Starch Degradation in Endosperms of Barley and Wheat Kernels During Initial Stages of Germination[1]

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ABSTRACT

During initial stages of germination, patterns of starch degradation in kernels of barley and durum wheat appeared to be similar as assessed by scanning electron microscopy. Degradation started at the ventral crease edge of the endosperm-embryo junction and moved along this junction to the dorsal edge. This suggests that the site of initial α-amylase synthesis in germinating cereal grains is the embryo and not the aleurone layer.

Modification of barley kernels during malting is a complex process that includes the synthesis and activation of hydrolytic enzymes, extensive breakdown of protein and cell wall material within the endosperm, and small but significant hydrolysis of starch granules (Briggs 1978). This hydrolysis is performed primarily by α-amylase (Sandstedt 1955), an enzyme that is present in only small quantities in sound barley but increases rapidly during malting (Briggs 1968). The site of synthesis of this enzyme in germinating kernels has been a controversial issue for many years.

Brown and Morris (1890), in their classic and thorough studies, reported that starch granules close to the embryo were the first to show signs of degradation in germinating barley kernels and concluded that the epithelium of the scutellum was the site of synthesis of diastatic enzyme (α-amylase). This finding was contested by Haberlandt (1890, 1914), who suggested that the aleurone layer rather than the scutellum was the major source of α-amylase in germinating barley kernels. Later work by Dickson and Shands (1941) suggested that the conclusions of Brown and Morris were correct, but much later, Briggs (1964) showed that both the aleurone layer and the scutellum of germinating barley kernels contain α-amylase. Therefore, both tissues appear to synthesize the enzyme.

The discovery that the hormone gibberellin activates aleurone tissue of cereal grains to synthesize α-amylase (Briggs 1963, MacLeod et al 1964, Paleg 1960, Yomo 1958) focused much research effort on α-amylase synthesis in isolated aleurone layers and in aleurone layers within intact kernels of cereal grains. Because of the obvious technological importance of α-amylase in malted barley, most research workers studied α-amylase synthesis in barley rather than in other cereal grains. No doubt now remains that barley aleurone layers under the stimulation of gibberellic acid are capable of synthesizing large amounts of α-amylase (Briggs 1963, Varner 1964, Varner and Ram-Chandra 1964) but this does not necessarily prove that aleurone-synthesized α-amylase is primarily responsible for starch degradation during malting (Palmer 1980). Recent evidence (Gibbons 1979, 1980; MacGregor 1980) suggests that the scutellar epithelium is mainly responsible for the synthesis of α-amylase during initial stages of germination. A similar conclusion was reached by Okamoto et al (1980) on the initial site of α-amylase synthesis in germinating kernels of barley, wheat, rye, oats, and maize.

A detailed examination of barley endosperms during germination should reveal the area of initial starch degradation and thus indicate the source of α-amylase. This article describes the results of such a study using scanning electron microscopy to examine endosperms of barley kernels germinated for various periods of time with and without prior steeping. In addition, samples of sound and field-germinated kernels of durum wheat were analyzed.

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MATERIALS AND METHODS

Grain Samples

Malt was prepared from the two-rowed barley cultivar Klages using steeping and germination units described previously (Bettner et al 1962). Samples were removed after steeping and after 24, 48, 72, 96, and 120 hr of germination. All samples were freeze-dried.

Kernels of Klages barley were soaked for 20 min in sodium hypochlorite solution (1.5%) and rinsed thoroughly with deionized, sterile water. These kernels were germinated at 18° C in sterile petri dishes, each containing three pieces of Whatman No. 1 filter paper, 4 ml of water, and 50 kernels. Samples were removed after 24, 48, 72, 96, 120, and 164 hr of germination and deep-frozen and freeze-dried after shoots and rootlets were removed.

The durum wheat used in this study was a farm sample containing a 5% level of sprout damage as assessed by visual examination. The criteria used for sprouting were a swollen germ with a definite break in germ end and an emerging or emerged rootlet. From this sample, sound, sprung, and sprouted kernels were selected. Criteria used for sprung kernels were that the germ end be swollen but not broken, and that the germ end have a distinctly floury appearance.

α-Amylase Activity

Freeze-dried kernels were ground in a Wiley mill through a 1-mm sieve. The fine grist was assayed for α-amylase activity using amylopectin β-limit dextrin as substrate (Briggs 1961, MacGregor et al 1971).

Scanning Electron Microscopy

Kernels were cracked open longitudinally through the crease, fixed to microscope stubs with Dotite silver paint (Fujikura Kasei Co. Ltd., Tokyo), and coated with gold. Samples were analyzed on

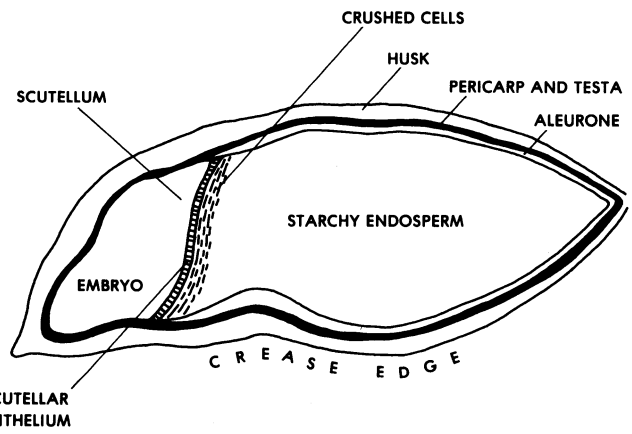

Fig. 1. Longitudinal section of a barley kernel cracked open through the crease edge.

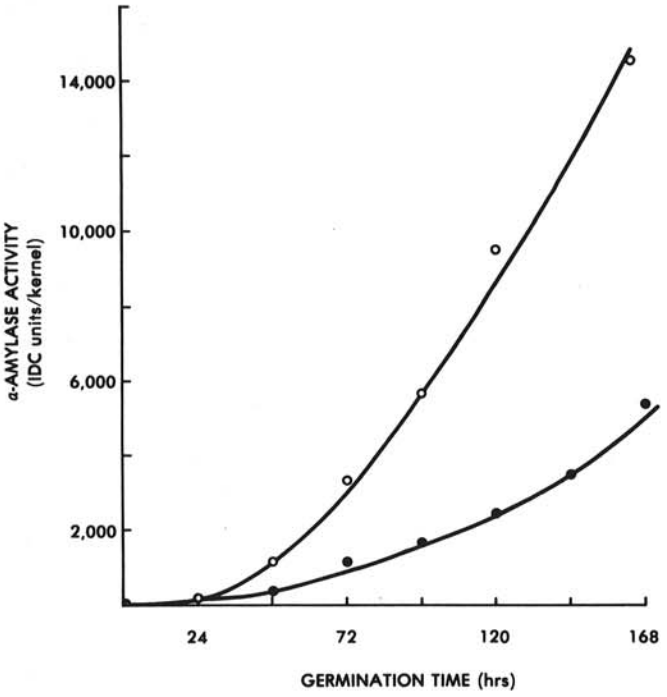

Fig. 2. Synthesis of α-amylase in barley kernels during germination. -o-o-o- germination in petri dish; -●-●-●- kernels steeped for initial period of 48 hr followed by germination (preparation of malt).

a JEOL 35C scanning electron microscope at an accelerating voltage of 10 kV. Photomicrographs were taken on Plus-X Pan Kodak film.

RESULTS AND DISCUSSION

All kernels used in this study were split open longitudinally through the crease, using a scalpel. Care was taken to crack open kernels rather than slice through them because the latter procedure tended to smear the cut surfaces, thereby distorting or even completely hiding structural features. Kernel areas of interest in this study are shown in Fig. 1, which depicts a longitudinal section through a barley kernel with the crease edge or ventral furrow at the bottom. This study focused on the area of the endosperm adjacent to the embryo. Lying between the starchy endosperm and the embryo is a layer of compressed or crushed cells that is part of the endosperm. During early development of the kernel, these cells were reputed to contain starch, but as the embryo grew, contents were depleted, and the empty cells were crushed between the growing embryo and endosperm tissues (Brown and Morris 1890). Between the crushed cells and the scutellum is a single layer of cells called the scutellar epithelium, which contacts the cells of the aleurone layer.

The increase in α-amylase activity of kernels of Klages barley germinated in petri dishes is shown in Fig. 2. Enzyme synthesis was relatively slow up to 48 hr of germination, but thereafter it proceeded rapidly and showed little sign of diminishing even after 164 hr. Kernels at different stages of germination were examined in the scanning electron microscope to determine the pattern of starch degradation in the endosperm. Preliminary studies (MacGregor

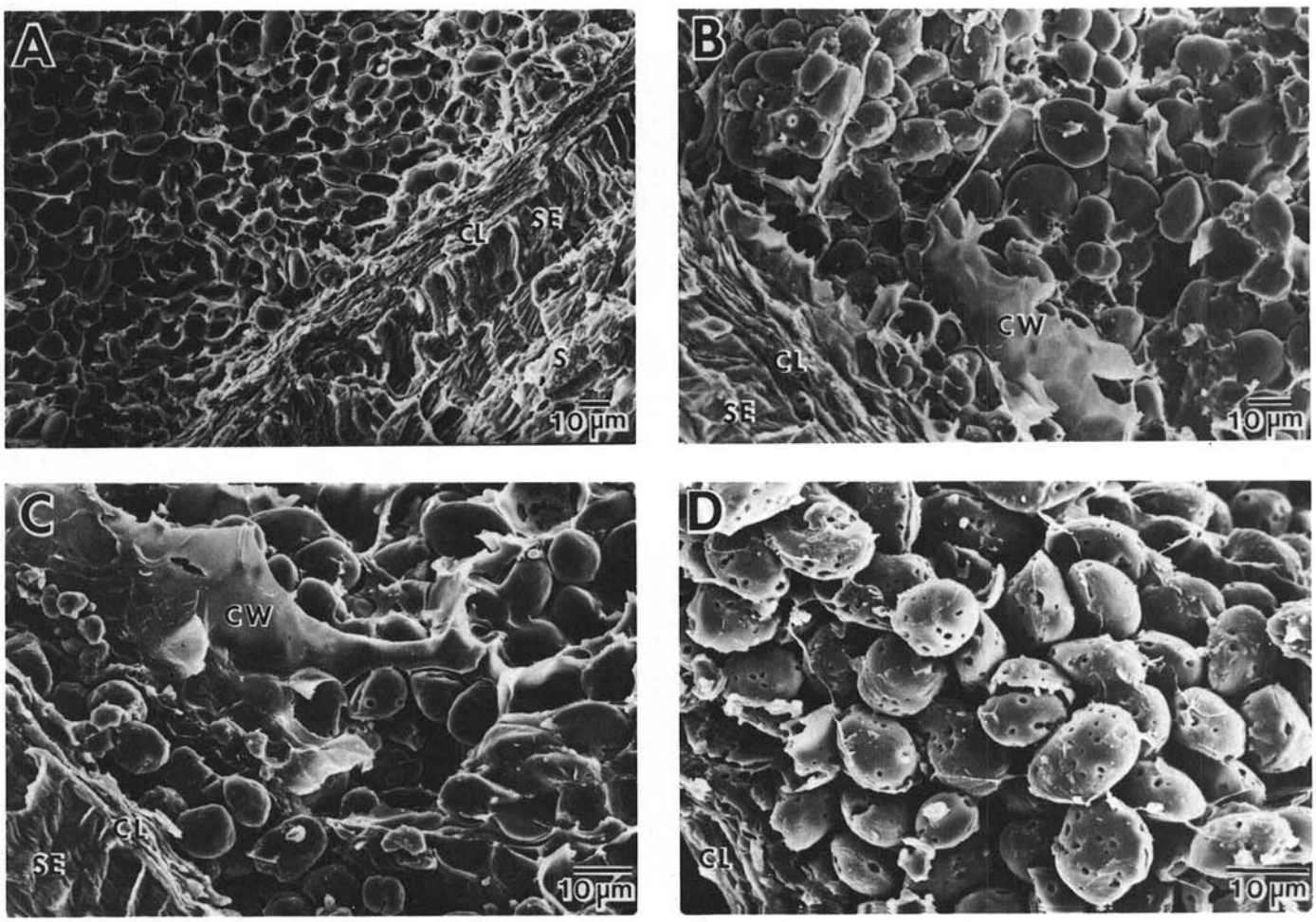

Fig. 3. Scanning electron photomicrograph of barley endosperms. **A, B,** sound, mature kernel; **C,** kernel germinated for 24 hr; **D,** 48-hr germinated kernel. **S** = scutellum; **SE** = scutellar epithelium; **CL** = crushed layer of cells; **CW** = cell wall.

1980) had shown that initial signs of starch degradation were found in that part of the endosperm adjacent to the embryo, so this study focused on the endosperm-embryo junction.

The structure of the endosperm-embryo junction in the central part of a typical kernel of Klages barley is shown in Fig. 3A. Structures such as the starchy endosperm, the crushed layer of cells, and the columnar cells of the scutellar epithelium are readily apparent. The protein matrix in which the starch granules are embedded varies widely in amount both within individual kernels and between kernels of the same cultivar. Sometimes the matrix is so dense that individual granules are barely visible (vitreous or steely areas); in other areas of the endosperm little or no matrix is apparent, and large numbers of free starch granules can be seen (starchy or floury areas). In Fig. 3A, the protein matrix is sufficient to bind the granules without completely covering them. This is typical of the kernels of Klages used for this study.

Many of the supposedly sound barley kernels studied showed small areas of starch degradation (Fig. 3B). These isolated spots of degradation were found beside the crushed layer of cells and were usually, but not always, close to the crease edge of the endosperm-embryo junction.

After 24 hr of germination, all kernels contained areas of starch degradation adjacent to the crushed layer of cells. These degraded areas were concentrated at the crease end of the endosperm-embryo junction and although they varied in size they did not extend far into the endosperm. Such a degraded area is shown in Fig. 3C. The protein matrix has been eroded, and some of the starch granules have been partially degraded. Attack appears to commence on many, but not all, granules at the equatorial groove, as noted by other workers studying barley and wheat (Evers and McDermott 1970, Jones and Bean 1972, Lineback and Ponpipom 1977, Maeda et al 1978, Palmer 1972).

Starch degradation after 48 hr of germination had become more extensive along the endosperm-embryo junction, but none was detected close to the dorsal edge. Degradation was still concentrated close to the crease edge, but it extended a little further into the endosperm (Fig. 3D). In this area, cell wall and proteinaceous material had disappeared, and many starch granules showed "pin-hole" damage as well as damage to the equatorial groove.

The extent of starch degradation along the endosperm-embryo junction after 72 hr of germination is shown in Fig. 4. In most kernels examined, starch degradation extended most of the way along the endosperm-embryo junction. By this stage many granules at the crease edge were highly degraded (Fig. 4A), and the internal ring structure of the granules described by other workers (Gallant and Guilbot 1973, Kiribuchi and Nakamura 1973a) can be clearly seen. The area of degradation extended much further into the endosperm and was appreciable even halfway along the endosperm-embryo junction (Fig. 4B). Here the gradation in starch granule degradation can be seen. Close to the embryo, the starch granules were almost completely destroyed, and degradation became less in granules further away from this area. Close to the dorsal edge were signs of granule attack (Fig. 4C). Many small granules had roughened surfaces typical of α-amylase attack (Kiribuchi and Nakamura 1973b, MacGregor and Ballance 1980), and large granules also showed evidence of attack.

Figure 4D shows the endosperm-embryo junction where it meets the aleurone layer on the dorsal edge of the kernel. Some starch degradation is visible, but it is not extensive. However, little

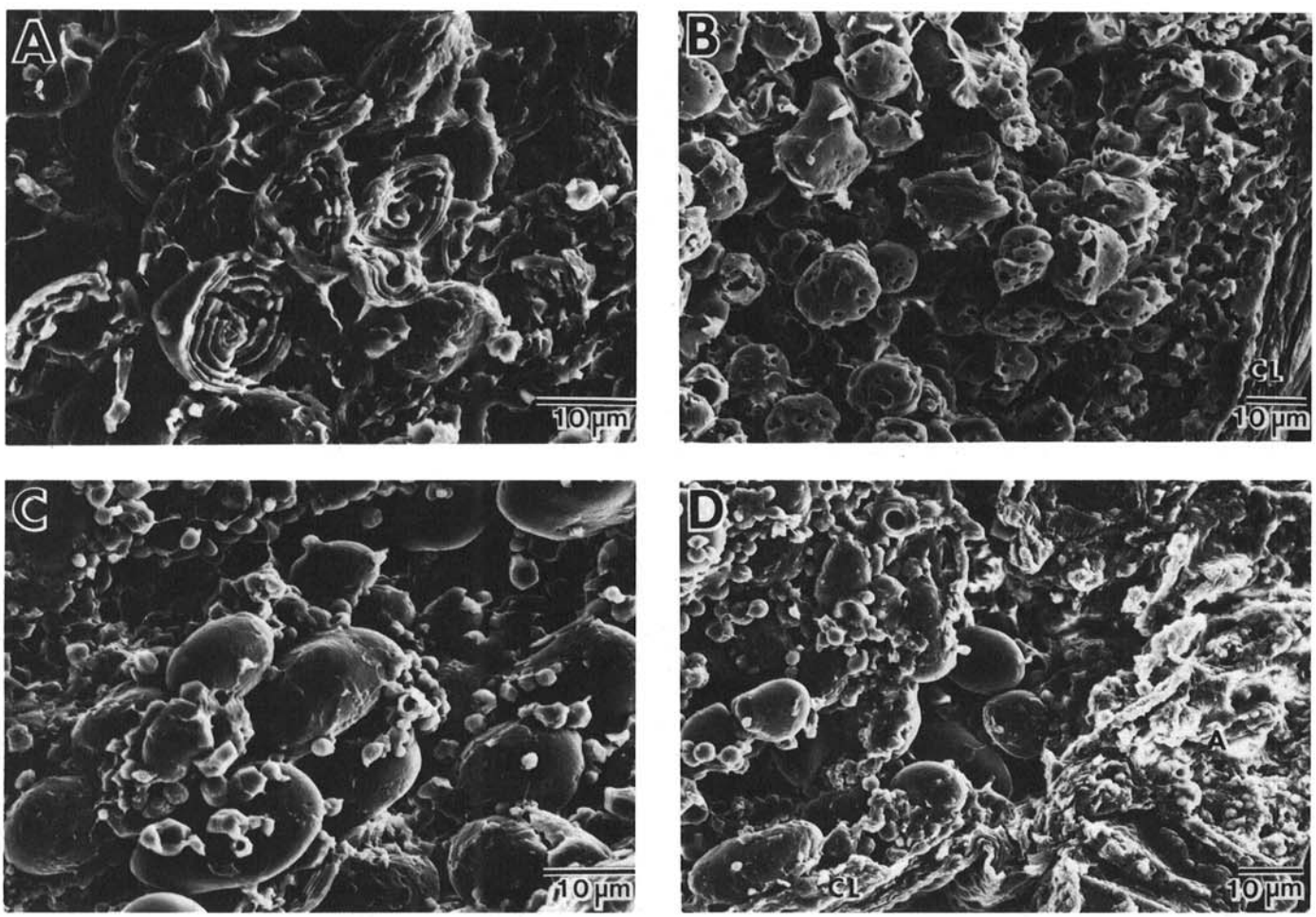

Fig. 4. Scanning electron photomicrograph of endosperms of 72-hr germinated barley. **A**, degraded starch granules close to endosperm-embryo junction and crease edge of kernel; **B**, halfway along endosperm-embryo junction; **C**, starch granules close to dorsal edge of endosperm-embryo junction; **D**, endosperm-embryo-aleurone layer junction at dorsal edge. **CL** = crushed layer; **A** = aleurone layer.

remains of the protein matrix and endosperm cell walls.

After 96 hr of germination, a narrow band of highly degraded granules extended the whole length of the endosperm beside the layer of crushed cells. At this stage, degradation had still not extended very far into the endosperm at the dorsal edge of the endosperm-embryo junction. Most of the detectable degradation in this area appeared to be radiating from the embryo. However, after 120 hr of germination extensive starch degradation was apparent in this area, and few intact starch granules remained close to the embryo or aleurone layers. These results suggest that at this stage α-amylase from the aleurone layer was playing a major role in granule degradation, but the degraded area did not extend very far into the endosperm. Large numbers of small starch granules were visible not far from the aleurone layer, again indicating small penetration of the aleurone α-amylase into the endosperm. Small starch granules are usually hydrolyzed faster than large granules by α-amylases from germinated barley (MacGregor and Ballance 1980).

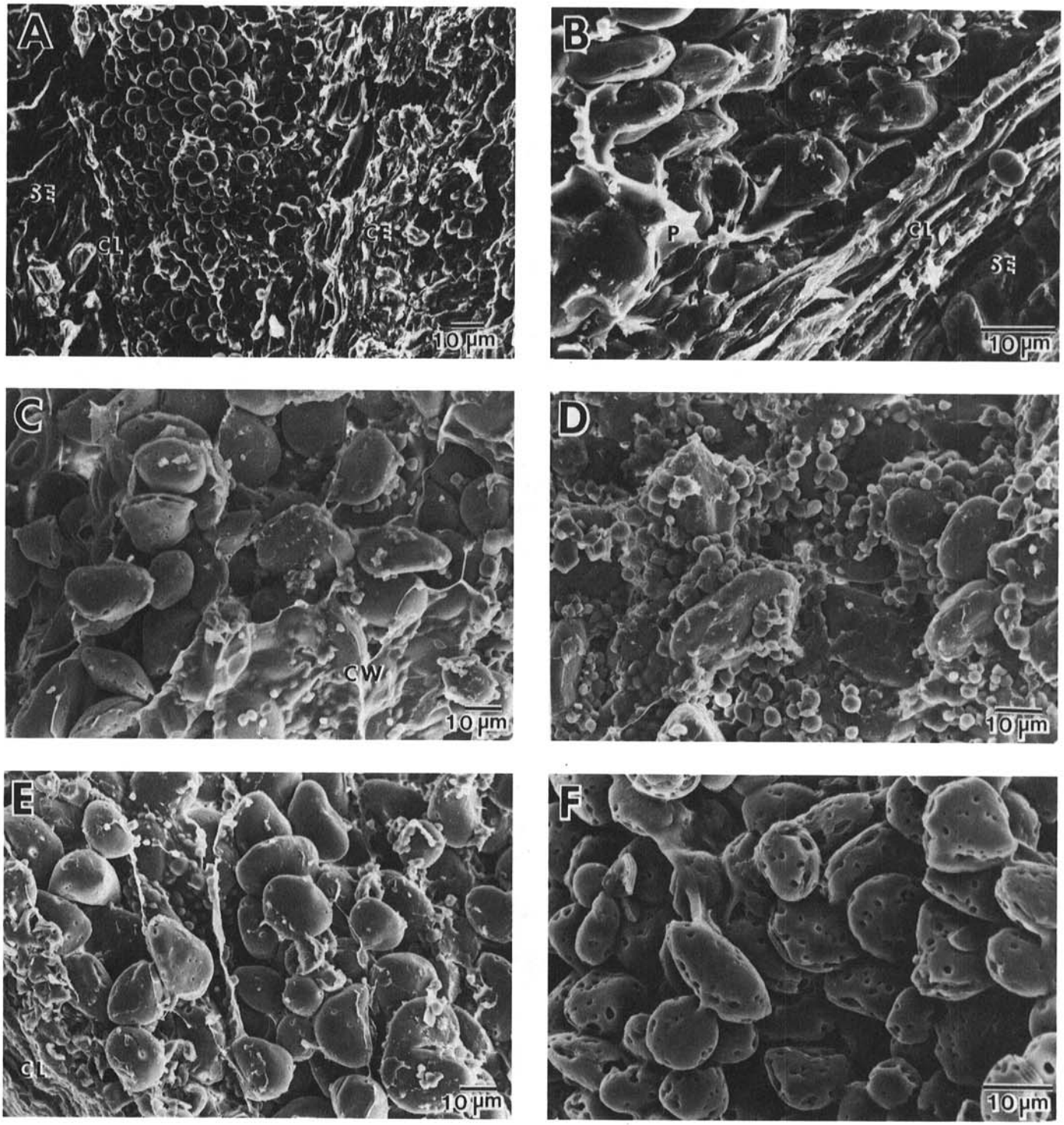

Fig. 5. Scanning electron photomicrograph of barley endosperms during malting. **A**, barley steeped for 48 hr, endosperm-embryo junction close to ventral crease edge; **B**, barley steeped for 48 hr, degraded starch granules at endosperm-embryo junction close to crease edge; **C**, steeped barley germinated for 48 hr, degraded starch granules halfway along endosperm-embryo junction; **D**, steeped barley germinated for 48 hr, starch granules close to endosperm-embryo-aleurone junction at dorsal edge; **E**, steeped barley germinated for 96 hr, degraded starch granules at the endosperm-embryo junction close to dorsal edge; **F**, same sample as 6E, highly degraded starch granules at endosperm-embryo junction close to ventral crease edge. **CL** = crushed layer; **SE** = scutellar epithelium; **CW** = cell wall; **P** = protein; **CE** = crease edge.

The pattern of starch degradation in the malted Klages kernels is shown in Fig. 5. After a steeping period of 48 hr, degraded starch granules were always found at the endosperm-embryo junction close to the crease edge (Fig. 5A). Further along this junction, pockets of degradation such as that shown in Fig. 5B were often observed, but no degradation was detected at the dorsal edge of the grain. After 48 hr of germination, extensive starch degradation was observed close to the crease and, even in the central endosperm, degraded granules were detected close to the embryo (Fig. 5C). Here the protein matrix had been largely hydrolyzed, but thin, filamentous sections of cell wall material remained. Close to the dorsal edge extensive cell wall and protein matrix hydrolysis had occurred, but little evidence of starch degradation could be found (Fig. 5D). Subsequently, in the samples germinated for 96 hr, starch hydrolysis was visible at the endosperm-embryo junction close to the dorsal edge (Fig. 5E), but at this stage very extensive granule disintegration was apparent at the crease edge of this junction (Fig. 5F). The starch granule hydrolysis apparently started at the crease edge of the endosperm-embryo junction and proceeded along this junction to the dorsal edge.

Sound, sprung, and sprouted kernels were hand-picked from a sample of durum wheat containing 5% sprout damage. The α-

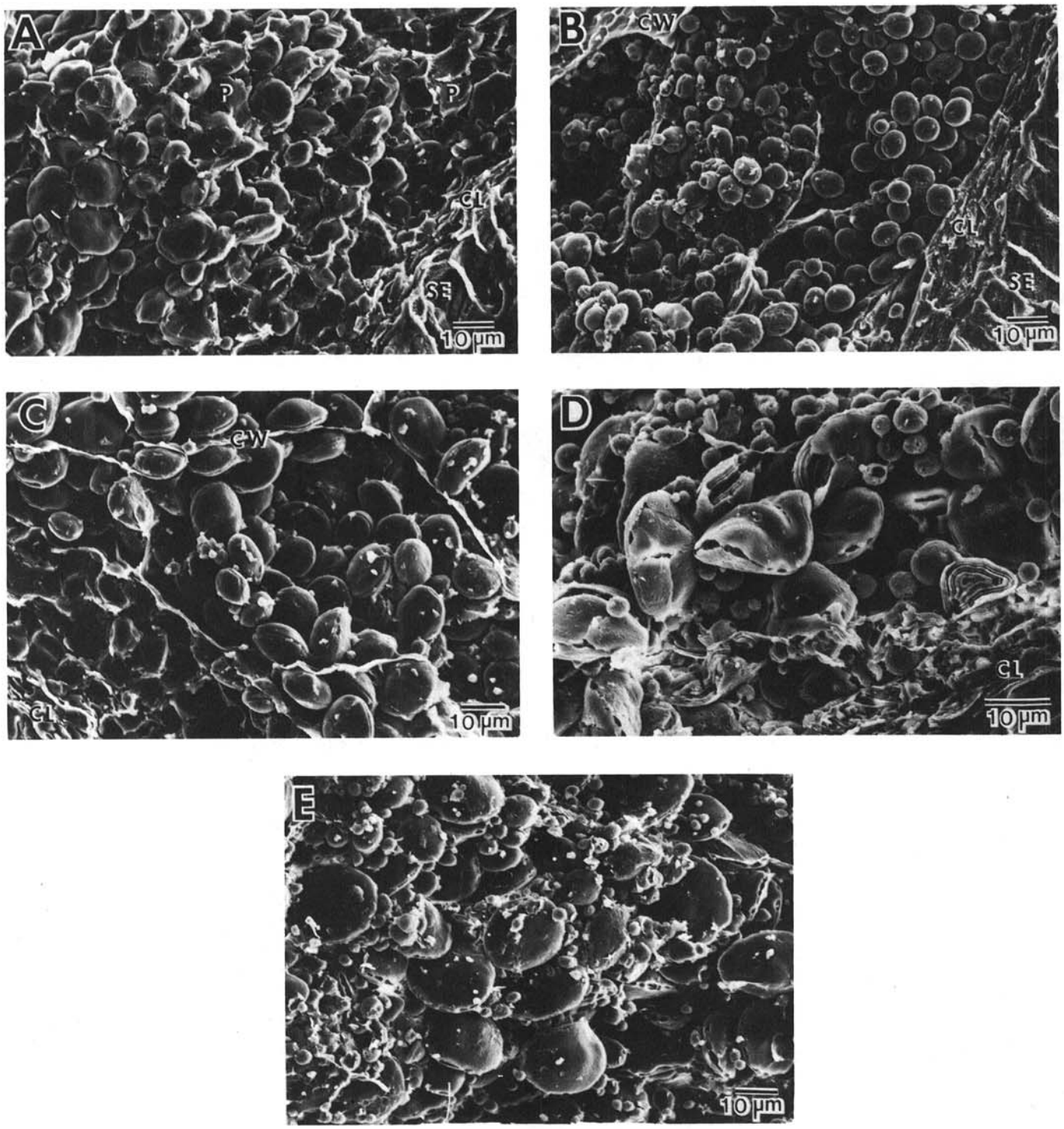

Fig. 6. Scanning electron photomicrograph of endosperms of durum wheat. **A,** sound, mature kernel; **B, C,** "sprung" kernels with degraded starch granules at the endosperm-embryo junction; **D**, sprouted kernel, extensively degraded starch granules at the endosperm-embryo junction close to the ventral crease edge; **E**, sprouted kernel, lightly degraded starch granules at the endosperm-embryo junction close to the dorsal edge.

amylase content of these samples is given in Table I. All samples contained detectable levels of α-amylase, and even the sound kernels contained higher levels of activity than is normally found in sound, mature kernels of durum wheat.[2] Sprung kernels contained appreciable amounts of α-amylase, suggesting that even at this very early stage of sprouting—as indicated by small physical changes in the kernel—extensive synthesis of hydrolytic enzymes had taken place.

The sequence of starch degradation in the endosperm of pregerminated durum wheat is shown in Fig. 6. Sound kernels with no physical signs of pregermination and small levels of α-amylase activity had only very small and infrequent areas of light starch degradation. Again, these degraded areas were found only close to the embryo. As in barley, a layer of crushed cells separated the endosperm from the layer (one cell thick) of the scutellar epithelium (Fig. 6A). Starch granules, large and small, were embedded well into a protein matrix. In the endosperms of vitreous kernels such as this, identifying individual cell walls can be difficult because of the heavy layer of protein. Endosperm cell walls are more obvious in floury kernels or in floury areas of vitreous kernels where the protein matrix appears to be thinner.

Most sprung kernels examined contained both α-amylase activity and areas of degraded granules. Again, degradation was first detected at the crease edge of the kernel, close to the embryo (Fig. 6B). Some cell wall material was apparent, but the starch granules appeared to be essentially free from the protein matrix. Whether the matrix had already been destroyed or whether it ever had existed in this area of the kernel was not clear. A typical section of the isolated areas of starch degradation found along the endosperm-embryo junction in sprung kernels is shown in Fig. 6C. Remnants of cell walls could be seen, and most of the fairly clean starch granules (little or no adhering protein) showed evidence of attack by α-amylase—especially at the equatorial groove. This area of cereal starch granules appears to be particularly susceptible to attack by α-amylase (Evers and McDermott 1970, Gallant and Guilbot 1973). In the sprung samples examined, starch degradation had not proceeded as far as the dorsal edge of the endosperm-embryo junction.

Sprouted kernels showed appreciably higher levels of α-amylase activity (Table I) and starch degradation (Fig. 6D and E). In some kernels degradation was visible along the whole length of the endosperm-embryo junction. Generally, extensive degradation was found close to the crease edge (Fig. 6D), but only lightly degraded granules were visible at the dorsal edge (Fig. 6E).

Several characteristics of degraded cereal starch granules, such as those shown in Fig. 6D, have been well documented. The equatorial groove is very susceptible to α-amylase attack, and some granules showing extensive degradation in this region can be seen. In some of these granules the internal ring structure, characteristic of cereal starch granules, is quite obvious. The interior of some granules has been hydrolyzed in preference to the granule surface and this, too, is quite characteristic. Despite extensive hydrolysis of large granules, many small granules still remain, and this differs from the normal situation in barley, where very few small granules are found in areas of the endosperm containing highly degraded large starch granules (Bathgate and Palmer 1973). This suggests that small granules of wheat starch are less susceptible to α-amylase attack than are small granules of barley starch. This finding is not inconsistent with earlier reports on the relative susceptibilities to α-amylase of large and small starch granules from wheat and barley (Dronzek et al 1972, Lineback and Ponpipom 1977, Palmer 1972). Many small granules had roughened surfaces resembling those of small granules of barley starch hydrolyzed in vitro by α-amylase (MacGregor and Ballance 1980). However, several small granules also contained large corrosion holes as if the α-amylase had entered the granule and preferentially hydrolyzed the interior portion, as described previously by Dronzek et al (1972) in studies on hard red spring wheat. This type of degradation was only occasionally observed in the barley endosperms analyzed.

Small starch granules from wheat, then, appeared to be hydrolyzed both by surface erosion and by interior hydrolysis via erosion channels from the granule surface. These different types of attack could be caused by different carbohydrase enzymes or by the fact that wheat might contain two different kinds of small starch granules, as suggested by Meredith (1981). Further work is required to clarify this problem.

During initial stages of germination in kernels of barley and wheat, similar physical changes were observed. Starch degradation started at the endosperm-embryo junction, usually close to the ventral crease, and moved along the junction to the dorsal edge of the kernel. This degradation was preceded by extensive breakdown of cell wall material and the protein matrix of the endosperm. The pattern of starch degradation does not necessarily follow the pattern of α-amylase synthesis or excretion into the endosperm, but the results of this study strongly support recent conclusions of Gibbons (1979, 1980) and Okamoto et al (1980) that synthesis of α-amylase in germinating cereal grains starts in the embryo. In recent years much research effort has been devoted to studying α-amylase synthesis in aleurone layers and the effect of gibberellic acid on this synthesis. These results make clear that much more investigation is required on the embryo as a source of α-amylase. This is particularly important for studies on the problems of sprouting or pregermination in cereal grains. Because little is known about the detailed biochemical interactions between the tissues of cereal kernels, such studies should include investigations on the kernel as a whole and not just on isolated tissues.

[2] Matsuo, Dexter, and MacGregor. Unpublished results.

TABLE I
α-Amylase Activities in Sound, Sprung, and Sprouted Kernels of Durum Wheat[a]

Sample	α-Amylase Activity (IDC[b] units/kernel)
Original (5% sprouted)	1,250
Sound	315
Sprung	1,640
Sprouted	9,600

[a] Sound, sprung, and sprouted kernels were hand-picked from a sample of durum wheat that contained 5% sprouted kernels.
[b] IDC = Iodine Dextrin Color.

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