Endosperm Structure of Barley Isogenic Lines[1]

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ABSTRACT

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Light microscopy (LM) and transmission electron microscopy (TEM) were used to compare two pairs of isogenic lines of barley: Hiproly (high protein-high lysine, CI 3947) vs high protein (CI 4362) and normal starch (JGM 57) vs waxy starch JGM 58). Hiproly and high protein (CI 4362) were compared with a widely grown cultivar, Firlbecks. The subaleurone regions of both Hiproly and high protein (CI 4362) were enlarged, but that of the high protein was irregular in thickness. Two major types of proteins were detectable by both LM and TEM. One type stained magenta with toluidine blue O and basic fuchsin and appeared homogeneous and electron-lucent in the TEM. The other type stained purple in the LM and was heterogeneous and electron-dense in the TEM. Hiproly, CI 4362, and Firlbecks differed in the amounts and shapes of the two protein types. In LM, the starch granules in the waxy starch line were distorted and irregular in shape. In TEM, the waxy starch granules appeared mottled and the normal starch granules were uniformly stained.

The best way to examine genetic differences in properties such as protein content, amino acid composition, and type of starch among cereals is to study closely related lines differing in only one property. These isogenic lines are assumed to differ by a single gene or a small block of genes. Mutants that have survived many generations of natural selection since the mutation are the most useful for studies of genetic differences (Wiebe 1968). Several pairs of isogenic lines have been used in studies of differences in barley (Pomeranz and Robbins 1976), but little information is available on differences in structure, specifically in ultrastructure, of isogenic pairs of barley. Such differences might help to explain the basis for the high protein content and/or the improved amino acid balance of some selections and the basis for the varying performances of barleys that differ in the proportion of amylopectin in their starch.

Several microscopic studies have compared high lysine isogenic lines of barley (Munck et al 1970; Tallberg 1973; Munck and von Wettstein 1976; Olsen 1974; Ingversen 1975, 1976; Miflin and Shewry 1979). Light microscopy (LM) and scanning electron microscopy (SEM) have been used in studies of mature barley caryopses (Munck et al 1970, Tallberg 1973, Olsen 1974, Munck 1972), whereas to our knowledge, transmission electron microscopy (TEM) has been used only in studies of developing caryopses (Munck and von Wettstein 1976, Miflin and Shewry 1979). Because changes (including loss of moisture, death of starchy endosperm cells, noncovalent binding in storage proteins, and most importantly, changes that affect end-use properties) continue to occur in grain after it reaches physiologic maturity, knowledge of the structure of the mature, dry caryopsis is desirable.

One important breakthrough in plant breeding was commercial development of cultivars with starches containing very high concentrations of amylose or amylopectin. Waxy starches, which are high in amylopectin, are used extensively in the production of foods in which high paste viscosity and low gel retrogradation are desired. The high amylopectin content permits more extensive starch granule swelling than a normal amylose content does, without loss of the starch granule integrity (Medcalf 1973). Little information is available on the microscopic differences between waxy and normal starches.

Until recently, difficulty in obtaining satisfactory thin sections for TEM has prevented examination of mature cereal endosperms. We modified embedding and sectioning techniques (Bechtel and Pomeranz 1978) and can now obtain thin sections of mature cereal caryopses. For this study we chose two pairs of isogenic barleys—high protein (CI 4362) vs high protein-lysine (Hiproly, CI 3947) and normal starch (JGM 57) vs waxy starch (JGM 58)—and compared them with a widely grown cultivar, Firlbecks.

[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 aproval to the exclusion of other products that may also be suitable.

MATERIALS AND METHODS

Plant Materials

All barleys were two-rowed, white aleurone types, of spring habitat. The barleys with normal starch (JGM 57) and waxy starch (JGM 58) are isogenic lines grown at Aberdeen, ID, during the 1971 growing season. Hiproly (CI 3947), CI 4362, and Firlbecks were grown during the 1973 season at Aberdeen, ID. The barleys are described in Table I.

Analytical Methods

Protein and moisture contents were determined by AACC approved methods 46-11 and 44-15A, respectively (1962).

Microscopy

Barley grains were cut into small pieces, which were fixed in a combination of (1.5–4.5% v/v) glutaraldehyde and (1.5–3.0% v/v) paraformaldehyde in 0.05*M* phosphate buffer at pH 6.7 for 4 hr at 21°C and 20 hr at 4°C. The tissue was washed in buffer until the smell of the fixative was gone and was postfixed at 21°C for 1–2 hr in 0.05*M* phosphate-buffered 1.0% osmium tetroxide. The pieces were dehydrated in a graded acetone series and infiltrated and embedded in Spurr's resin (Spurr 1969). Sections 1 μm thick were cut with a glass knife on a Porter-Blum MT-2b ultramicrotome; then, silver-gold thin sections were cut with a diamond knife. Thick sections were stained with Paragon (Paragon C. & C. Co., Inc., Bronx, NY). Thin sections were stained for 3 min in lead citrate (Reynolds 1963). A short staining time was essential because the lead citrate precipitated on the aleurone lipid bodies and also leached out the lipid-body centers. Thin sections were viewed at 60 kV in a Philips EM 201 electron microscope. Proteins were histochemically stained with Coomassie Brilliant Blue (CBB) as previously described (Bechtel and Pomeranz 1978). The aleurone cells of all five barleys were structurally indistinguishable.

TABLE I
Some Characteristics of Barleys

Cultivar or Selection	Type	Kernel Weight (mg)[a]	Moisture (%)	Protein (N × 6.25) (%)[b]
Firlbecks	covered	44.1	12.0	10.4
CI 4362	naked	53.5	10.7	16.1
Hiproly	naked	38.9	10.4	17.8
Normal starch	covered	36.8	12.1	11.1
Waxy starch	covered	36.8	11.7	12.0

[a] As-is moisture basis.
[b] Dry matter basis.

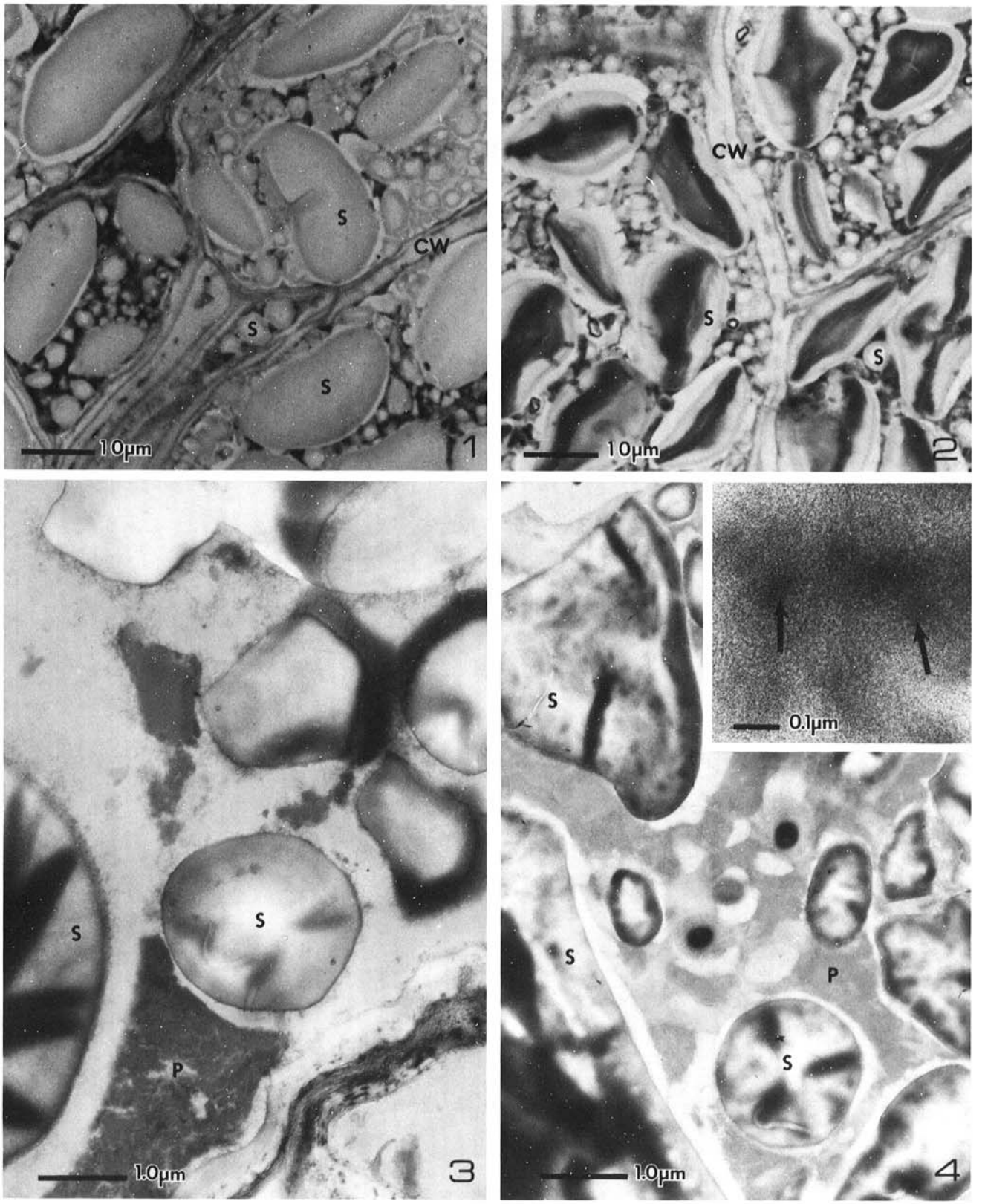

Fig. 1. Light microscopy (LM) of normal starch (isogenic line) central region showing starch **(S)** and cell wall **(CW)**. (×1,350) **Fig. 2.** LM of waxy starch (isogenic line) central region showing **S** and **CW**. (×1,350) **Fig. 3.** Transmission electron microscopy (TEM) of normal starch (isogenic line) granules **(S)** embedded in protein **(P)** in central endosperm. (×15,500) **Fig. 4.** TEM of waxy starch (isogenic line) granules **(S)** in central endosperm embedded in **P.** Note mottled appearance. (×15,500) **Inset.** High magnification of mottled portion (arrows) of starch granule. (×77,900)

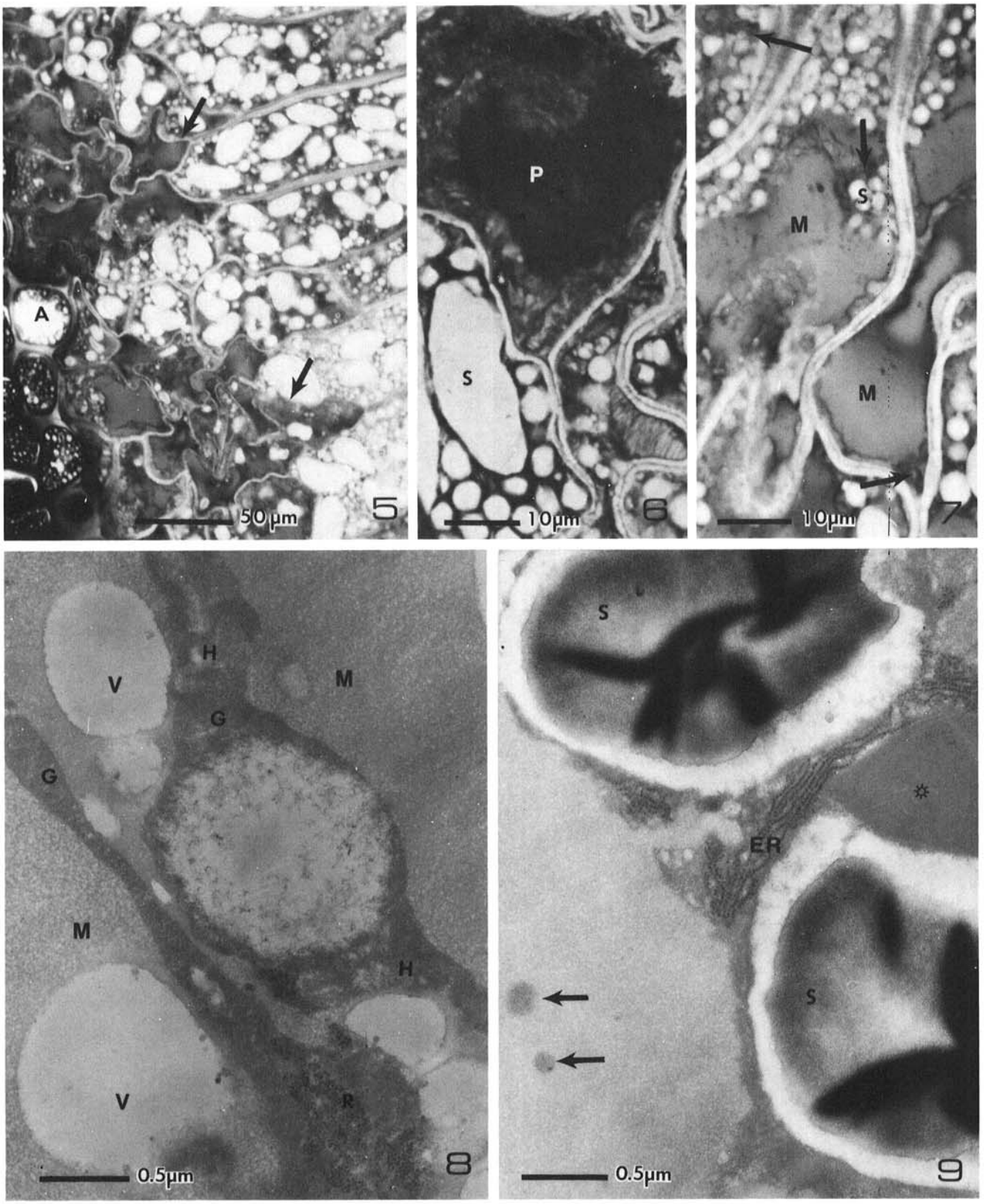

Fig. 5. Light microscopy (LM) of CI 4362 aleurone **(A)** and irregular subaleurone (arrows). (×340) **Fig. 6.** LM of CI 4362 stained with Coomassie Brilliant Blue. Protein **(P)** stained blue whereas starch **(S)** did not stain. (×1,350) **Fig. 7.** High magnification LM of CI 4362 subaleurone showing large central magenta-stained protein **(M)**, **(S)**, and purple-stained protein (arrows). (×1,350) **Fig. 8.** Transmission electron microscopy (TEM) of CI 4362 subaleurone protein showing voids **(V)** in **M**. The heterogeneous purple protein **(H)** contains granular regions **(G)** and ribosomes **(R)**. (×31,000) **Fig. 9.** TEM of CI 4362 protein with small dense inclusions (arrows), endoplasmic reticulum **(ER)**, angular protein (☼), and starch **(S)**. (×30,000)

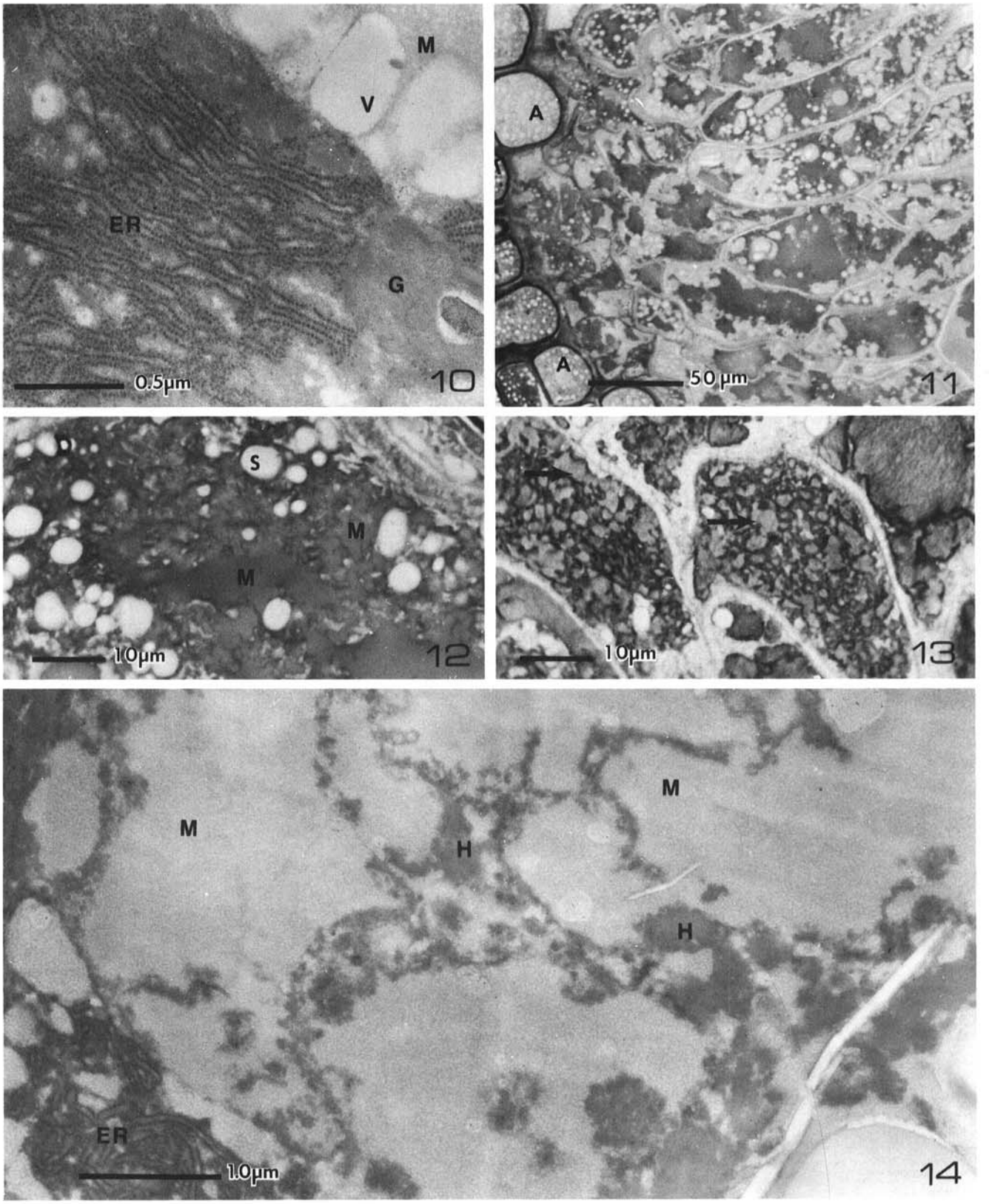

Fig. 10. Endoplasmic reticulum **(ER)** and granular region **(G)** in heterogeneous protein. Magenta protein **(M)** contains void **(V)**. (×38,700) **Fig. 11.** Light microscopy (LM) of Hiproly aleurone **(A)** and large subaleurone region. (×340) **Fig. 12.** LM of Hiproly subaleurone showing **M** and starch **(S)**. (×1,350) **Fig. 13.** LM of Coomassie Briliant Blue-stained Hiproly subaleurone region with magenta protein (arrows) surrounded by densely stained purple protein. (× 1,350) **Fig. 14.** Transmission electron microscopy of Hiproly subaleurone cell showing **M** and heterogeneous protein **(H)** containing **ER**. (×25,600)

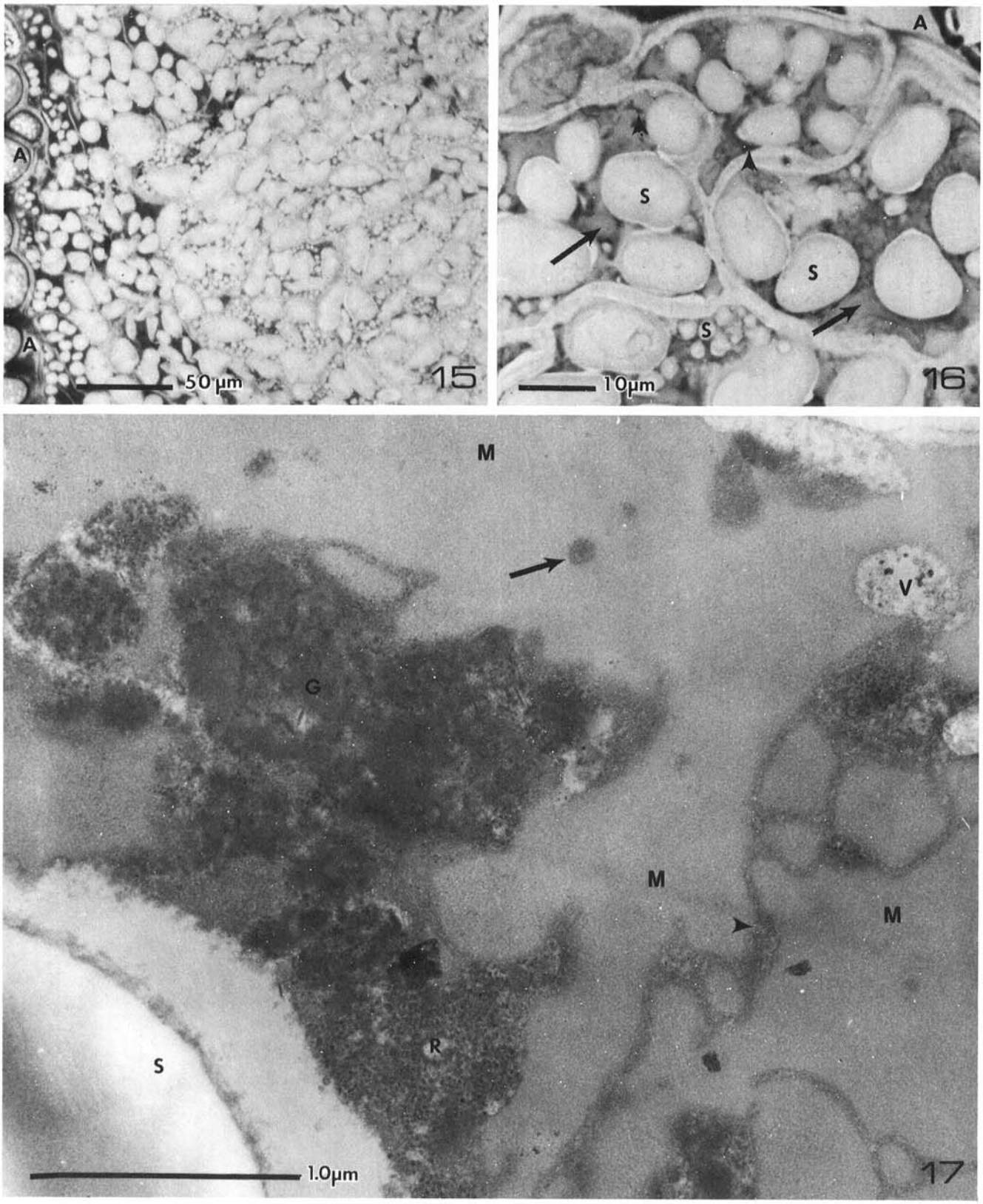

Fig. 15. Low magnification light microscopy (LM) of Firlbecks aleurone **(A)**, subaleurone, and central regions. (×340) **Fig. 16.** High magnification LM of Firlbecks subaleurone region. Magenta-staining protein (arrows) were more lightly stained than the purple-staining protein. Note large number of starch granules **(S)**. (×1,350) **Fig. 17.** Transmission electron microscopy of Firlbecks subaleurone region showing homogeneous magenta proteins **(M)** containing dense inclusions (arrow) and voids **(V)**. Heterogeneous protein contains ribosomes **(R)** and granular material **(G)**. Magenta protein regions are separated by thin bands of heterogeneous protein (arrowhead). (×48,000)

RESULTS

Normal vs Waxy Starch

The starchy endosperm of cereals is typically divided into the subaleurone region, located just beneath the aleurone layer, and the central endosperm, which comprises the rest of the starchy endosperm. Comparison of isogenic lines containing normal and waxy starch revealed little difference in the subaleurone region, either in the protein components or in the starch (not shown). Large differences in the appearance of the starch were observed in the central endosperm region. At the LM level, normal starch granules stained uniformly, whereas waxy starch granules appeared highly distorted and stained densely (Figs. 1 and 2). Analysis of the ultrastructure showed that the waxy starch granules were more irregular than the normal ones (Figs. 3 and 4). Also, both the large and small waxy starch granules had light and dark regions that gave the starch granules a mottled appearance in the TEM (Fig. 4). The normal starch granules stained uniformly (Figs. 3 and 4).

Endosperm of Isogenic Lines

Comparison of the endosperms from high protein CI 4362, high protein-lysine Hiproly, and cultivar Firlbecks showed differences only in the subaleurone region.

CI 4362. The subaleurone region of the high protein CI 4362 was composed of numerous larger cells clustered below a few small cells. This arrangement gave the subaleurone region a highly irregular appearance (Fig. 5). The subaleurone cells contained few starch granules. Histochemical analysis of 1-μm thick plastic sections of glutaraldehyde-fixed tissue treated with CBB showed that protein stained blue (Fig. 6). Plastic thick sections of TEM material stained with basic fuchsin and toluidine blue O showed heterochromatic staining of the protein; one stained magenta and the other purple. The magenta-stained protein predominated in the central part of the subaleurone cells and appeared as a single irregular mass (Fig. 7). The purple-stained proteinaceous material was limited to the cell periphery (Fig. 7). TEM of these cells revealed that the magenta-colored material was typically granular (Fig. 8). Two types of inclusions were in the magenta-stained protein: small electron-dense inclusions (Fig. 9) and circular electron-lucent voids (Fig. 8). The purple-stained protein was a heterogeneous material composed of ribosomes (Fig. 8), rough endoplasmic reticulum (ER, Figs. 9 and 10), electron-dense granular material (Figs. 8 and 10) and a limited number of angular protein bodies (Fig. 9).

Hiproly. The Hiproly (CI 3947) barley had a larger subaleurone region than the high protein CI 4362 barley (Figs. 5 and 11). The region was of uniform thickness, in contrast to the uneven appearance of the region in CI 4362. These subaleurone cells contained even fewer starch granules than did cells of CI 4362. The magenta-stained protein was composed of small irregular regions surrounded by the purple-stained material (Fig. 12). Staining with CBB clearly showed the numerous small irregular regions (Fig. 13). These two protein types (magenta and densely stained) were ultrastructurally similar to the corresponding types in CI 4362 (Figs. 8–10 and 14), except that the magenta-stained protein lacked the voids and the small dense inclusions.

Firlbecks. This barley (Figs. 15 and 16) and the isogenic lines with normal and waxy starch (not shown) had a limited subaleurone region one to several small cells thick that contained large numbers of starch granules (Figs. 15 and 16). Similarly, in Firlbecks and in the two starch isogenic lines, much of the subaleurone protein consisted of small regions of magenta-stained and purple-stained protein that was not well resolved with the light microscope (Fig. 16). The magenta-stained protein of those cells contained voids and dense inclusions (Fig. 17). The heterogeneous protein contained ribosomes, ER (not shown), and granular material (Fig. 17). The bands of heterogeneous protein separating the regions of magenta-stained protein were thinner than those in Hiproly and CI 4362 (Fig. 17).

DISCUSSION

Earlier LM (Munck et al 1970) and SEM (Munck 1972) studies failed to reveal significant differences between Hiproly and its isogenic sister line CI 4362. Minor differences between Hiproly and CI 4362 or a low lysine cultivar included an enlarged subaleurone region in Hiproly (Munck et al 1970, Olsen 1974); lack of small starch granules in the subaleurone region in Hiproly (Munck et al 1970, Olsen 1974, Munck 1972); presence of crumbled, large starch granules and greater adherence of protein to the starch granules in Hiproly (Munck et al 1970); and lack of difference in the protein bodies (Munck et al 1970, Olsen 1974). Tallberg's study (1973) showed no difference in either number or size of starch granules. We found an enlarged subaleurone region for both Hiproly and CI 4362. However, this region was smaller and more irregular in CI 4362 than in Hiproly. In addition, both CI 4362 and Hiproly contained fewer starch granules than did barleys of normal protein content.

Major structural differences observed between protein bodies of normal and high lysine (opaque-2) maize (Wolf et al 1967) have not been observed for barley. Indeed, only minor structural differences are expected for barley because, unlike maize, barleys differ very little in their protein fraction composition. Protein fractions in normal and high lysine maize vary greatly. For example, proteins in normal and opaque-2 maize contain, respectively, 55 and 25% prolamine and 39 and 55% glutelin (Pomeranz 1975). Such major protein fraction differences in barley have not been observed. Only changes of a few percent are observed in barley, and consequently only minor structural differences are expected. The differences in protein among Firlbecks, CI 4362, and Hiproly seemed to be in quantity rather than in quality. The qualitative similarity among the proteins of these barleys contrasts with the major differences between protein bodies of Bomi and Risø 1508 (Munck and von Wettstein 1976, Miflin and Shewry, 1979). Risø 1508 is not a natural mutant, however, but one that was induced by ethyleneimine treatment.

The irregular appearance of waxy starch granules by LM and TEM is difficult to assess. The appearance was reproducible in six separate fixations. The irregular shape of the waxy starch granules contrasts with the round shape reported for two other waxy starch lines, Sumiremochi and Mochimugi (Banks et al 1970). Because TEM clearly differentiated between the normal and waxy starches, it may be useful for detecing other waxy starches.

Differences in protein content and the protein and starch compositions of barley selections were accompanied by gross morphological differences in kernel size, structure, and degree of shrinkage and by structural differences that could be detected at the LM and TEM levels. As yet, we do not know whether these differences in structure and composition are related in a causal or a causative manner or, if causative, which is the primary and which the secondary factor. Regardless of that relation, findings of structural differences may prompt modifications in the processing of barley (in milling or in starch production) and hence may ultimately affect the yields of the processed products, their storability, and their end-use properties. Finally, differences in fine structure may be used as markers to differentiate among isogenic lines for genetic studies.

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