Submicroscopic Structure of Wheat Flour and Gluten Lipoprotein Components

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ABSTRACT

Protein fractions of gluten, obtained either by the Osborne separation method or by acetic acid solubilization followed by chromatography on Sephadex G-100, were observed by electron microscopy. Gliadin and fractions with a high gliadin content have a smooth, compact, and nearly electron-lucent structure; glutenin and fractions with a high glutenin content present a granular and fibrillar structure. The fibrils have a diameter of 100 to 200Å and form compact networks. Albumins and globulins have a structure which is more like glutenin than gliadin. In gluten there is a smooth, compact matrix similar to gliadin which, however, encloses zones of structure similar to glutenin, albumins, and globulins. Flour proteins also present two kinds of structure: fragments of relatively compact and nearly electron-lucent proteins, and granular zones and fibrillar felting which adhere to both starch granules and protein fragments.

There is no clear agreement concerning the submicroscopic structure of wheat flour and of the protein and lipoprotein components of gluten. In immature wheat

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endosperm cells, Buttrose (1) and Morton et al. (2) observed numerous protein bodies formed by storage proteins. However, Shkvarkina et al. (3) described a fibrillar structure of the wheat endosperm proteins. Hess (4), who first studied flour structure with a shadowing technique by electron microscopy, concluded that the flour proteins are composed of fragments of "wedge" proteins without characteristic structure, and fibrillar networks of "adhering" proteins around the starch granules.

Recently Seckinger and Wolf (5) found an undifferentiated, amorphous protein in flour sections between and around the starch granules after osmic fixation. In a previous work (6) we observed fragments of smooth, compact proteins as well as fibrillar proteins adhering to these fragments and to the surface of starch granules in flour. Numerous lipid inclusions are associated with the fibrillar proteins.

Few studies have been undertaken on the structure of gluten. Grosskreutz (7) suggested a structural model of gluten based on studies by X-ray diffraction and electron microscopy. In this model the proteins are considered to exist in the form of platelets about 70 Å thick.

Seckinger and Wolf (8) observed morphological variation^ between purified fractions of gliadin and glutenin spread on water as a surface dispersion. Grosskreutz (9) also examined samples of the gliadin and glutenin fractions of wheat by shadowing and negative staining of dilute solutions and found structural differences between these fractions. Kasarda et al. (10) examined samples of purified α-gliadin by the same methods.

In order to better understand the structure of flour and gluten proteins, we have observed relatively simple fractions of gluten proteins, obtained by Osborne's separation method (11) and by dextran gel chromatography. On the basis of these observations we have attempted to explain the structure of flour and gluten protein.

MATERIALS AND METHODS

The flours were obtained from soft French wheat varieties of various baking qualities. From these flours gliadin, glutenin, albumins, and globulins were prepared by Osborne's method, modified according to Feillet (11). In addition, after gluten was washed out, different fractions of gluten were obtained by solubilization in 0.01N acetic acid, followed by centrifugation and chromatography on Sephadex G-100 (12,13). Although molecular sieving is the essential factor in this separation, the application of three eluants is necessary because of adsorption and ion-exchange effects. It is particularly important to finish by ammoniacal elution for a quantitative recovery. The solubilized proteins are thus divided into

TABLE I. PROTEIN DISTRIBUTION BETWEEN FRACTIONS OF SEPHADEX EFFLUENT

Variety	F_0 %	F_1 %	F_2 %	F_3 %	F_4 %
Champlein	12.1	44.3	27.7	9.0	9.6
Magdalena	15.4	24.4	43.9	5.7	7.0
Rex	22.1	25.1	42.4	5.2	6.7

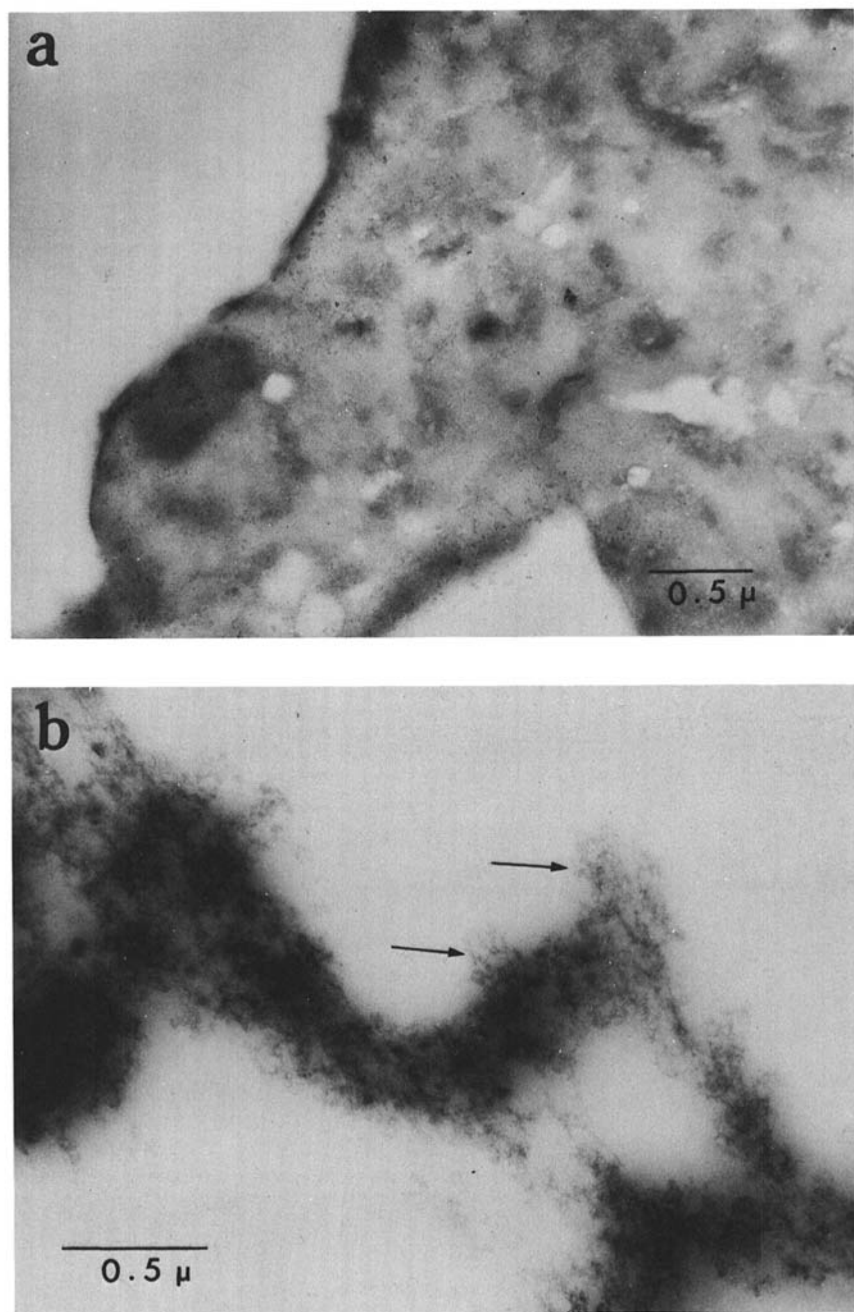


Fig. 1. Gluetnin. a) (top) fragment of glutenin with heterogeneous structures; b) (bottom) periphery of a glutenin fragment, showing fine fibrils (arrows). Fixation OsO_4

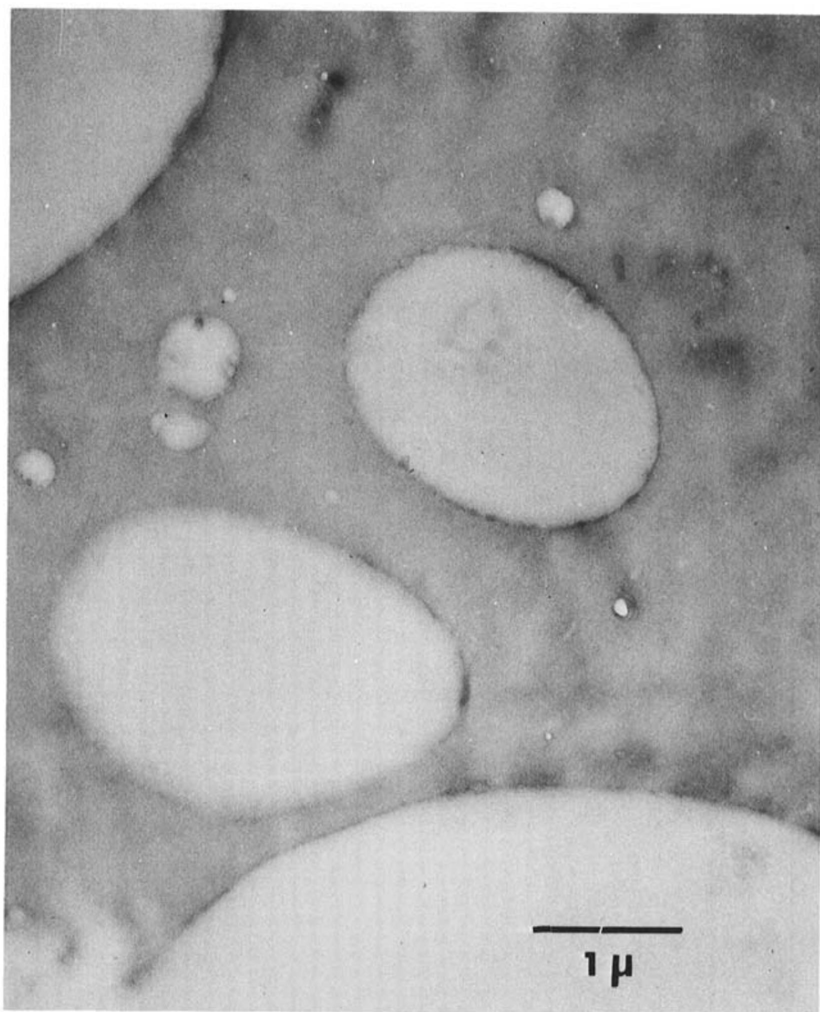


Fig. 2. Fragment of gliadin with smooth and compact structure, stained with OsO_4.

four fractions. Including the insoluble fraction, the total proteins of gluten are divided into five groups as shown in Table I.

Previous chemical and physicochemical analyses (12,13) indicate that each fraction comprises aggregates of different proteins: glutenin is the major constituent of the insoluble F_0 as well as F_1 and F_4 fractions; gliadin is the major constituent of the F_2 fraction.

For electron microscopic examination, the fractionated samples were freeze-dried and ground. All the samples were fixed 1 hr. in a 15% formol solution buffered at pH 7 at room temperature, washed carefully with the buffer solution at pH 7, and post-fixed 30 min. in a 1% osmic acid solution at the same pH and temperature. They were washed again, then dehydrated by passage through 80, 95,

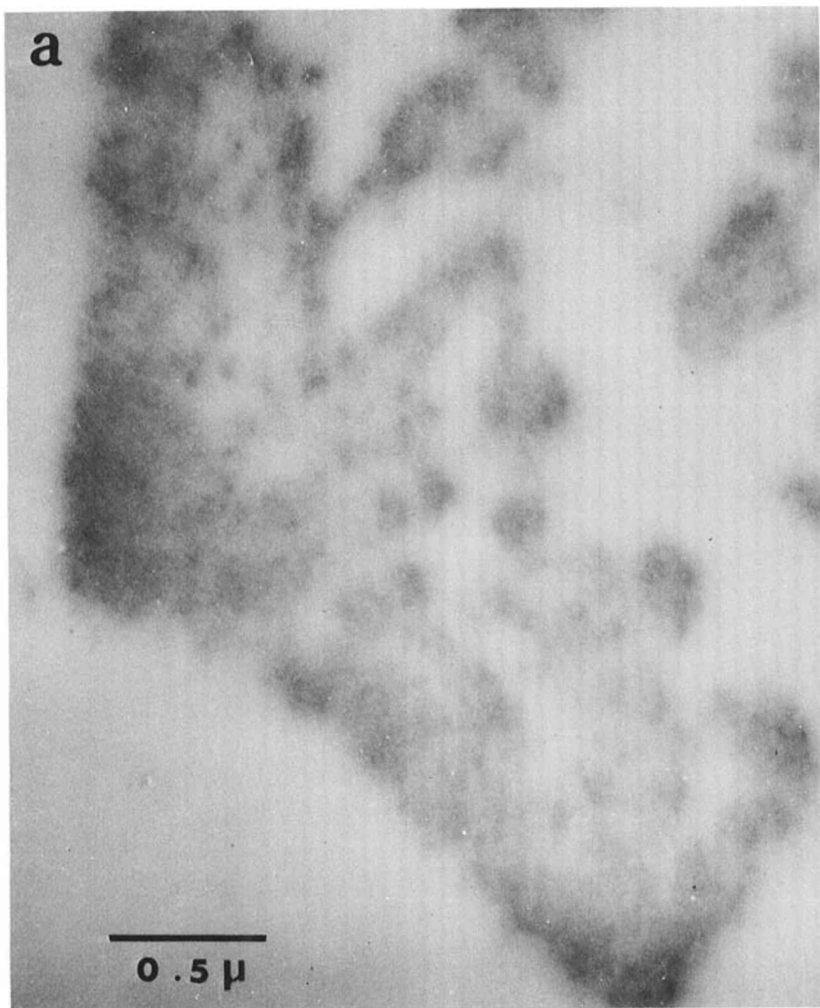
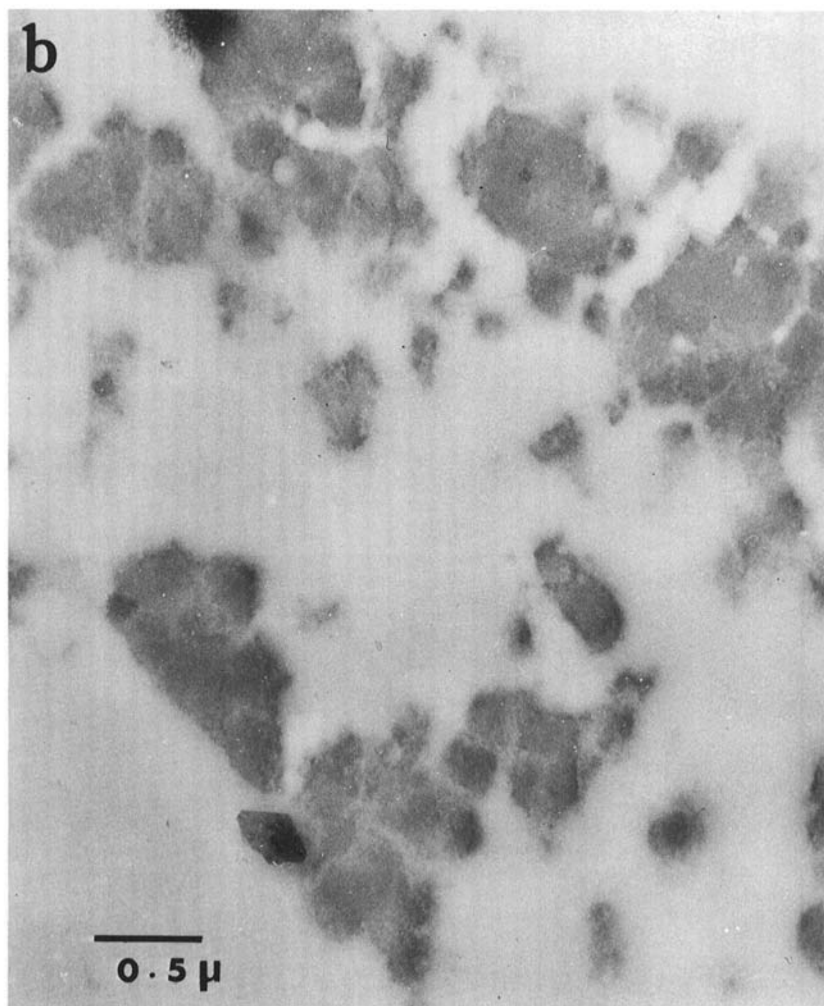


Fig. 3. Albumins (a, above) and globulins (b, facing page) obtained by Osborne's method, stained with OsO_4.

and 100% ethanol. After methacrylate embedding, ultrathin sections were observed with a Siemens Elmiskop I electron microscope.

RESULTS

The gliadin and glutenin fractions of flour present characteristic structures. Glutenin (Fig. 1) is heterogeneous: the protein fragments are relatively compact (Fig. 1a) but there are numerous zones of granular structure, sometimes with fine fibrillar felting, and are particularly visible at the fragments' periphery where the protein section is thinner (Fig. 1b). Numerous more electron-dense granulations are



also visible, which are probably lipid inclusions. The fibrils are 100 to 200 Å in diameter. Gliadin, on the other hand (Fig. 2), appears as homogeneous, smooth, compact, and relatively electron-lucent protein fragments.

Albumins and globulins present a structure more similar to some aspects of glutenin than gliadin (Fig. 3). In the gluten protein fractions obtained by chromatography there is again the same structural difference between fractions of high gliadin content and the glutenin fractions. Figure 4 shows a section of the F_2 fraction which presents a smooth and compact structure similar to the gliadin structure. F_0 and F_1 fractions of gluten with a high glutenin content present the characteristic fibrillar and granular structure of glutenin (Fig. 5).

In whole gluten (Fig. 6) a smooth, compact matrix encloses fibrillar felting and granular zones. Numerous lipid inclusions (L) are associated with these zones, as has

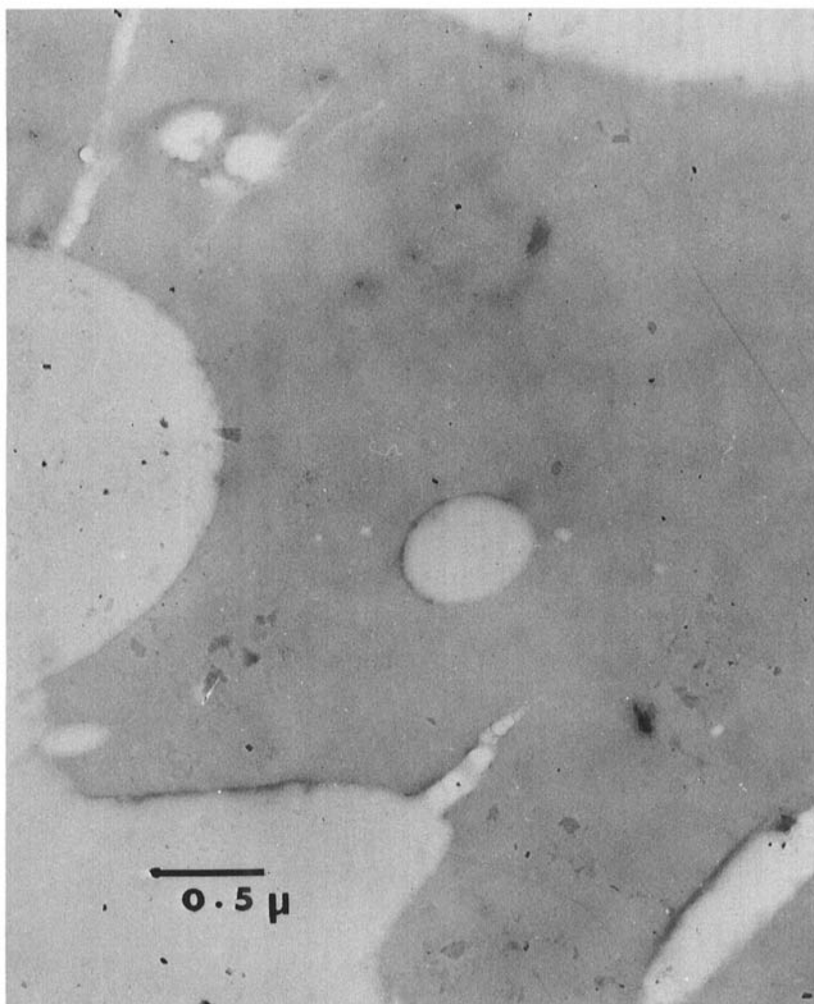


Fig. 4. F_2 fraction of gluten proteins obtained by chromatography fixed by OsO_4.

been shown elsewhere (6). In flour proteins, this heterogeneous structure can also be found.

In the section of a flour particle (Fig. 7), protein fragments with a smooth and compact structure can be seen between the starch granules. At the periphery of these fragments and sometimes around the starch granules there are zones of fibrillar felting proteins (see arrows) with which are associated numerous lipid globules. According to further, unpublished studies, it appears that lipid distribution in the form of globules is an artifact caused by OsO_4 reaction with unsaturated fats[1].

[1]Partly presented at the Colloque Annuel de la Société Française de Microscopie Electronique, Caen, May 1971.

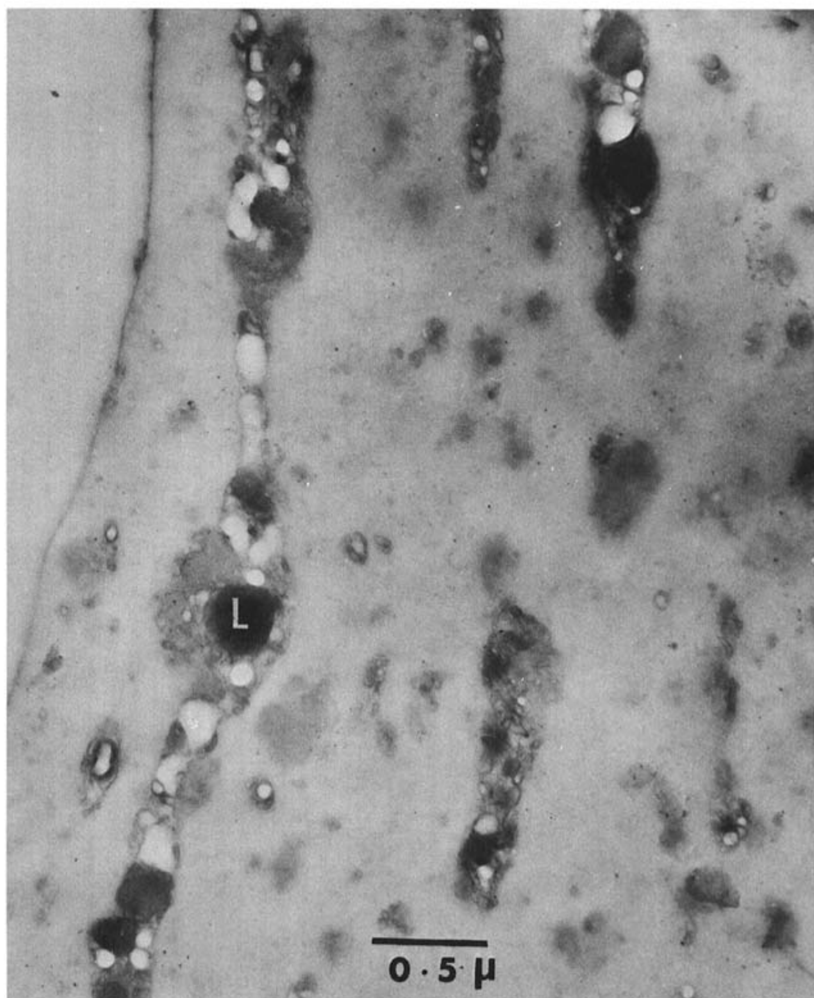


Fig. 6. Whole gluten proteins stained with OsO_4 showing a smooth compact matrix and fibrillar and granular zones to which are associated numerous lipid inclusions (L).

DISCUSSION

These results show that it is possible to differentiate the two major constituents of gluten proteins, gliadin and glutenin, by electron microscopy after osmic fixation. Differences of structure between these components have been observed previously by other authors using other techniques of preparation. Thus, Seckinger and Wolf (8) found that gliadin forms a film when it spreads on the surface of water, whereas glutenin forms strands in the same conditions. These results show some similarity with those that we have obtained on sections. In our study the structure of gliadin is smooth and compact while that of glutenin is fibrillar and granular.

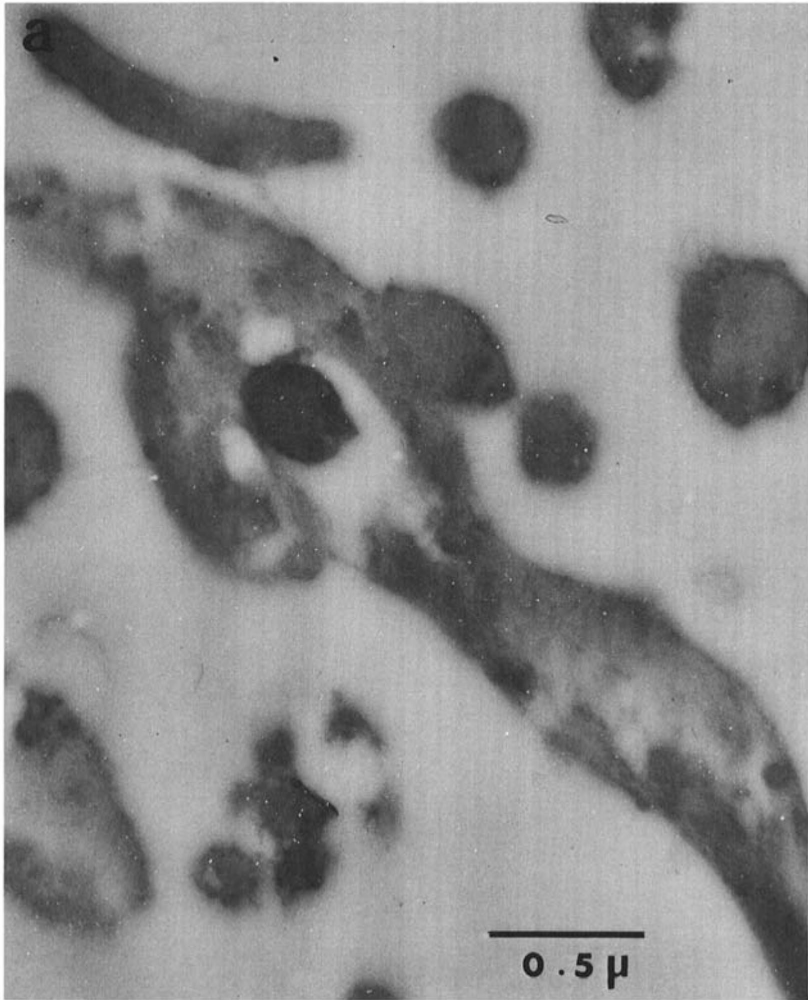
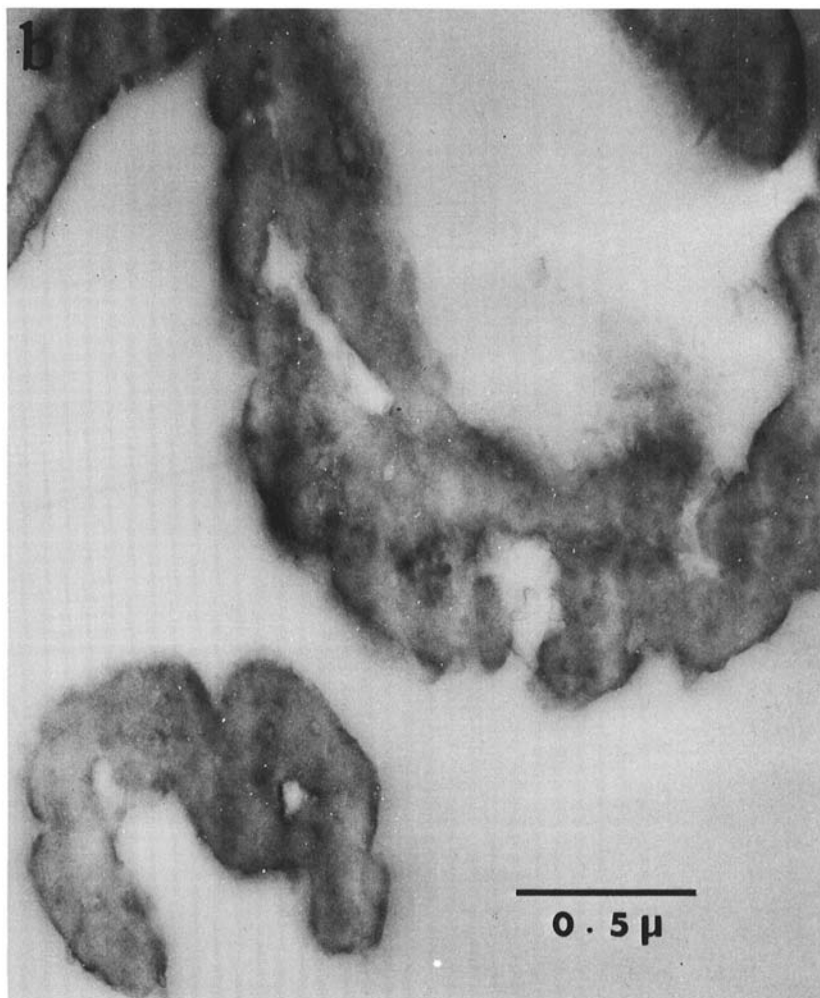


Fig. 5. F_0 (a, above) and F_1 (b, facing page) fractions of gluten proteins obtained by chromatography, stained with OsO_4.

Present in flour proteins, under two distinct forms, are protein fragments with an amorphous gliadin-like structure and fibrillar and granular proteins which adhere to both these fragments and to the starch granules. These results are different from those obtained by Seckinger and Wolf (5), who found an undifferentiated and amorphous structure for all flour proteins observed on sections after osmic fixation. However, these authors investigated a hard wheat variety while we have studied only soft wheats. Moreover, because many starch granules have been lost from their sections, perhaps by a swelling in water, it is possible that fibrillar and granular protein fractions can be lost along with the starch granules. Shkvarkina et al. (3) noticed that fibrillar proteins about 200 to 300 Å thick adhere to the surface of



starch granules. According to these authors, the proteins between starch granules present a periodic, striated structure and could be formed by linkage of numerous protein fibrils. In some cases, we have also observed a striated structure in flour or in gluten protein fragments, but it was considered to be an artifact caused by sectioning.

Hess (4) concluded, from his study with a shadowing technique, that in flour "wedge" amorphous protein fills the space between starch granules and "adhering" lipoprotein forms a fibrillar network on the starch granule surface. This differentiation has been disputed by other authors (3,5). Indeed we have also observed fibrillar felting of proteins at the periphery of protein fragments and of starch granules. According to Morton et al. (2), proteins occur in two distinct forms in immature wheat endosperm cells: protein bodies and proteins of different

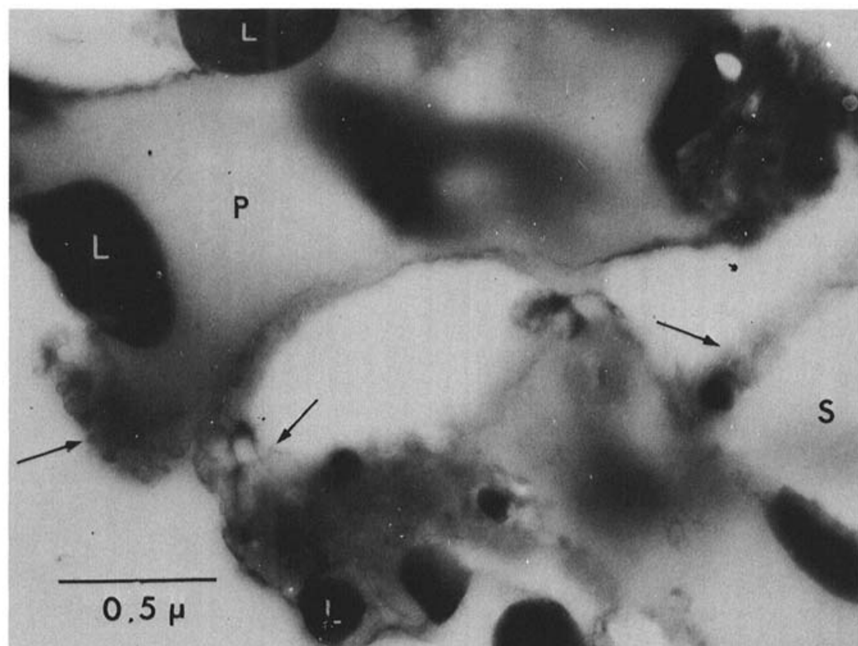


Fig. 7. Sample of wheat flour stained with OsO_4 showing both compact (P) and fibrillar (see arrows) proteins, lipid inclusions (L), and starch (S).

origins. The protein bodies are sites of storage protein accumulation; they seem to have a compact and relatively smooth structure after osmic fixation. The other proteins, and particularly lipoproteins, seem to have a fibrillar or granular structure and are associated with the protein bodies. Buttrose (1) also observed protein bodies with a homogeneous structure, in which he found fine granules about 100 Å thick. Therefore the heterogeneous structure observed in gluten preexists in flour and probably in the endosperm cells, and the possibility of a different origin for the protein fractions is not excluded.

To conclude, it appears that two characteristic types of structure can be found in wheat flour and in gluten proteins fixed by OsO_4: smooth and compact in gliadin, fibrillar and granular in glutenin, albumins and globulins being more like glutenin than gliadin.

(Note: During the printing of this work a study by D. H. Simmonds, "The Ultrastructure of the Mature Wheat Endosperm", was published in CEREAL CHEMISTRY, volume 49, page 212 (1972). The protein structures described by this author may be compared with our observations.)

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