A Microscopic Study on the Development of a Layer in Barley That Causes Hull-Caryopsis Adherence[1]

R. L. GAINES,[2] D. B. BECHTEL,[2,3] and Y. POMERANZ[3]

ABSTRACT

The cementing layer in barley that causes the hull to adhere to the caryopsis was investigated using transmission electron microscopy and cytochemistry. The cementing substance appeared on the surface of the pericarp two days after flowering prior to the physical contact between the caryopsis and the hull. The cementing layer increased in thickness throughout kernel development. The substance is secreted solely by the pericarp epidermis, as no cementing material is found associated with the hull until after pericarp-hull contact. Cytochemical tests for protein and carbohydrate gave negative results for the cementing layer. Thickness of the cementing layer in mature kernels is highly variable, ranging from 130 to over 600 nm. The cement has a distinct morphology, being nearly electron-lucent and bearing faint lamellar striations. The junction between the hull and cement is smooth and distinct, whereas the junction between the cement and pericarp is very irregular. Mechanical separation of the hull from the caryopsis usually results in the cementing layer remaining attached to the pericarp.

Cereal Chem. 62(1):35–40

Most barley harvested in the Western world today is covered, ie, the innermost glumes (lemma and palea) adhere firmly to the caryopsis. This botanical characteristic is important for the use of barley in malting and brewing. The hull serves several functions in malting. It protects the coleoptile during processing and results in more uniform germination. The hull also provides a filtration bed in separation of soluble wort materials during malt processing. Yet, as important as hull adherence is in barley processing, little work has been published on the mechanism of hull-caryopsis attachment. Harlan (1920) described the appearance of a sticky, cementing substance 10 days after flowering that caused the glumes to adhere to the pericarp. He attributed the production of a sticky substance to the caryopsis rather than the glumes. We describe here an ultrastructural and cytochemical investigation of the formation and nature of this cementing layer.

MATERIALS AND METHODS

Covered barley cultivar Kanby, a Kansas winter feed barley, was field-grown during the 1978, 1980, 1981, and 1982 growing seasons. Kanby and naked barleys CI 4362 and Hiproly were prepared for light and electron microscopy as previously described (Bechtel and Pomeranz 1979, Bechtel and Gaines 1982). Enzyme digestions and the periodic acid-thiocarbohydrazide (PI-TCH)-silver protein procedure for carbohydrate localization were carried out according to the procedure of Bechtel and Pomeranz (1981). The carbohydrate localization procedure was modified to use sodium borohydride as an aldehyde blocking agent instead of dimedone (Lillie and Pizzolato 1972, Craig 1974). Freshly made sodium borohydride was made up in a 0.1% aqueous solution and the appropriate sections (according to the localization scheme) were placed in a drop of the borohydride for 30 min at room temperature, followed by washing in double-distilled water.

RESULTS

Initiation of the Cementing Layer from the Caryopsis

The cementing layer is absent on the day of flowering (Fig. 1). The only modification of the pericarp outer epidermal cell wall at this stage is an electron-dense layer 15-25 nm thick (Fig. 2). The pericarp epidermal cells are still growing and depositing cell wall material one day after flowering (DAF) and there is still no modification of the outer cell wall (Figs. 3 and 4).

A distinct cementing layer appears two DAF, at which time the outer pericarp epidermal cells have obtained their maximum cell wall thickness (Fig. 5). The cement forms a layer 65–100 nm thick on the outermost part of the epidermal cell wall (Fig. 6). The cementing layer is unique to the epidermal cells and is not formed by the specialized epidermal trichome cells present early in development (Figs. 7 and 8). The cement is a thin, electron-lucent layer in which tendrils of denser cell wall material are embedded. This layer is unique to covered barleys; comparison of the pericarp outer epidermis of covered and naked barleys clearly shows that the cement is present only in the covered barley. Mature naked barleys (CI 4362 and Hiproly) lack the distinctive cementing layer. Instead, a very thin (35–50 nm) cuticle is present on the pericarp outer epidermal cell wall in the mature kernel (Fig. 9).

Six Days After Flowering

At six DAF, the caryopsis is still not large enough to contact the glumes. Both the lemma (dorsal, outer glume) and palea (ventral, inner glume) are at least 150 μm from the pericarp surface. The inner surfaces of the lemma (Figs. 10 and 11) and palea (Figs. 12 and 13) show no sign of any cementing material or structural modification. A threadlike electron-dense substance is inconsistently present at six DAF and is separated from the cell wall by a 15–50 nm space (Figs. 11 and 13). The cementing layer of the pericarp is 100–150 nm thick at six DAF and does not possess any internal structure (Figs. 14–17). The outer surface of the cementing layer is smooth, but its interface with the pericarp cell wall retains the irregular nature of earlier stages.

Ten Days After Flowering

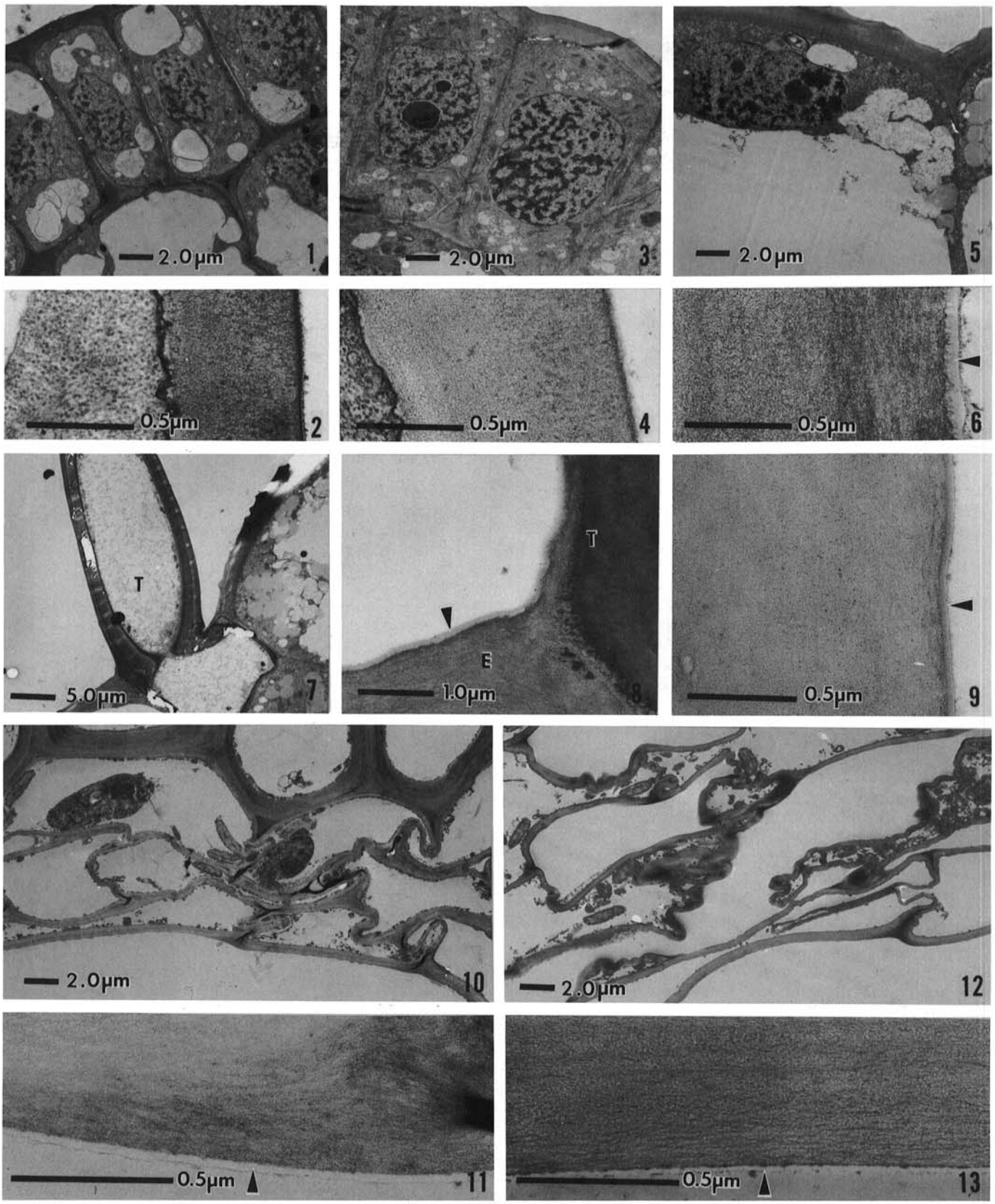
By 10 DAF, the caryopsis has grown enough that the pericarp is in contact with the glumes (Fig. 18). The cementing layer between the glumes and pericarp is 100–600 nm thick, with an average thickness of 400 nm. It is extremely variable in appearance and sometimes does not completely fill the gap between pericarp and hull (Figs. 19 and 20). The cementing layer at some locations is amorphous (Figs. 19 and 20) and at others displays a definite fibrillar or laminar structure (Fig. 21). The fibrils are 12–14 nm thick and are usually arranged closely parallel to the pericarp. The interface between the cementing layer and pericarp retains the irregular appearance (Figs. 20 and 21).

Twenty-one Days After Flowering

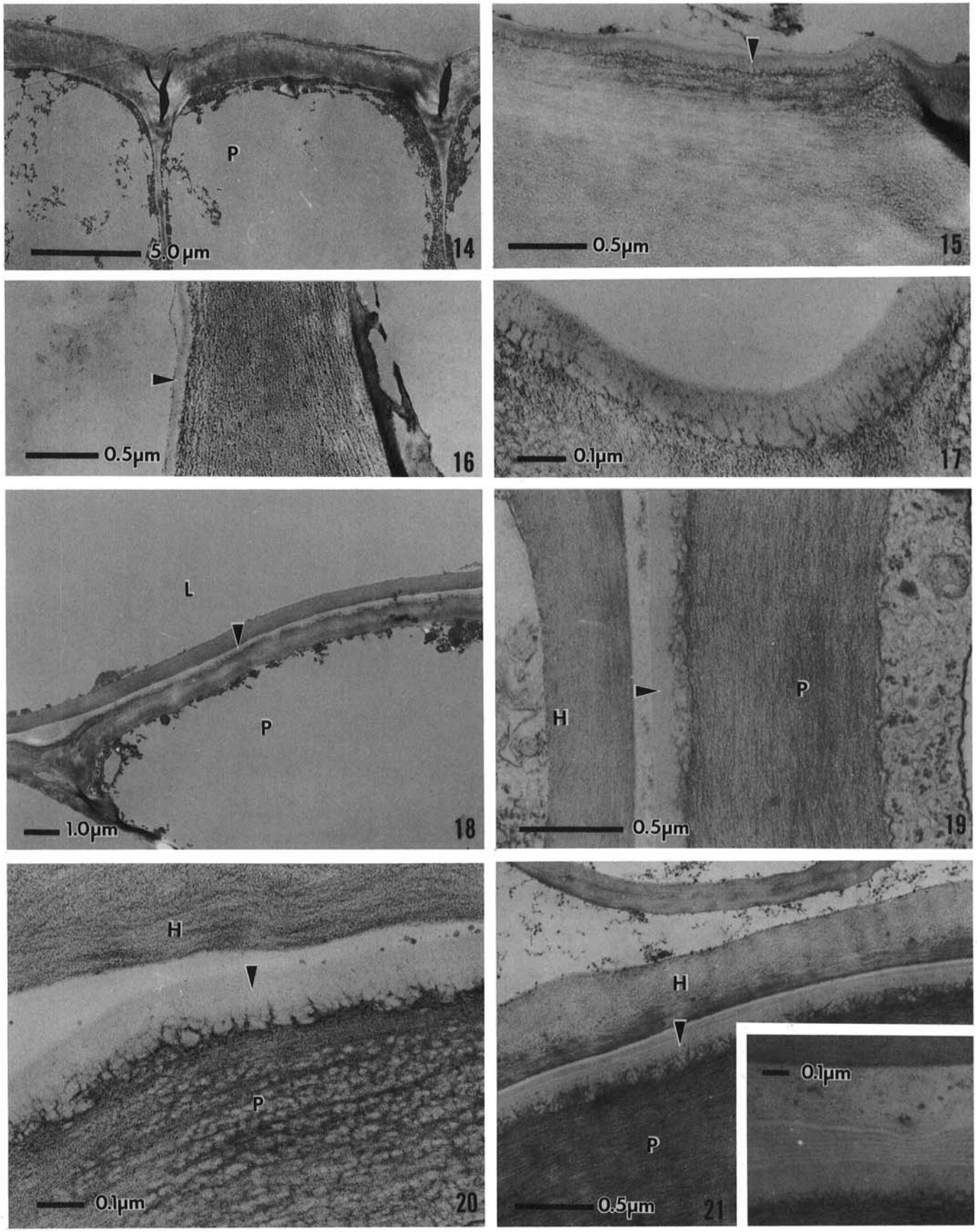
The cementing layer is always present on the pericarp cell wall rather than the glume wherever a separation between the two occurs (Figs. 20 and 22–24). This is particularly evident at points

[1]Mention of firm names or trade products does not constitute endorsement by the USDA over others not mentioned.

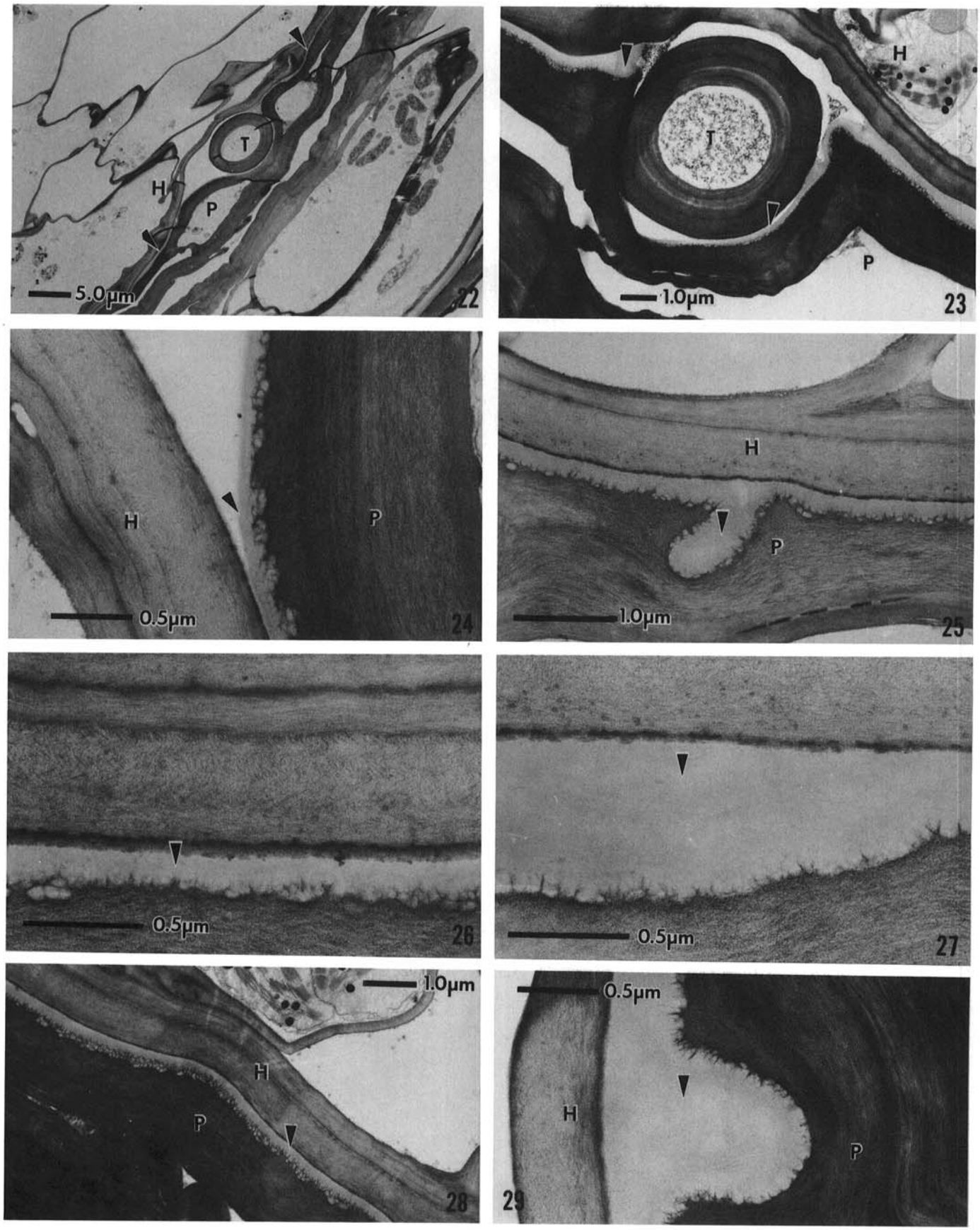
[2]Division of Biology, Kansas State University, Manhattan, KS 66506.
[3]U.S. Grain Marketing Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 1515 College Avenue, Manhattan, KS 66502.



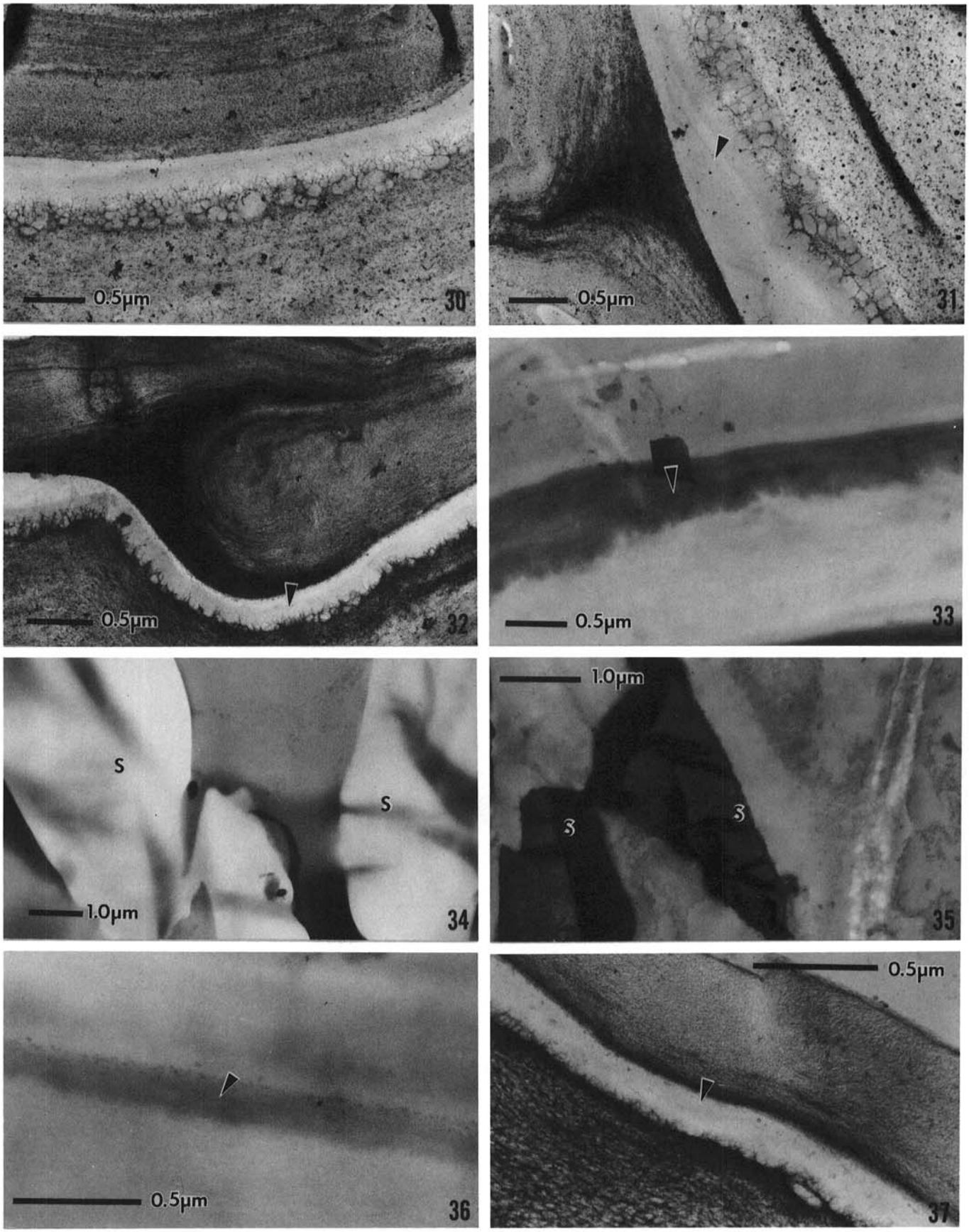
Figs. 1–13. 1, Low magnification of Kanby barley pericarp epidermis on day of flowering. ×3,100. **2,** High magnification of day-of-flowering Kanby pericarp epidermal cell wall showing absence of cementing layer. ×39,100. **3,** One-day-after-flowering (DAF) Kanby pericarp epidermis depicting cellular growth from previous day. ×3,100. **4,** High magnification of cell wall of one-DAF Kanby pericarp epidermis showing lack of cementing layer on wall surface. ×39,100. **5,** Low magnification of greatly enlarged epidermal cell from two-DAF Kanby caryopsis. ×3,100. **6,** Enlargement of two-DAF Kanby cell wall of pericarp epidermis showing the distinct cementing layer (arrow). ×39,100. **7,** Trichome (T) emanating from the epidermis of two-DAF Kanby caryopsis. **8,** High magnification of junction between Kanby epidermal cell wall (E) and trichome cell wall (T) showing that cementing layer (arrow) extends only to the trichome cell wall. ×13,800. **9,** Cell wall of the pericarp epidermis of mature naked Hiproly barley showing only a thin cuticle (arrow). ×38,100. **10,** Low magnification of six-DAF inner epidermis of Kanby lemma. ×3,100. **11,** High magnification of six-DAF Kanby epidermal cell wall of lemma showing lack of cementing layer on the wall surface (arrow). ×58,800. **12,** Low magnification of six-DAF inner epidermis of Kanby palea. ×3,100. **13,** High magnification of six-DAF Kanby palea epidermal cell wall showing lack of cementing layer on its surface (arrow). ×59,200.



Figs. 14–21. 14, Pericarp (P) of six-DAF Kanby caryopsis showing thickened epidermal cell wall. ×4,280. **15,** High magnification of cementing layer (arrow) of six-DAF Kanby caryopsis. ×29,400. **16,** Six-DAF cementing layer (arrow) depicting variation in thickness. ×26,600. **17,** High magnification of six-DAF cementing layer revealing internal structure. ×90,800. **18,** Lemma (L) and pericarp (P) cemented together in 10-DAF Kanby caryopsis. Note contact of cementing layer (arrow). ×6,200. **19,** Ten-DAF Kanby caryopsis does not have entire cementing layer (arrow) in contact with hull (H). P = pericarp. ×37,700. **20,** High magnification of cementing layer (arrow), hull (H), and pericarp (P) showing separation of layer and hull. ×82,000. **21,** Cementing layer in 10-DAF Kanby caryopsis showing internal structure (arrow). H = hull, P = pericarp. ×29,600. (Inset) High magnification showing internal lamination of cementing layer. ×45,400.



Figs. 22–29. 22, Low magnification of 21-DAF Kanby caryopsis showing relationship among hull (H), tubelike cell (T), and pericarp (P). Note cementing layer (arrows). ×1,560. **23,** High magnification of tubelike cell (T) in 21-DAF caryopsis showing cementing layer (arrow) on the pericarp side (P) but not on the hull side (H). ×6,900. **24,** Cementing layer (arrow) in 21-DAF Kanby caryopsis always separates from the hull (H) and remains intact on the pericarp (P). ×29,600. **25,** Invagination of pericarp wall (P) filled with cement (arrow) in 21-DAF caryopsis. H = hull. ×19,100. **26,** Very thin cementing layer (arrow) in 21-DAF caryopsis. ×45,400. **27,** Very thick cementing layer (arrow) in 21-DAF Kanby caryopsis. ×45,400. **28,** Cementing layer (arrow) at physiological maturity showing tight bonding between pericarp (P) and hull (H). ×9,900. **29,** High magnification of hull (H), cementing layer (arrow), and pericarp cell wall (P) at maturity. ×29,600.



Figs. 30–37. 30, Control section of protease digestion experiments stained in lead citrate after incubation in buffer at 37° C. ×22,000. **31,** Cementing layer (arrow) intact after digestion with protease VI. ×22,700. **32,** Cementing layer (arrow) intact after digestion with pepsin. ×24,100. **33,** Section treated with hydrogen peroxide and left unstained shows no effect on cementing layer (arrow); cement is darker than cell walls because section is unstained. ×22,800. **34,** Starch in endosperm used as internal control shows no reaction product on starch (S) in control section. ×9,800. **35,** Treated section shows electron-dense carbohydrate reaction product only on starch (S). ×14,700. **36,** Unstained cementing layer (arrow) in control section. ×47,300. **37,** Section treated with periodic acid-thiocarbohydrazide-silver protein shows heavily stained cell walls but unstained cementing layer (arrow). ×46,200.

where tubelike cells are located (Figs. 22 and 23) and at other gaps that appear occasionally (Fig. 24). The tubelike cells may represent remnants of the trichomes that have been trapped between the pericarp and hull. Invaginations of the pericarp cell wall are sometimes filled with cement (Fig. 25) but are rare and probably contribute little to the bonding of hull and pericarp.

The thickness of the cementing layer still varies from 130 to over 600 nm (Figs. 26–29), with the pericarp tendrils extending up to 150 nm from the cell wall (Fig. 28). The cementing layer is amorphous in some regions (Fig. 28) and shows fibrils that are not consistently organized parallel to the cell walls (Fig. 29). A survey of sections taken all over the caryopsis showed little pattern of variation in thickness or structure. The inner margin of the glume cell wall shows an electron-dense layer 40–50 nm thick (Figs. 24–29).

Cytochemistry

Thin sections of unosmicated tissue from 10-DAF and 21-DAF caryopses were enzymatically digested with proteases or specifically stained for carbohydrates. Results of the protease digestion experiments are shown in Figures 30–33. Figure 30 shows an untreated, stained control section for comparison with protease-digested tissue. The cementing layer was unaffected by either protease VI (Fig. 31) or pepsin (Fig. 32). Sections pretreated with hydrogen peroxide to ensure that the enzymes were able to contact embedded proteins also yielded negative results (Fig. 33, unstained). Storage protein from the endosperm on the same sections were digested by both proteases and unaffected by the control treatments.

Results of the carbohydrate localization experiments are shown in Figures 34–37. Endosperm starch was used as an internal control. Unstained starch was electron-lucent (Fig. 34), whereas starch stained with the PI-TCH-silver protein method was granular and electron-dense (Fig. 35). The cementing layer in unstained control sections was more electron-dense than either the pericarp or glume cell wall, owing to the lack of staining by the cell wall (Fig. 36). In PI-TCH-silver protein stained sections, however, the cementing layer failed to react with the reagents, whereas the carbohydrates of both cell walls stained strongly (Fig. 37). The cementing layer also failed to react with the conventional periodic acid-Schiff stain for carbohydrates and Coomassie Brilliant Blue for proteins at the light microscopy level (results not shown), nor does it stain with the toluidine blue-basic fuchsin routinely used to stain thick sections.

DISCUSSION

The thinness of the cementing layer (usually less than 500 nm) and its failure to stain with a variety of reagents suggests why it has not been noticed in other microscopic studies on the barley kernel. The cementing layer is a unique, very strong adhesive, and its chemical composition remains unknown. It seems to be neither protein nor carbohydrate (PI-TCH staining such as pectins, hemicellulose, cellulose), although the specificity of the reagents used does not rule out some related complex component. The presence of a cuticle on naked barleys suggests that the composition of the cement may be cuticular in origin. This possibility is currently under investigation. The cement is produced by the undifferentiated pericarp epidermis and is not secreted by epidermal-derived trichomes. The mechanism(s) for secretion has not been elucidated, but the material must be synthesized in the pericarp epidermis and pass through the cell wall to the site of deposition. The cementing layer is present much earlier than Harlan (1920) noted, being formed by the pericarp two days after flowering, long before the pericarp contacts the glumes. In fact, the trichomes that are present at this stage would prevent such contact. The cementing layer is initially amorphous, but after it contacts the hull, many regions have a fibrillar or laminar appearance, the significance of which is yet to be determined. The irregular surface of the pericarp adds mechanical strength to its bonding with the cementing layer, but the major force of attachment is probably chemical adhesiveness because the interface between the cementing layer and the palea or lemma is smooth.

LITERATURE CITED

BECHTEL, D. B., and GAINES, R. L. 1982. Presence of protease-digestible material in Golgi vesicles during endosperm development of selected cereals. Am. J. Bot. 69:880.

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

BECHTEL, D. B., and POMERANZ, Y. 1981. Ultrastructure and cytochemistry of mature oat (*Avena sativa* L.) endosperm. Cereal Chem. 58:61.

CRAIG, A. S. 1974. Sodium borohydride as an aldehyde blocking reagent for electron microscope histochemistry. Histochemistry 42:141.

HARLAN, H. V. 1920. Daily development of kernels of Hannchen barley from flowering to maturity at Aberdeen, Idaho. J. Agric. Res. 19:393.

LILLIE, R. D., and PIZZOLATO, P. 1972. Histochemical use of borohydrides as aldehyde blocking reagents. Stain Technol. 47:13.

[Received April 6, 1984. Accepted August 8, 1984.]