Some Novel Observations by Scanning Electron Microscopy on the Seed Coat and Nucellus of the Mature Wheat Grain

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ABSTRACT

The seed coat and nucellar epidermis of wheat caryopses were investigated by scanning electron microscopy. The generalized concept of these tissues previously available from light microscopical studies has proved inadequate to describe the considerable variation present in cell morphology and orientation. An amorphous layer, previously unreported in mature grains, was shown to exist between the nucellar epidermis and aleurone layer. The layer is absent over the embryo and thinner on the dorsal side of the grain than on the flanks of the crease. The relationship between its variation in thickness and the rate of water penetration into the grain is noted.

Cereal Chem. 65(2):81-85

The endosperm and embryo of the mature wheat caryopsis are enveloped by a series of tissues originating from the carpel wall; together they constitute the pericarp or fruit coat. Within such tissues lie two further envelopes: the testa, or seed coat, which is closely associated with the innermost pericarp tissues, and the remains of the nucellus, lying next to the aleurone layer of the endosperm. The nucellar epidermis, which is all that is thought to remain of the nucellus around most of the endosperm, is sometimes referred to as the hyaline layer. These tissues completely surround the endosperm except in the chalazal region at the inner extremity of the crease. The margins of both, however, insert into specialized tissues at this point, the testa into the pigment strand and the nucellar epidermis into the nucellar projection. Many studies have been performed with the assistance of the light microscope to reveal the structural details of the outer layers, and MacMasters et al reviewed these in 1971. In light microscope studies, information on cell morphology was limited owing to the need for complete separation of tissues for successful examination. As this is difficult in some cases, only small fragments could be characterized, and the extent to which these were representative of the whole could not be established.

Because the requirements of preparation for scanning electron microscopy (SEM) are different, needing, not complete separation of the layer in question, but merely the exposure of one of its faces, SEM can be performed on grains or parts of grains from which those tissues inside or outside the required layer have been removed, allowing examination in situ. This less exacting technique is admirably suited to surveying the conditions at different sites on the surface.

For pericarp tissues, SEM examination added little to knowledge acquired by light microscopy and already published. For seed coat and nucellus however, previously unrecognized characteristics were exposed, and it is these findings, established in many exploratory and confirmatory dissections, which are reported here.

MATERIALS AND METHODS

Grains of the U.K. cultivar Avalon were used. For examination of the outer surfaces of the tissues surrounding the endosperm, grains were dissected in a dry condition. Successive tissues were exposed by removal of overlying layers using forceps and a dissecting needle. For examination of the inner surfaces, fragments of tissues were folded back or, alternatively, grains were soaked in water overnight to soften endosperm, which could then be removed from the split grain by gentle scraping with the dissecting needle under a drop of water. Several washings were required to remove starch granules and glutens. Aleurone tissue could be gently lifted by inserting the blade of the needle parallel to its outside face and gently prying rather than cutting. A similar technique was used to remove other tissues in an outward progression. For some purposes scraping was more successful than attempts at prying. Following rinsing, wet tissues were allowed to dry on 10-mm cover glasses to which they adhered. All samples were coated with gold by sputtering before examination in a Cambridge Instruments S600 scanning electron microscope at 7.5 kV. Photographs were taken on Polaroid type 55 film.

RESULTS AND DISCUSSION

Both testa and nucellar epidermis are cuticularized on their outer surface (Morrison 1975). During grain maturation the cuticle of the testa becomes closely associated with the tube cells and cross cells outside it. The tube cells lie immediately outside the cuticle of the testa, but as they do not form a complete layer, the cross cells, forming the next complete layer, contact the cuticle through the spaces among tube cells. Morrison (1976) reported a substance that cemented tube cells to the testa cuticle, and an adcrustation of this also appears to lie between cuticle and cross cells (Fig. 1). The cuticle of the testa is thicker than the cellular remains of the integument itself which, although referred to as the color layer (singular), is in fact two layers deep (Fig. 2). When seen together either by light or electron microscope, the cellular structure of each is confused with that of the other, as the long axes of cells in the respective layers run in different directions. They have been described as crossing at less than right angles, with neither being parallel to the long axis of the grain (Bradbury et al 1956), and this was considered to be applicable over the whole of the grain surface. However, our observations have shown this concept to be inadequate since the relationship of each layer to the other and to the grain's axis varies. Even within a small area of one layer, various cell orientations may be encountered, as in Figure 3, which shows a bold imprint in the cuticle of cells in the outer cellular layer of the seed coat.

The seed coat inserts into the pigment strand in the shallow trough that runs along the inner extremity of the crease. In this region the long axis of the cells of the inner layer, in the many grains examined, ran at near right angles to the grain's axis (Fig. 4). The cells of the inner layer had a wrinkled profile where they turned down over the ridges on either side of the pigment strand (Fig. 5). The long axis of cells in the outer layer, an impression of which can be detected through the inner layer, ran almost parallel to the axis of the grain. Our findings confirmed that most testa cells are flattened, having been subjected to compression from the expanding endosperm during grain maturation. When exposed by dissection, their contents were seen to be globular or granular (Fig. 6), reminiscent of the adcrustations of the pigment strand cells described by Zee (1975). Cells in the crease region suffer less pressure than most, and those at the brush end in particular retain a rounded profile (Fig. 7) that makes them difficult to identify as seed coat cells. Their relationships with other tissues and continuity with more typical testa cells leave no doubt as to their identity.

Pressure during development is also relatively low over the embryo, and indeed a cavity exists between the embryo and the

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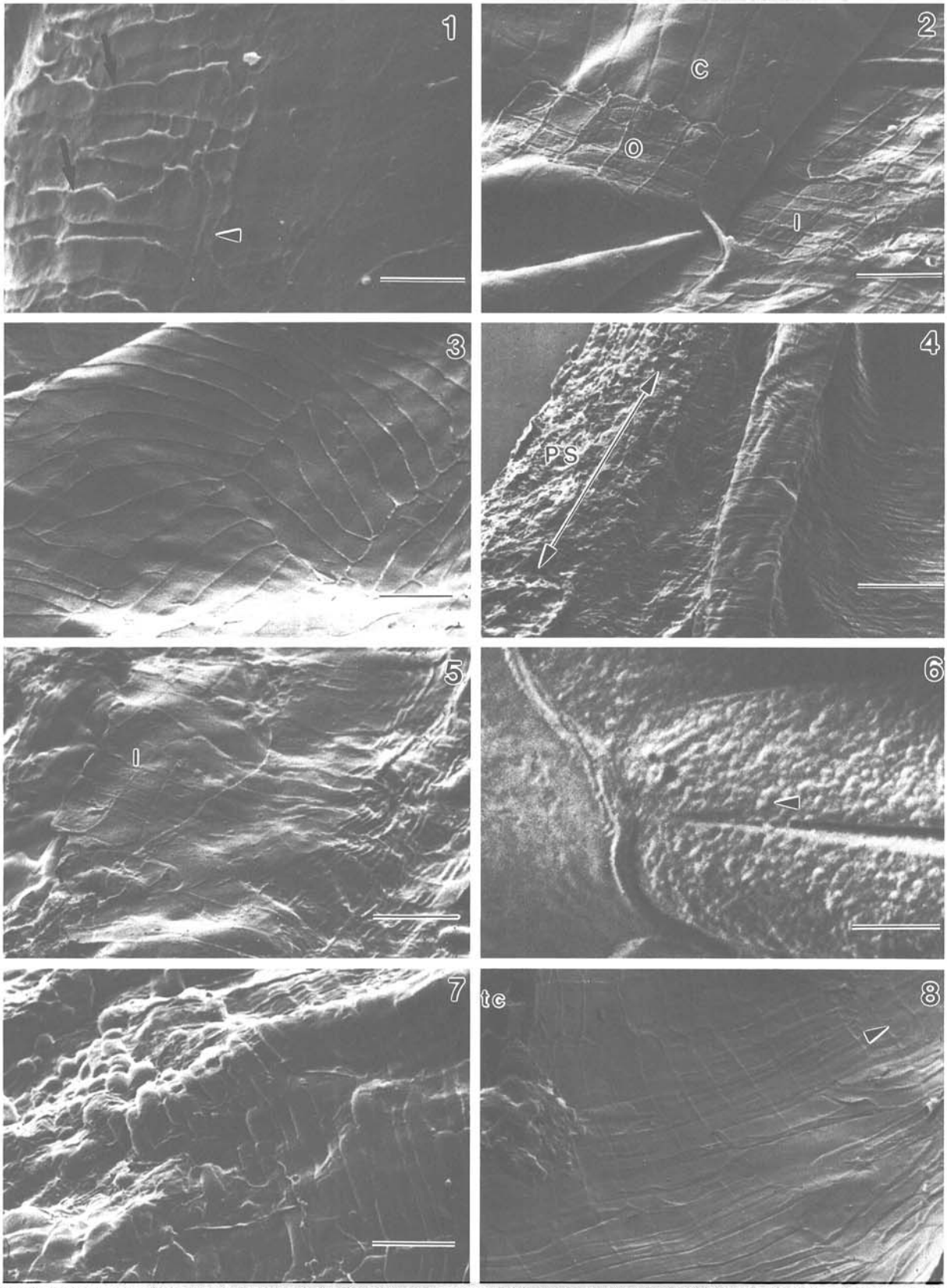

Fig. 1. Outer surface of seed coat cuticle, exposed by removal of pericarp tissues. Impressions left by tube cells (small arrow) and cross cells (large arrows) may be deposits of cementing material. Bar represents 20 μm. **Fig. 2.** The three component layers of the testa. The outermost, the cuticle (**C**), has been lifted to reveal impressions of the cellular layers on its inner face. Part of the outer cellular layer (**O**) has remained with the cuticle, revealing an impression of its own cell walls, and the walls of the inner layer (**I**). The two cell layers lie at near right angles one to the other. Bar represents 40 μm. **Fig. 3.** The pattern of cells at one point on the surface of the outer cellular layer of the testa. The pattern is seen on the inner face of the cuticle. The orientation of cells is variable. Bar represents 40 μm. **Fig. 4.** The inner surface of the seed coat where its margin inserts into the pigment strand (**PS**). The long axis of the cells of the inner cell layer lies at right angles to the axis of the pigment strand (arrow). Bar represents 100 μm. **Fig. 5.** Wrinkled walls of cells of the inner testa layer (viewed from the endosperm side) turning down over the ridge lying alongside the pigment strand. Cells of the inner cellular layer (**I**) are exposed on the left of the micrograph. Bar represents 40 μm. **Fig. 6.** Globular deposits (arrow) exposed by the removal of outer walls of seed coat cells. Bar represents 4 μm. **Fig. 7.** The inner face of the testa near the margin that inserts into the pigment strand at the brush end of the grain. The cells have a rounded profile, which contrasts with the flattened appearance of testa cells found elsewhere. Bar represents 40 μm. **Fig. 8.** The inside surface of the loose tissue overlying the embryo. The cell walls of the inner testa layer run at right angles to those of the outer layer, which converge towards the micropyle lying in the direction indicated by an arrow. Note the exposed portions of tube cells (**tc**) each with a square section. Bar represents 40 μm.

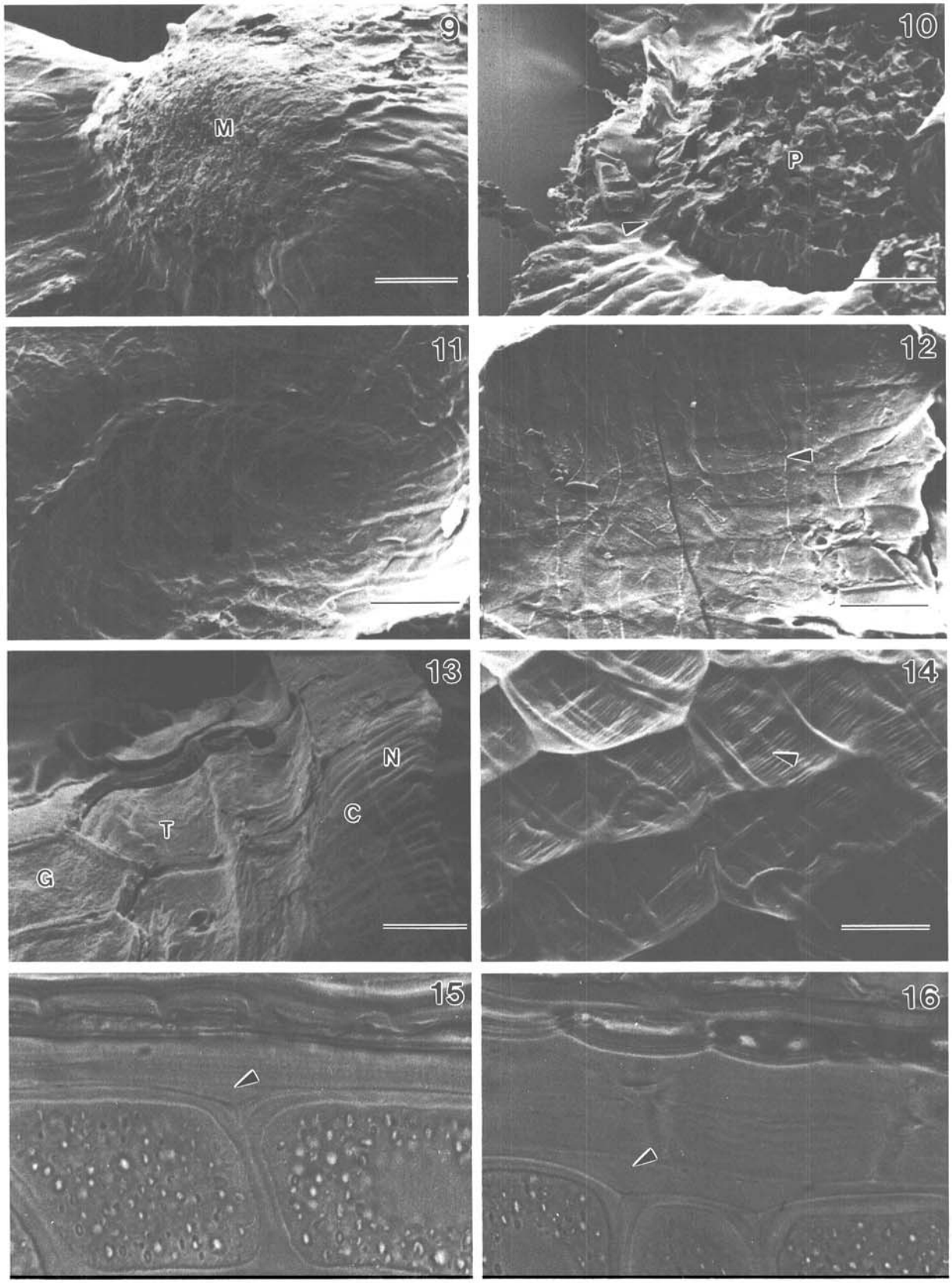

Fig. 9. The outer face of the micropylar region. The spongy, possibly porous disk (**M**) has been exposed by careful removal of closely adherent tube cells. It is surrounded by tissues that have been splayed out to demonstrate the convergence of cells of the outer testa layer. Bar represents 40 μm. **Fig. 10.** Fracture face of the tip of the coleorhizal papilla (**P**) showing its intimate association with the micropylar area. Some cellular continuity (arrow) is apparent between the papilla and the surrounding tissue. Bar represents 40 μm. **Fig. 11.** Inner face of the micropylar region. Cellular structures of individual tissues are indistinguishable, but no modified aleurone cells are present in the region in or around the area (*) where contact between papilla and micropyle was established. Bar represents 40 μm. **Fig. 12.** Vestiges of nucellar epidermal cells (arrow) on the inner surface of the loose tissue overlying the embryo. Through the thin nucellar layer, the shape of cells of the inner testa layer is visible. The long axes of the two layers lie at right angles. Bar represents 40 μm. **Fig. 13.** A sequence of seed coat and nucellar tissues exposed by progressive removal of overlying tissues from the outside of the grain: globular contents of testa (**G**), inner wall of inner cellular layer of testa (**T**), cuticle of nucellar epidermis (**C**), and outer face of nucellar epidermis cell (**N**). Bar represents 10 μm. **Fig. 14.** The inner face of the nucellar epidermis on the dorsal side of the grain. The cellular outline and convoluted surface (arrow) can be seen beneath an imprint of aleurone cells. The imprint occurs in a previously unrecognized amorphous layer. Bar represents 20 μm. **Fig. 15.** Light micrograph of nucellar lysate (arrow) on the dorsal side of a longitudinal section of a wheat grain 35 days after anthesis (× 1,000). **Fig. 16.** As 15, but on the flank of the crease where the layer of lysate (arrow) is considerably thicker (× 1,000).

tissues overlying it. The direction of the seed coat cell axes followed a definite and consistent pattern here. The lateral walls of the outer layer converged towards the micropylar region (Fig. 8), whereas those of the inner layer lay approximately at right angles to them, giving rise to a concentric disposition (Fig. 9). We were unable to detect whether the inner layer extended to the micropylar tip. In fact, the structure at the micropyle itself was not clear. Cleaning the inside of the micropyle was difficult because of its close association with the coleorhizal papilla (Symons et al 1984). The intimacy of this union is clear from a light micrograph used as Figure 14 by MacMasters et al (1971), and apparent cellular continuity of coleorhizal papilla and its covering layer is shown here in Figure 9. Attempts to reveal the outer surface of the micropyle were also thwarted because its extreme delicacy made clearing of the overlying and closely adherent tube cells difficult. Our cleanest preparation uncovered a sponge-like, possibly porous structure (Fig. 10). No thick cuticle was present over or around the porous disk.

The difficulties in separating the coleorhizal papilla from the inner surface obviously impair exposure not only of the testa, but also the nucellar epidermis in the micropylar region. Doubt has been expressed over the presence or absence of this tissue over the embryo (Bradbury et al 1956). We have found in some grains, vestiges of nucellar epidermal cells (Fig. 11) but, as with the inner testa layer, it is not clear how closely they approached the micropylar region. Modified aleurone cells, appearing as loosely attached scales, were encountered on the inner face of the loose tissue overlying the embryo, but the interpretation by Norstog (1972) for barley, that the entire embryo becomes surrounded by modified aleurone cells, is not endorsed by our observations because none were encountered on either face of the micropyle/coleorhizal papilla junction in cases where separation was possible.

The vestigial nature of those nucellar epidermal cells that overlie the embryo was not typical of cells found elsewhere in the layer. Although variable in thickness, the layer is in most locations thicker in section than the complete complement of testa components. The typical appearance of cells on the dorsal side is shown in Figure 13, in which a sequence of tissue components can be seen as they are progressively revealed from left to right of the micrograph. The innermost layer is the cellular component of the nucellar layer. It is partly overlain by the nucellar cuticle, which in turn is overlain by a single cellular layer of the testa. Removal of its outer wall revealed granular contents as already described. The convoluted nature of the nucellar epidermal cell wall has not been reported previously, but it was regularly observed in the present study. Neither is it confined to the outer wall, as can be seen in Figure 14, which shows the inner face of tissue that lay adjacent to the aleurone layer before being peeled back for display. In addition to the characteristic shape and wrinkled surface of nucellar epidermal cells, additional contours, corresponding to those of the aleurone layer are also in evidence. The pattern is not embossed on the cellular layer itself but on a previously unrecognized amorphous layer lying between the epidermis and the aleurone layer. Although no reference has previously been made to this amorphous layer, it is nevertheless recognizable under the light microscope in grain sections, from which it is clear that its thickness is greater on the flanks of the crease (Fig. 15) than on the dorsal side of the grain (Fig. 16). The amorphous layer probably represents the remnants of the inner cells of the nucellus, the disintegration of which has been described by several authors including Norstog (1974), who drew attention to a "nucellar lysate" in developing barley caryopses but was not aware of its persistence in the mature grain.

One consequence of the greater thickness of the lysate on the flanks has been to increase the difficulty in examining the cellular structure of the nucellar epidermis by SEM in that region. The problem is well illustrated in Figure 17, in which the relationship among testa, epidermis, and lysate is shown. The micrograph shows the lysate with its deep impressions of aleurone cells, partially removed from the inner surface of the nucellar epidermis on the cells of which transverse convolutions can be detected. Where it has been possible to remove lysate on the flanks of the crease it has been found that the orientation of the nucellar epidermal cells has been different from their orientation on the grain's dorsal side. In the crease region, the long axis of the cells is at right angles to that of the grain, whereas on the dorsal side the two axes are parallel. We have not been able to detect the manner in which the direction changes because of difficulties in removing lysate in the critical areas. No lysate has been found at the junction

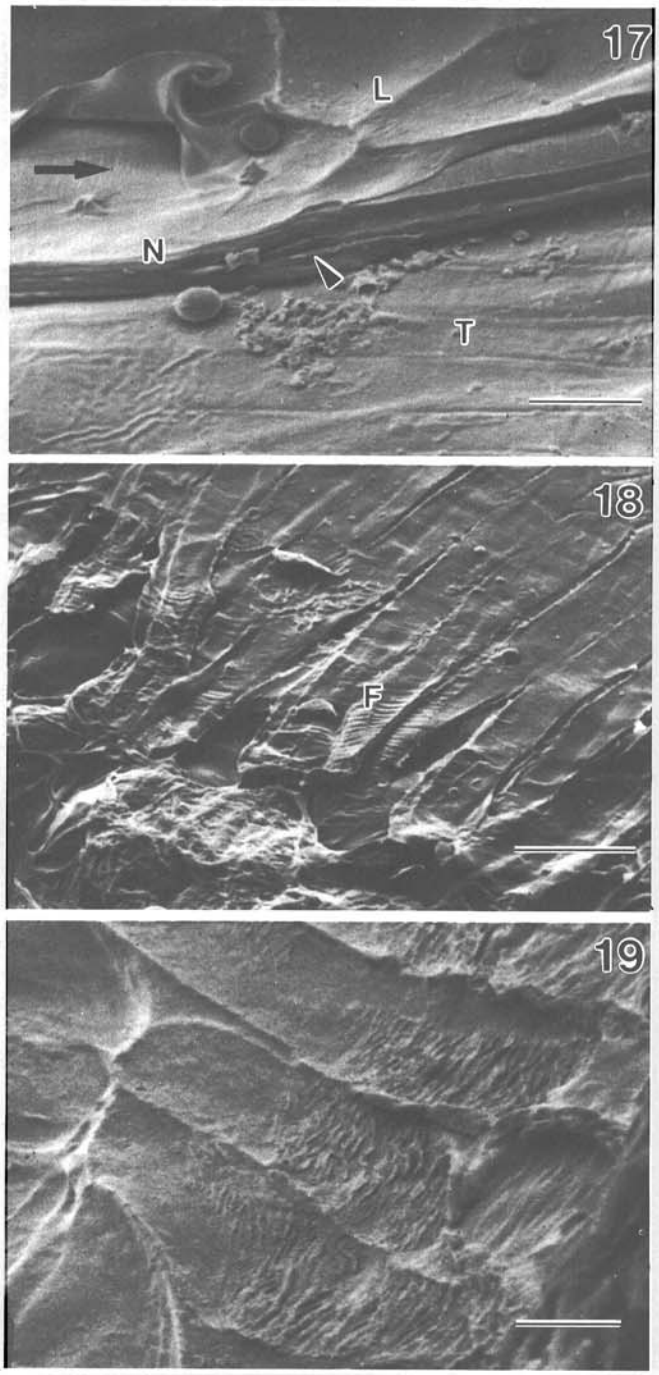

Fig. 17. Nucellar lysate (**L**) bearing imprints of aleurone cells, overlying nucellar epidermis (**N**) on the surface of which convolutions can be seen (large arrow). Removal of part of the nucellar tissues has revealed a cut edge of the epidermis, expanded where a cross-wall occurs (small arrow) and the inner cell layer of the testa (**T**). Bar represents 20 μm. **Fig. 18.** Nucellar epidermal cells in the crease region. The fringe of cells (**F**), separating laterally at the tips, inserts into the nucellar projection (removed by dissection). Bar represents 40 μm. **Fig. 19.** Wall proliferations on the inner wall of nucellar epidermal cells in the crease region. The internal structure of the cells was exposed by dissection. Bar represents 10 μm.

of the epidermis with the projection, probably because no lysis has occurred here. Epidermal cell surfaces have thus been seen clearly in examinations facilitated by removal of endosperm. Examples of such cells are shown in Figure 18. Lateral separation of the individual cells which insert at near right-angles to the projection produces a fringe-like margin. The ribbed appearance of the cells' surfaces is consistent with their appearance elsewhere in the grain; it appears to become emphasized towards the tips of the cells that insert into the nucellar projection, and this may be related to their internal structure. This has been seen only in a few cases where inner cell walls have fortuitously been removed. In Figure 19 internal proliferation on the inner surfaces of outer walls can be seen. Such a structure is compatible with the findings of Smart and O'Brien (1983) and the notion that cells in the nucellar projection perform a transfer function (Cochrane 1983).

This study has been concerned with the revelation of new histological information. The possible implications of these findings in relation to caryopsis maturation and technologically important characteristics have not yet been investigated. We note, however, that Stenvert and Kingswood (1976) found that water penetration occurred most readily over the embryo (where we found no lysate) and least readily on the flanks of the crease—the area in which lysate was thickest. Although Hinton's (1955) experiments on carefully dissected grains indicate the testa as the critical determinant of water entry, and indeed the presence of a thick cuticle on this tissue adds plausibility to this conclusion, the nondestructive experiments of Stenvert and Kingswood (1976) indicate that a tissue lying inside the testa exerts control. In view of these conflicting results and the coincidence of anatomical and behavioral characteristics, the functions of the lysate layer are worthy of investigation.

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[Received January 12, 1987. Accepted November 3, 1987.]