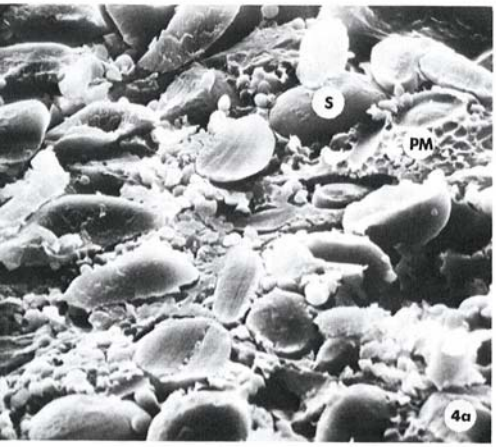
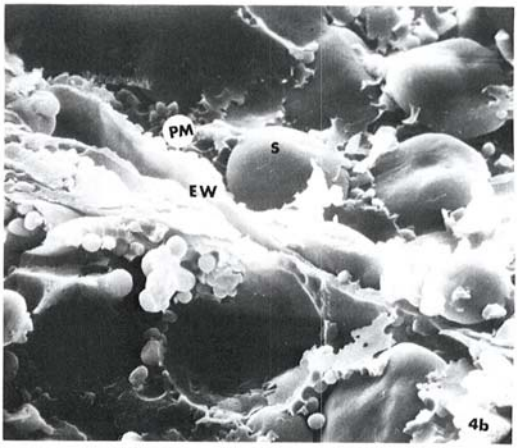
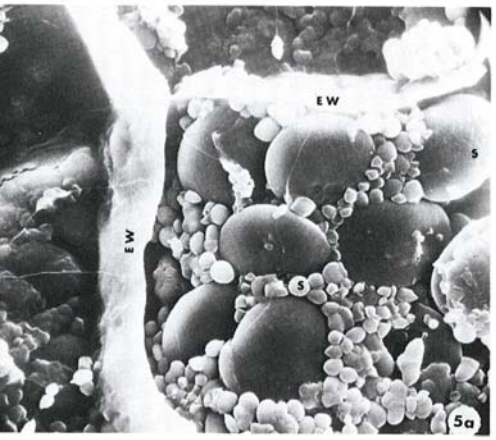


Fig. 3. Aleurone grains in steeped barley (9,700×).







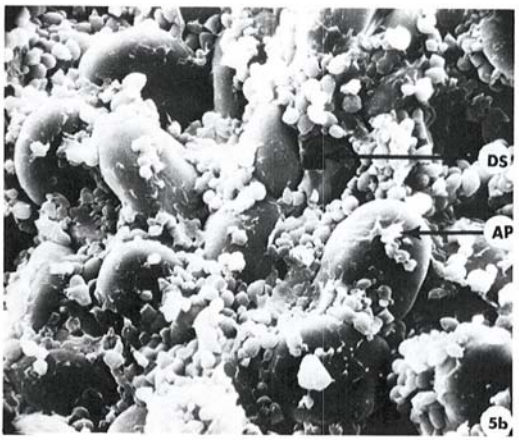


Fig. 4. Starchy endosperm of steeped barley. a) Subaleurone layer (800X); S = starch granules, PM = protein matrix. b) Central endosperm (860X); EW = cell-wall, PM = protein matrix, S = starch granules.

Fig. 5. Central endosperm of: a) Malted and freeze-dried barley (800X); EW = cell-wall, S = starch granules. b) Malted and kilned barley (810X); AP = adhering "proteinaceous" material, DS = highly damaged starch granules.

Scanning Electron Microscopy of the Oat Kernel

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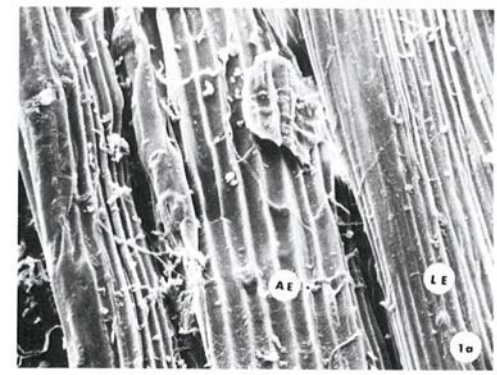


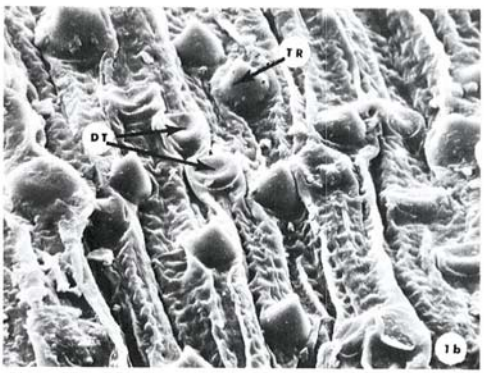


Fig. 1. Ventral view of an oat kernel. a) Secondary floret rachilla segment (AE) and lemma (LE) (magnification, 200X). b) Surface of an infertile third floret with intact (TR) and damaged (DT) trichomes (475X).

Fig. 5. Appearance of the inside of the palea. a) Longitudinal and cross ridges covered with hairs (300X). b) Fungal mycelium (630X). c) Plaque with microbial growth (650X).

Fig. 6. Conidium (C) and conidiophores (CP) on the seed coat in the crease of an oat groat (34X).

Fig. 7. Appearance of a dehulled oat kernel (groat). a) Hairs on the ventral side — upper tip and in the crease (14X). b) Hairs on the dorsal side, showing embryo and scutellum (16X).

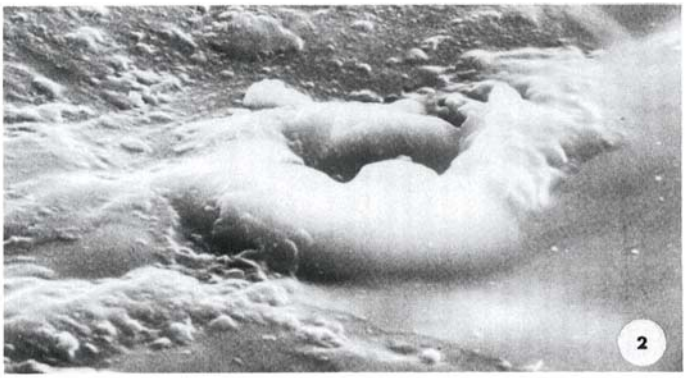
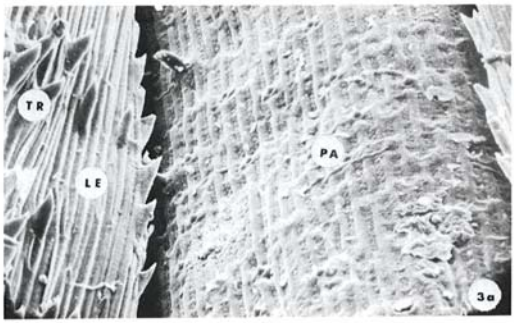


Fig. 2. Oblique view of a damaged trichome on the surface of the lemma of an oat kernel (3,000X).



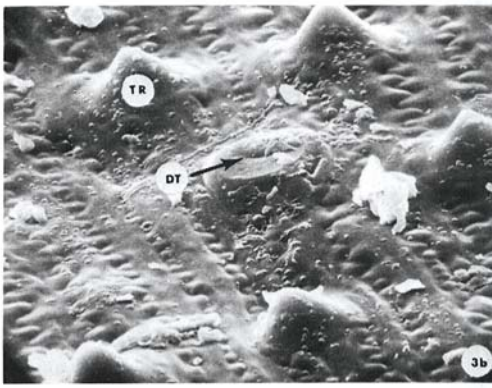


Fig. 3. Ventral view of a primary oat kernel. a) Appearance of the lemma (LE) with trichomes (TR) and palea (PA) in the basal part of the kernel (160X). b) Epidermal cells, and intact (TR) and damaged (DT) trichomes in the central part of the palea (920X).

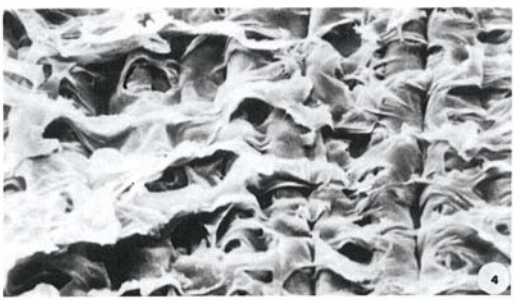


Fig. 4. Appearance of inside of the lemma (460X).

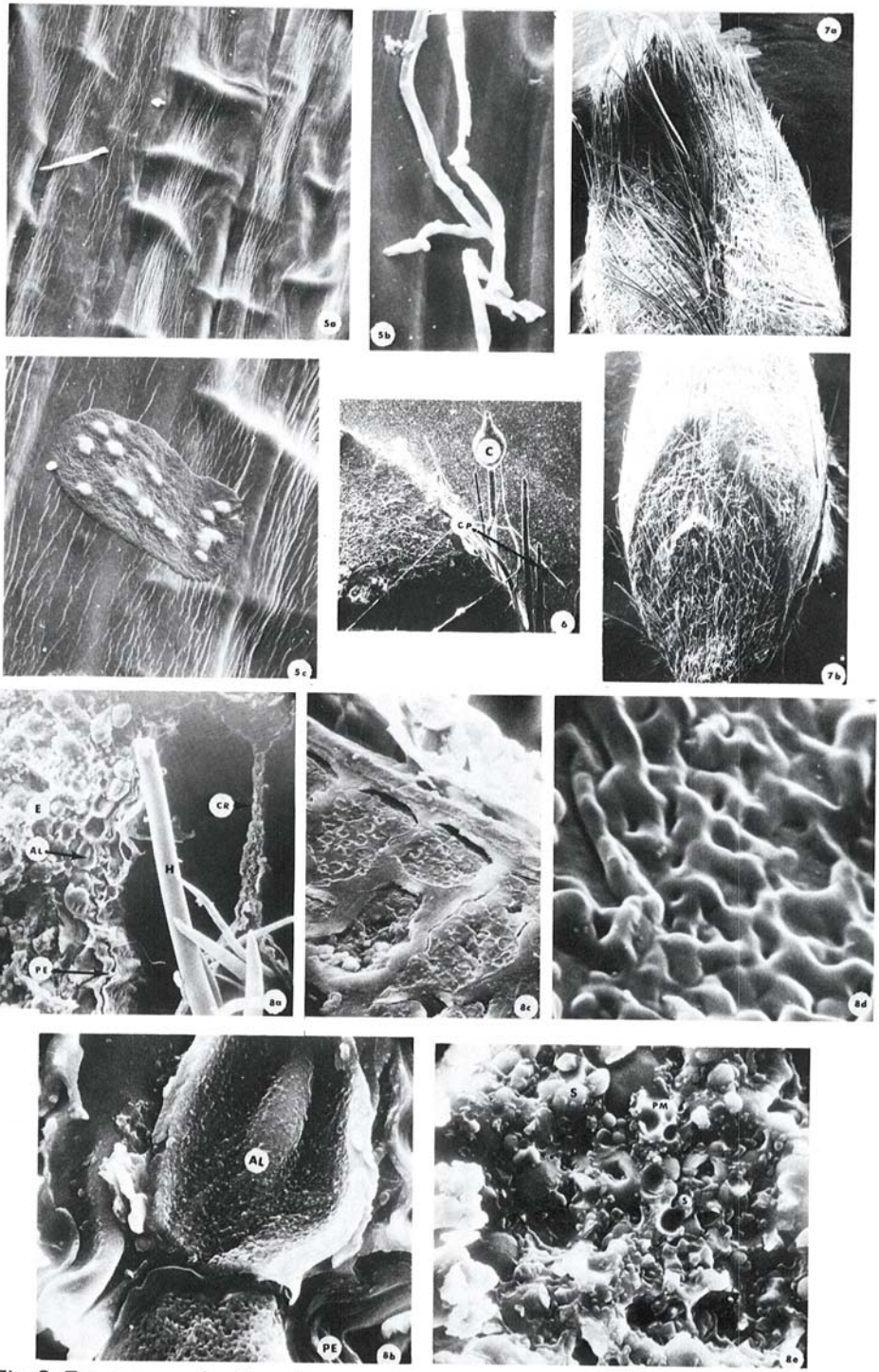
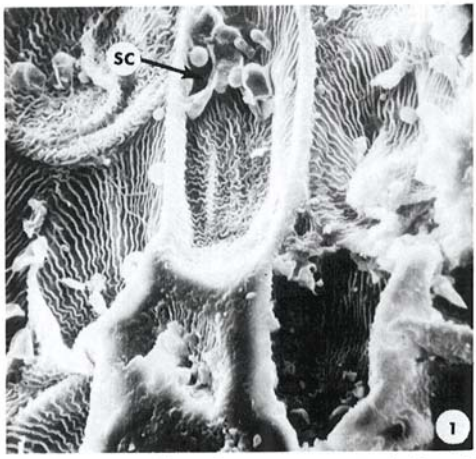


Fig. 8. Transverse section through groat. a) Low magnification (150X) showing crease (CR) with hairs (H), pericarp layers (PE), aleurone layer (AL), and starchy endosperm (E). b) High magnification of aleurone cells from Fig. 8a; AL = aleurone cell, PE = pericarp (1,480X). c) Section through aleurone layer in the distal side of the caryopsis (740X). d) Structure of the aleurone cell wall (3,750X). e) Central endosperm; PM = protein matrix, S = starch granules (750X).

Scanning Electron Microscopy of the Buckwheat Kernel

Y. POMERANZ and I. B. SACHS *(Refer to page 23 for text)*



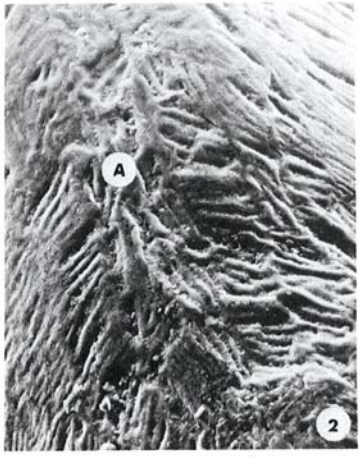
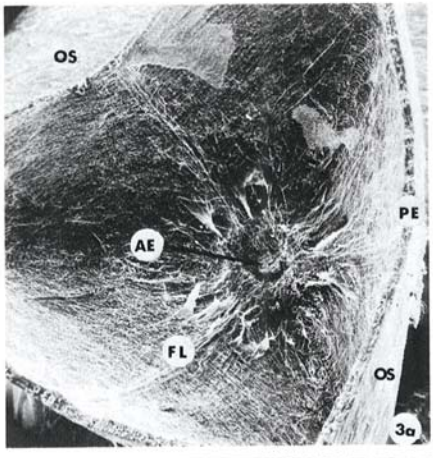
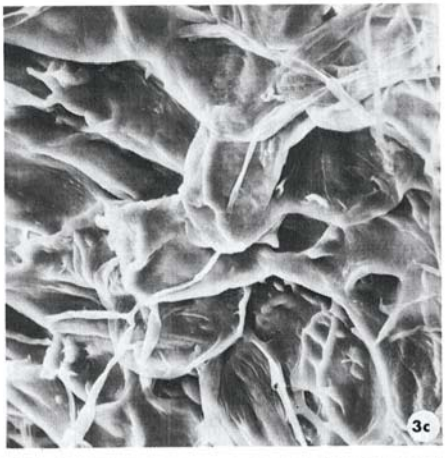
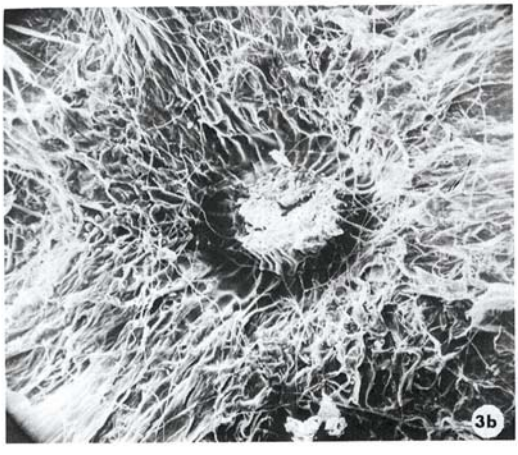
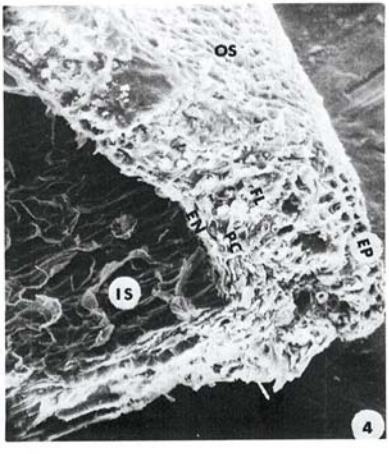


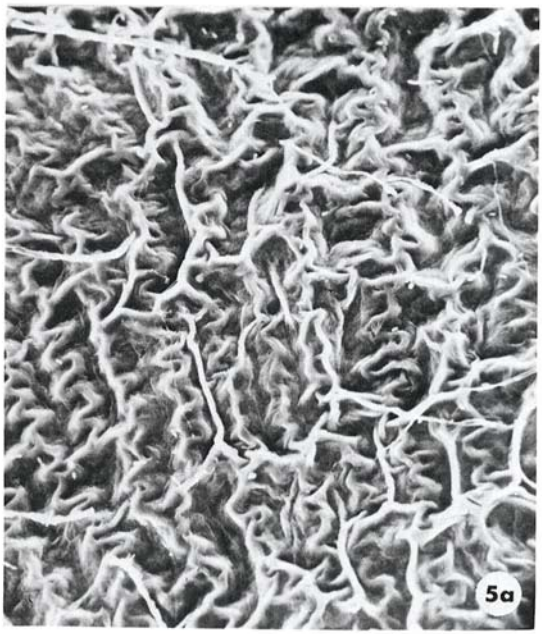
Fig. 1. Structure of a calyx segment attached to the hull of buckwheat (490X); SC = Stone cells. Fig. 2. Outersurface of hull at angle A (180X).











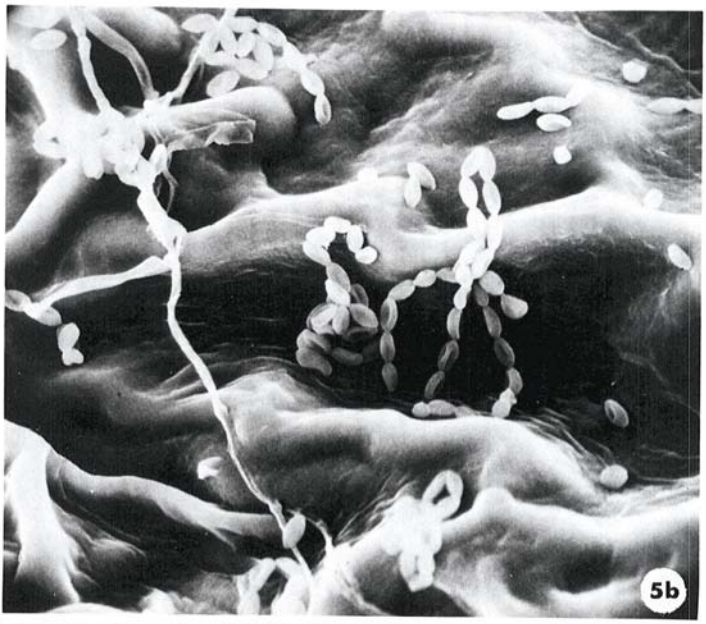


Fig. 5. a) Spermoderm surface (285X). b) Conidia and fungal hyphae on the spermoderm surface (1,300X).

Fig. 3. a) Section through hull (PE) showing outer surface (OS), fiber layers (FL), and attachment end (AE) at the inner surface (18X). b) Higher magnification of attachment end and of fiber layers at the inner surface of the hull (95X). c) High magnification of fiber layers at the inner surface of the hull (470X).

Fig. 4. Cross-section through the hull at the angle showing outer surface (OS), epicarp (EP), fiber layers (FL), parenchyma cells (PC), endocarp (EN), and inner surface (IS) (155X).

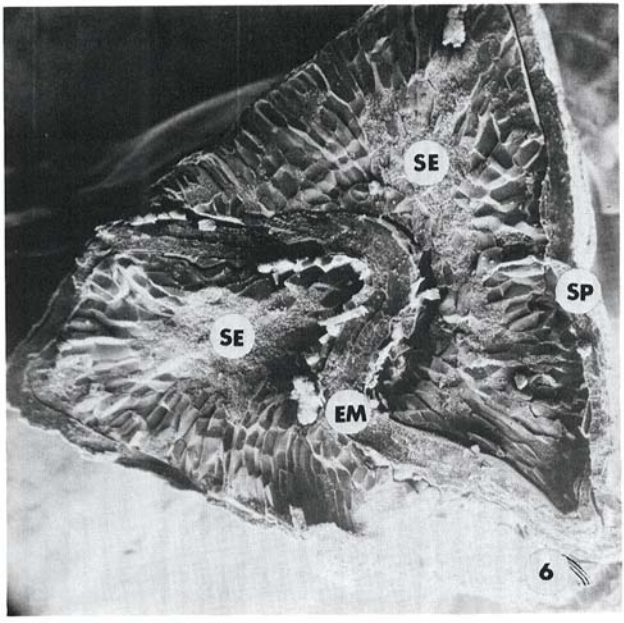


Fig. 6. Cross-section through a dehulled buckwheat achene showing spermoderm (SP), embryo (EM), and starchy endosperm (SE) (25X).

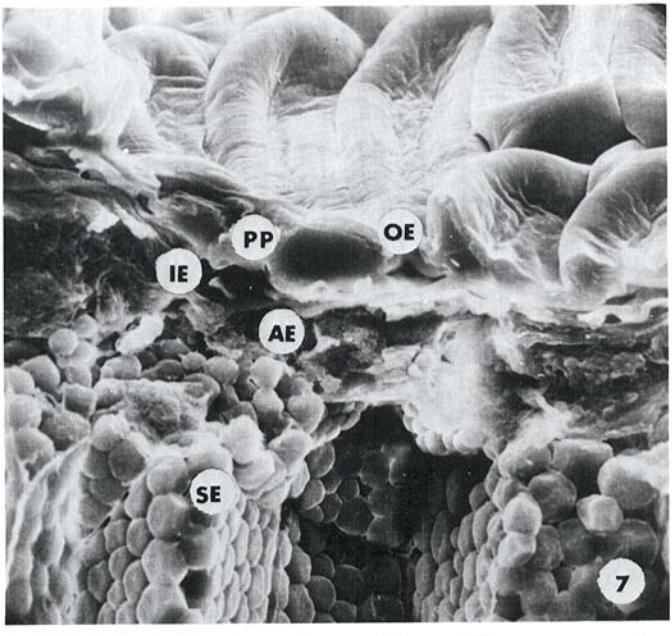


Fig. 7. Cross-section through spermoderm (outer epiderm = OE, spongy parenchyma = PP, and inner epiderm = IE), aleurone layer (AE), and starchy endosperm (SE) (1,170X).

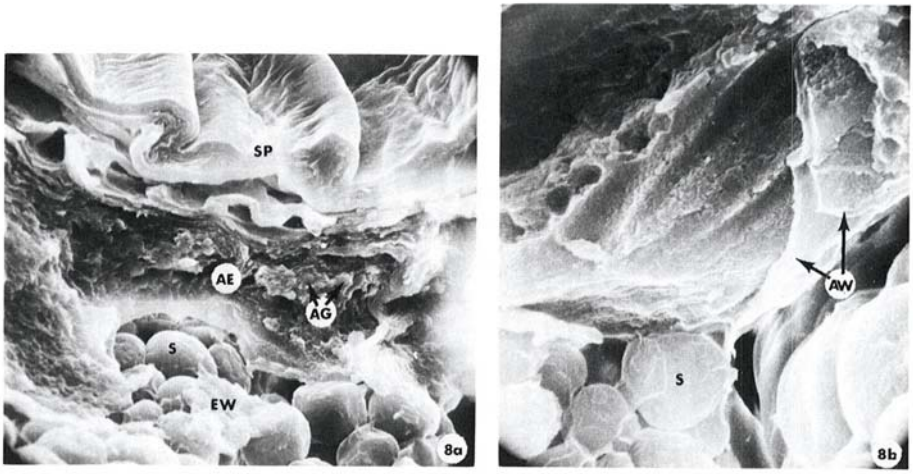


Fig. 8. a) Cross-section through spermoderm (SP), aleurone layer (AE) with aleurone grains (AG), subaleurone endosperm with starch granules (S), and endosperm cell wall (EW) (1,410X). b) Wall of two adjacent aleurone cells (AW) and starch granules (S) of subaleurone endosperm (3,700X).

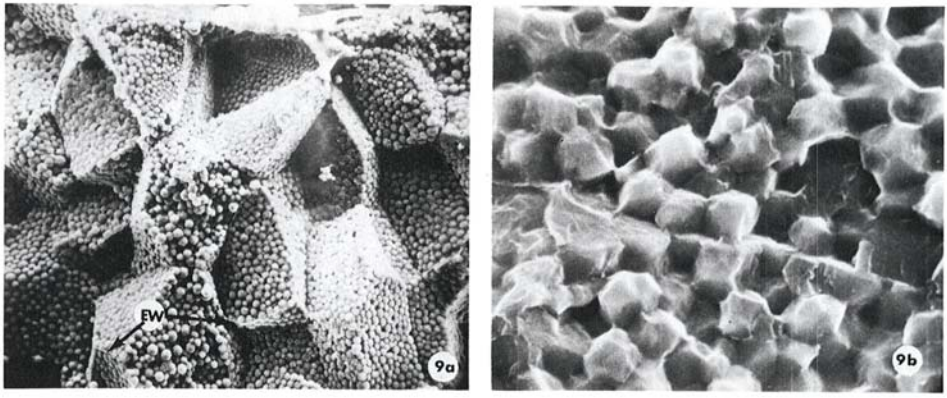


Fig. 9. a) Starch granules in center of endosperm (185X), EW = cell wall of starchy endosperm. b) High magnification of starch granules from center of endosperm (1,310X).

(continued from page 6)

fivefold magnification of Fig. 2, b. The aleurone grains have pitted, irregular surfaces. Malting had no effect on the appearance of the aleurone grains, but kilning somewhat distorted their spherical shape. Jones (9) found that within 2 hr. of gibberellic acid treatment of aleurone cells, the aleurone grains had lost the spherical appearance characteristic of aleurone cells incubated in water and buffer alone. This swelling increased with increased exposure of the cells to gibberellic acid.

Early in malting, proteolytic enzymes are elaborated and distributed throughout the kernel. Their attack renders about 40% of the total protein soluble in water or dilute salt solutions upon mashing (10). The rigid structure of the barley endosperm is in part owing to the protein present; degradation of the proteins (mainly hordein, a barley prolamine, and glutelin) contributes to the physical modification of the kernel (10). The conventional view is that starch granules, rendered more accessible by the action of cytolytic enzymes on cell-walls in malting, are attacked by amylases (1). According to Pollock (11), shortly after cell-walls of the barley endosperm near the embryo have been acted upon by cytolytic enzymes, the starch granules within those cells show evidence of erosion, i.e., characteristic pitting. At the end of malting, almost 20% of the starch in the malt was degraded (12). However, Dickson and Shands (3) reported that "the structural changes in the starch granules during malting appear to be slight. . . .While the starch granules are freed from the imbedding matrix during the germination period of malting, there is little indication of structural changes in the granules, except immediately adjacent to the scutellum."

Recently, it has been suggested (13) that proteases are capable of rapid movement in malt because the cementing materials between and through endosperm cells appear to be proteinaceous. It was postulated that α-amylase moves in association with proteases and that hemicellulases follow more slowly. Similar conclusions were reached in another review of recent work (14) which stated that the proteolytic activity during barley germination may be more significant in modification than has been realized previously. Thus, when purified papain was applied to endosperm slices, the individual cells separated. However, hemicellulase and α-amylase had no effect, contrary to predictions based on previously published work. It was suggested (14) that degradation of protein in the interstices of the endosperm cells might be a prerequisite to cell-wall degradation. The aleurone cells of barley are known to be interconnected by plasmodesmata (15), which could facilitate movement of proteolytic enzymes without degradation of hemicelluloses in the cell-walls.

The degradation of carbohydrates was reexamined recently by Barrett and Kirsop (16). It was shown that previous reports were based on studies in which, apparently, the amylolytic enzymes of malt were not inactivated prior to assay of mono- and oligosaccharides in malt. When the amylolytic enzymes of malts were inactivated, the malts contained only very small amounts of starch breakdown products. The findings reported here are in agreement with the results of the recent investigations.

The appearance of the subaleurone layer (Fig. 4, a) and central starchy endosperm (Fig. 4, b) shows starch granules embedded in a protein matrix and a

rather thick cell-wall (Fig. 4, b). Some of the starch granules shown in Fig. 4, a, were apparently damaged during cutting of the barley kernel. The protein matrix comprises, as expected, a larger fraction of the endosperm in the subaleurone layer (Fig. 4, a) than in the center of the starchy endosperm (Fig. 4, b). The starch granules in the subaleurone layer (Fig. 4, a) are more uniform in size than in the interior of the endosperm (Fig. 5, a). Whereas there was some degradation during malting of cell-wall material in the subaleurone layer, a substantial amount of cell walls was retained in the center of the grain (Fig. 5, a); in the kilned grain (Fig. 5, b), degradation of cell-walls was very extensive even in the center of the endosperm. While some structural modification of starch appears to have taken place after malting (Fig. 5, a), the extent of modification in the center of the grain is rather small. The strongest and most consistent impression from comparing the central endosperm of steeped and malted grain was the dissolution of the protein matrix resulting in "freeing" the small starch granules. In the kilned malt (Fig. 5, b), the additional breakdown of the cell-wall material and of the protein matrix is accompanied by what would appear to be heat-coagulation of some of the protein and extensive degradation of the larger starch granules.

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