

A LIPOPROTEIN MODEL OF WHEAT GLUTEN STRUCTURE¹

J. C. GROSSKREUTZ²

ABSTRACT

Electron micrographs and X-ray studies of gluten have shown the proteins to exist in platelet form of the order of 70A thick. Extraction of the phospholipids does not affect the basic platelet, but does seriously impair their ability to bond into sheets capable of sustaining large plastic deformations. X-ray evidence of the phospholipid structure in gluten favors the assumption that there exist well-oriented bimolecular leaflets of the type found in myelin figures. On the basis of the information at hand, a lipoprotein model is postulated which occupies some 2 to 5% of the elastic gluten structure. Consideration of the nature and strength of the bonds present in a hypothetical gluten sheet shows that the proposed lipoprotein model is capable of providing gluten with the plasticity necessary for optimum baking characteristics.

The structure responsible for the unique plastoelastic properties of wheat gluten has been investigated, using both X-ray and electron-microscopic techniques capable of structural detection on the macromolecular level. The details of the techniques used have been described in an earlier publication (3) and will not be repeated here. Gluten samples were prepared from two unbleached, untreated flours obtained as pure strains from Kansas State University. One strain (Pawnee) was characterized by a short dough-development time; the other (an experimental strain) by a long development time. Since no significant differences in the physical microstructure between the two glutes from these flours were found (3), no distinction will be made between samples in this paper.

X-Ray and Electron-Microscopic Evidence Concerning Gluten Structure

Previous work (3) carried out at this laboratory has demonstrated that the proteins in gluten consist of folded polypeptide chains in the α_1 -helix configuration arranged into flat platelets of the order of 70A thick. Figure 1 shows the typical X-ray scattering at small angles from moist gluten and its excellent agreement with the theoretically predicted platelet scattering. Two diffraction peaks which are visible in the moist gluten curve are interferences produced by the phospholipid component. Their significance will be discussed in detail later in this paper. Also shown in Fig. 1 is the scattering obtained from lyophilized,

¹ Manuscript received June 6, 1960. This work was supported in part by the C. J. Patterson Company, Kansas City, Mo.

² Midwest Research Institute, Kansas City, Mo.

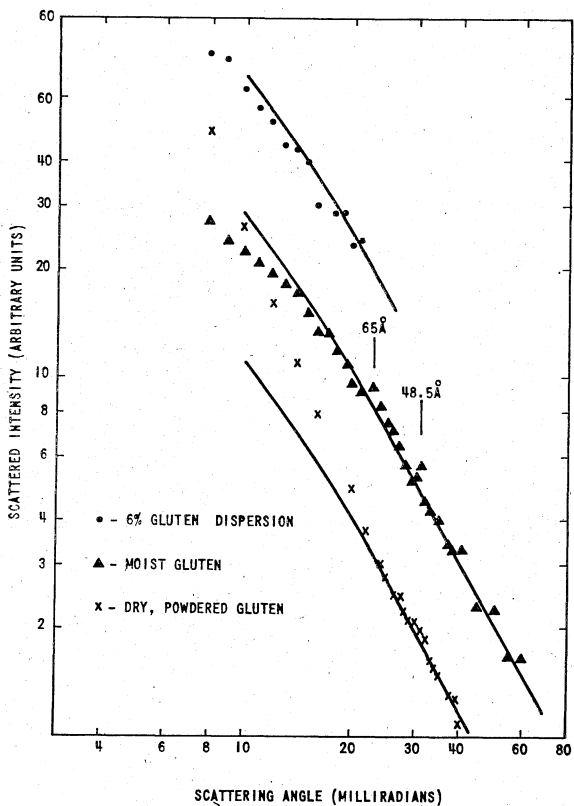


Fig. 1. X-ray scattering at small angles from moist and powdered glutens. Solid curves are the theoretical scattering from platelets.

powdered gluten which shows serious departure from the theoretical platelet scattering, especially at very small angles. This behavior indicates that the moist gluten platelets have, on drying, agglomerated

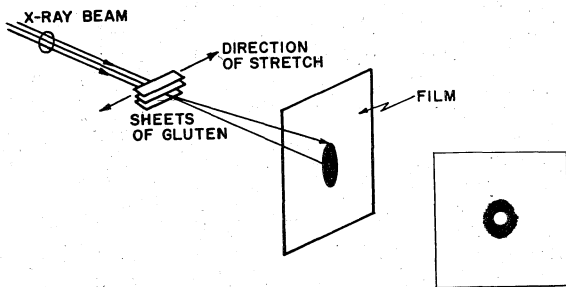


Fig. 2. X-ray scattering at small angles from stretched, freeze-dried gluten showing oriented scattering pattern.

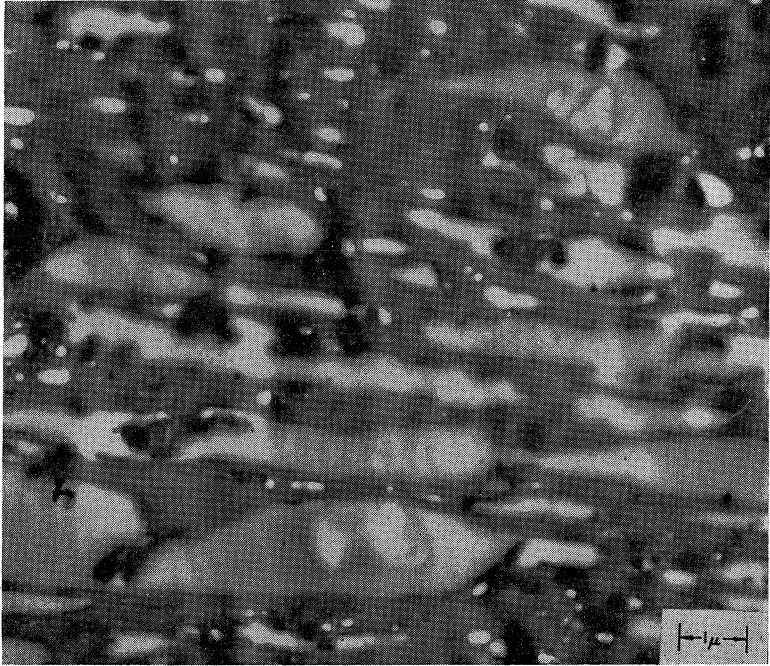


Fig. 3. Cross-section of stretched, dried gluten fixed with (OsO_4) . Thin section cut perpendicular to plane of stretch and along direction of stretch. Direction of stretch horizontal, magnification as shown.

into thicker lamellae.

To better understand this structure, consider the scattering obtained from stretched, freeze-dried gluten (3). Figure 2 depicts the oriented scattering pattern as recorded on photographic film. The angular dependence of this scattering, however, is the same as the powdered gluten curves given in Fig. 1. Thus, one may conclude that the units giving rise to the scattering in the powdered sample have, when stretched, arranged themselves into sheets parallel to the plane of stretch. Confirmation of these X-ray findings is afforded by electron micrographs of freeze-dried, gluten thin sections which were cut perpendicular to the plane of stretch and along the direction of stretch. Figure 3 shows the oriented sheetlike structure of a stretched, freeze-dried sample. The thickness of these sheets ranges from a few hundred to several thousand angstroms. Because it is not possible to obtain sections of moist gluten directly, it is impossible to say how closely Fig. 3 approximates the structure of moist gluten. However, since the X-ray evidence certainly indicates considerable agglomeration of the basic protein platelets on drying, one may assume that the

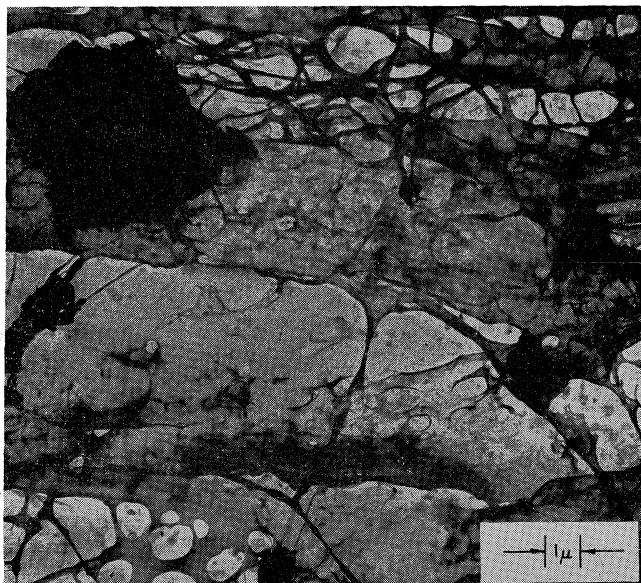


Fig. 4a. Surface replica of stretched, freeze-dried gluten. Direction of stretch horizontal.

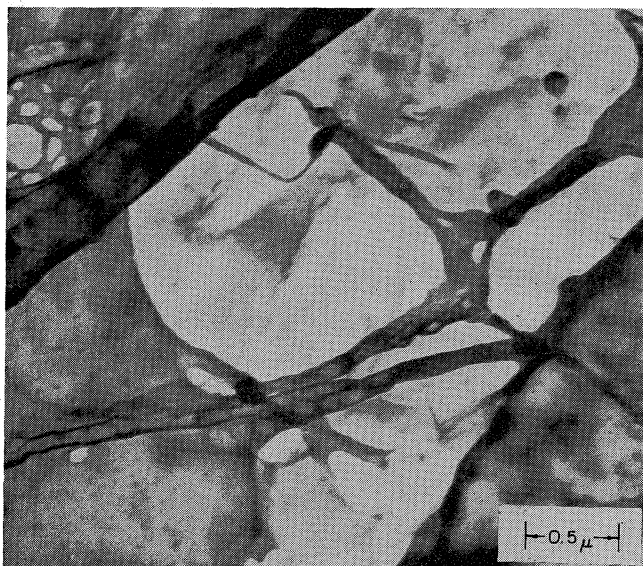


Fig. 4b. Surface replica of stretched, freeze-dried gluten showing three layers of gluten sheet.

picture in Fig. 3 is a distorted one.

The behavior of gluten sheets under strain has been observed by

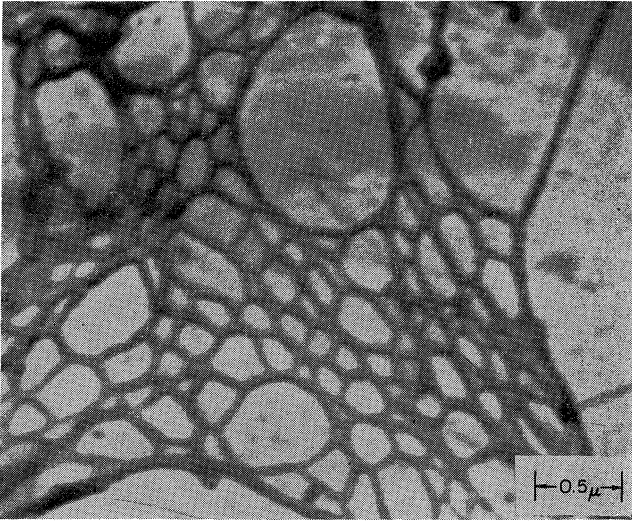


Fig. 4c. Surface replica of stretched, freeze-dried gluten showing extreme example of ruptured sheet.

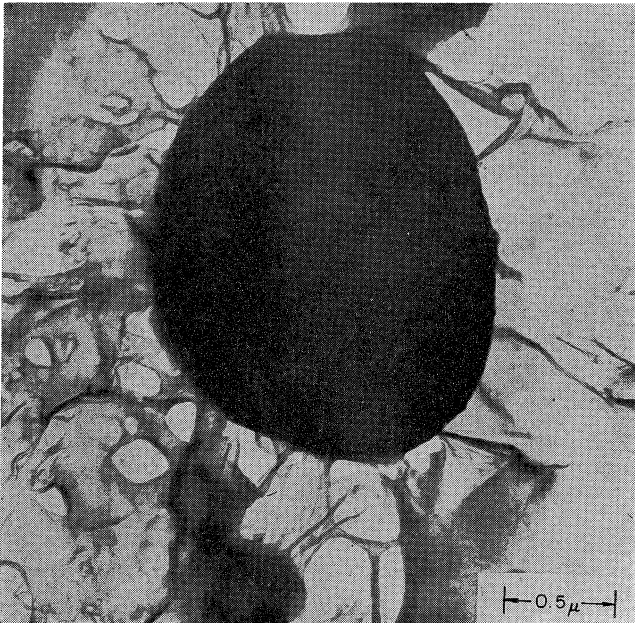


Fig. 5. Surface replica of stretched, freeze-dried dough showing starch grain (large black mass) occluded in matrix of gluten sheet.

means of electron micrographs taken of surface replicas of freeze-dried stretched gluten. Figures 4a, b, and c show different stages in the rupture of a strained sheet into filaments. These tiny filaments measure approximately 200–300 Å in diameter.

Of great practical interest is the actual structure of a dough on the macromolecular level. Figure 5 is an electron micrograph of a surface replica of stretched, freeze-dried dough which was mixed to peak consistency. A starch grain is shown held in place by a gluten sheet which has been strained to rupture in certain areas.

The Place of Lipids in Gluten Structure

Background. The effect of lipids, especially phospholipids, on gluten behavior has been a subject of interest and investigation for many years. Working (12,13) found that the presence of phosphatides caused gluten to be soft and plastic. Prolonged washing in water removed a portion of the phospholipids and caused the gluten to stiffen. Adding back wheat phosphatides caused glutes to become softer and more pliable. He concluded that the phosphatides formed a film over the gluten mesh and lubricated it, causing a loss of cohesiveness. Furthermore, he showed that oxidizing agents "liberated" phosphatides and improved their lubricating action. Geddes and Larmour (2) found a close relationship between phosphatide content and the baking behavior of a flour-germ mixture. The statement is made that there appears to be an optimum amount or concentration of phospholipid. An excess results in a soft gluten, a deficiency in a harsh, tough, inelastic gluten. Recently Mecham and Mohammad (6) have shown that lipid extraction with water-saturated n-butanol yields a flour which requires a fourfold increase in development time. Experiments in this laboratory have confirmed the latter observation; it was necessary to enclose the doughball in a nylon bag during washing in distilled water to retain a gluten mass. The coherency of this gluten under strain is very small.

Obviously, then, the presence or nonpresence of lipids, especially phospholipids, has a marked effect on the "quality" of the gluten structure in dough. To understand this effect, it is necessary to inquire more deeply into the structure of phospholipids. Stoeckenius (9) has shown that the pure phospholipids, lecithin and cephalin, extracted from human brain tissue form highly ordered molecular aggregates in aqueous media. Figure 6 illustrates the model proposed by Stoeckenius to account for his electron-microscope observations and the X-ray observations of others. This bimolecular leaflet consists of two phospholipid molecules with their hydrophobic ends inwardly oriented

PHOSPHOLIPID BI-MOLECULAR STRUCTURE

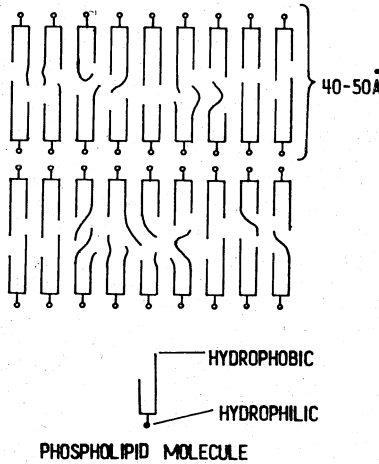


Fig. 6. Phospholipid bimolecular leaflet structure.

and their hydrophilic, phosphoric acid groups outwardly oriented into the aqueous media. The most commonly observed width of the leaflet is 42Å.

X-ray Evidence of Lipid Structure in Gluten. The X-ray scattering curve from moist gluten shown in Fig. 1 possesses diffraction maxima characteristic of the phospholipid bimolecular leaf dimensions. It has been shown (3,4,11) that extraction of phospholipids reduces the intensity of these diffraction lines in gluten, and further that the extracted lipids show the same diffraction maxima. The phospholipids must, moreover, be wetted in order to develop the bimolecular leaflet structure. Figure 7 shows the X-ray diffraction pattern of the lipids extracted from dry flour (where presumably no water was available to the lipids) and from developed gluten (in which hydration has occurred thoroughly during mixing).

The author, therefore, assumes that a bimolecular phospholipid leaflet structure exists in dough or glutes. Since the characteristic diffraction lines persist even in dried glutes (3), the structure is fairly well stabilized in the developed gluten. This is completely in agreement with the observations of Olcott and Mecham (7), who demonstrated the difficulty of lipid extraction, especially phospholipids, after doughing.

Effect of Lipid Removal on Platelet and Sheet Structure of Gluten. A simple manual inspection of the gluten extracted from doughs made from flour defatted with n-butanol reveals that the gluten is extremely

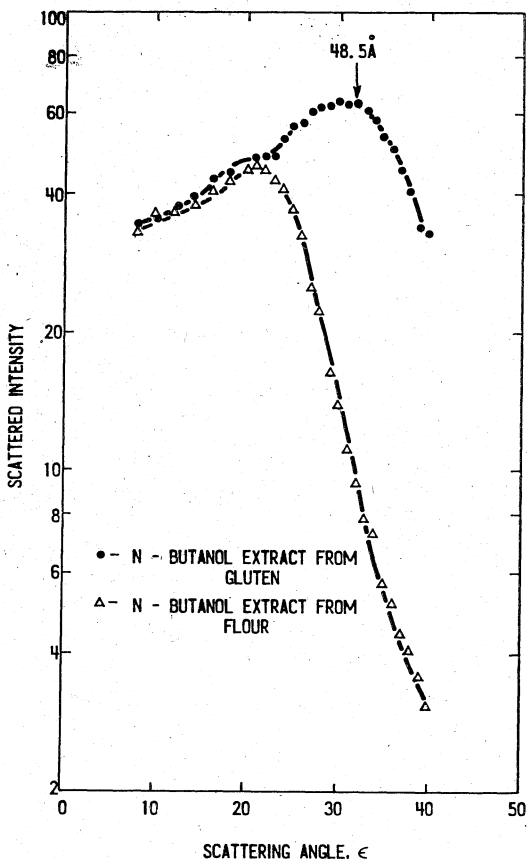


Fig. 7. X-ray scattering at small angles from lipids extracted from flour and developed gluten.

fragile. It appears to be elastic, but with a very low elastic limit, i.e., it ruptures easily. Most important, there is no plasticity. That is, it is not possible to induce large permanent deformations without rupturing. This distinction between elasticity and plasticity in gluten is a necessary one which is not always carefully made. Both properties are necessary for a dough of optimum baking quality.

More sophisticated examination of the lipid-free glutens³ has shown that the platelet nature of the X-ray scattering persists. That is, scattering curves from lipid-free glutens are identical to those shown in Fig. 1, with the exception of the sharp diffraction peaks. Moreover, it is not possible to develop good gluten sheet structure in stretched,

³ In the following discussion, all lipid-free samples were extracted after the procedure of Mecham and Mohammad (6).

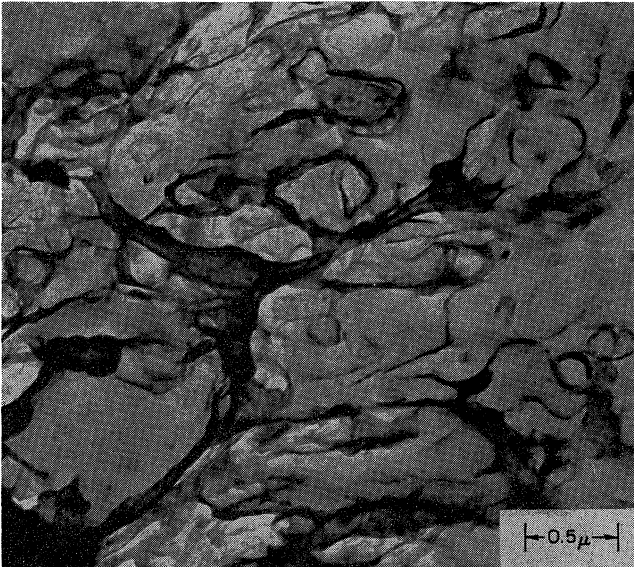


Fig. 8. Surface replica of stretched, freeze-dried, lipid-free gluten.

lipid-free glutens. Two observations support this latter statement. It is not possible to obtain an oriented X-ray scattering pattern such as that shown in Fig. 2, nor do electron micrographs of surface replicas show a well-developed sheet structure (Fig. 8).

It may be concluded that the removal of phospholipids does not destroy the basic protein platelet, but that it does seriously affect the ability of these platelets to bond into coherent sheets capable of large plastic deformation.

Discussion

The Protein Platelet. The experimental evidence discussed in the last paragraph has shown that the platelet structure as detected by X-ray scattering is a property of the protein alone and is not dependent on the presence of lipids. The X-ray data permit a determination of the approximate thickness of the platelet (3), but give no direct evidence as to its lateral extent. However, a comparison of the experimental curve with those calculated theoretically by Schmidt (8) for polydisperse ellipsoids of revolution has shown that the ratio of thickness to diameter is of the order of 0.01.

This protein platelet is most probably formed in the presence of water during the doughing process, and the author assumes that it consists of coiled (perhaps randomly) polypeptide chains with their hydrophilic side chains oriented outward and their hydrophobic side

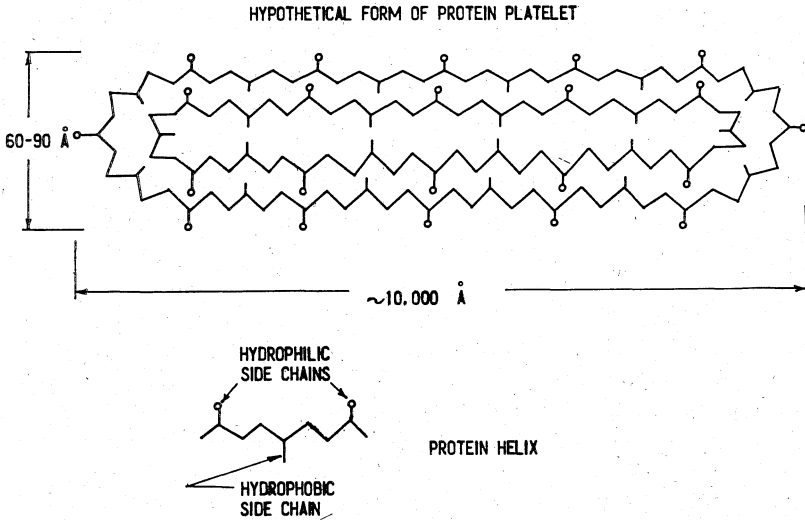


Fig. 9. Proposed model of protein platelet structure.

chains oriented inward. Figure 9 is a hypothetical, highly idealized model of such a protein platelet.

Lipoprotein Linkage between Platelets. Stoeckenius (9) found that mixing pure phospholipids in an aqueous solution with globin produced a lipoprotein complex whose structure was best described by

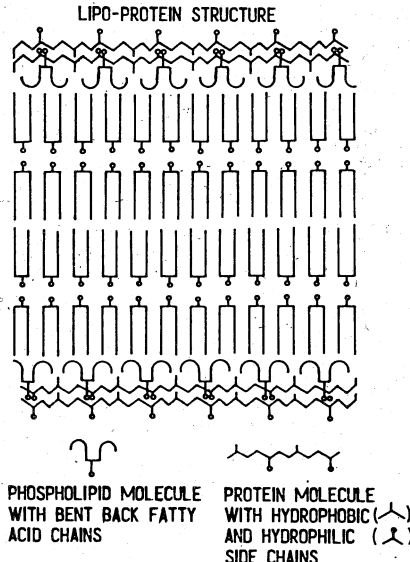


Fig. 10. Lipoprotein structure.

the molecular scheme shown in Fig. 10. At least two (and perhaps more) protein chains are bound to the outer edge of a phospholipid bimolecular leaflet array, probably by saltlike linkages between the acidic groups of the phospholipid and the basic protein groups. Note also that the hydrophobic side chains of the protein penetrate into the lipid bimolecular leaflet—a configuration not easily broken in water solution. On the other hand, the hydrophilic protein side chains stick out ready to bond with other proteins or simply to absorb a layer of water.

The question arises as to the probability of the existence of such a lipoprotein complex in gluten. Several widely separated pieces of information exist, which show, at least, that such a configuration is not inconsistent with the facts: Balls, Hale, and Harris (1) determined the nitrogen-phosphorus (N/P) ratio in a lipoprotein extracted from flour with petroleum ether. This ratio was found to be 8.05:1. Pence and Mecham⁴ find N/P ratios of this same order of magnitude in some fractions of the normal butyl alcohol extracts of flour. The α -helix of protein contains 3.6 residues per turn and, hence, an equal number of nitrogen atoms per turn, exclusive of the nitrogen in the side chains. The majority of amino acids found in wheat contain one additional nitrogen atom in the side chains, and hence the total nitrogen per turn of the helix is on the average of the order of 7. Each phospholipid molecule contains one phosphorus atom. Hence, it would not be inconsistent with the N/P ratios quoted above to postulate that a phosphoric acid end group exists for every turn or so of the polypeptide helix. If one counts up the phosphorus atoms per turn depicted in the hypothetical scheme of Fig. 10, there is approximately a 1:1 ratio. Thus, it is not inconsistent with the data at hand to propose that a lipoprotein complex of the type depicted in Fig. 10 exists in gluten.

On the basis of the known percentages of lipid phosphorus found in gluteins (6,10), the lipoprotein complex just proposed would amount to about 2½% by weight of the total protein present. If one further assumes that the glutenin fraction is responsible for most of the elastic properties (5,7), then the lipoprotein complex would amount to about 5% of the total elastic structure.

A model of gluten structure based on the evidence at hand may now be proposed. Because the major proportion of protein present in gluten is in platelet form, the continuous sheets observed in the electron micrograph must be formed mostly by the linkage of one

⁴Pence, J. W., and Mecham, D. K. Private communication.

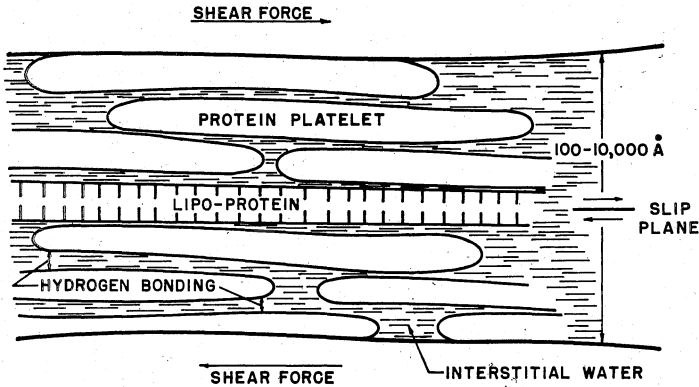


Fig. 11. Proposed model of gluten sheet structure.

platelet with another through an aqueous phase, probably by hydrogen bonding. Five per cent of the elastic sheet, however, must consist of the lipoprotein, which can also bond to the protein platelets through hydrogen or saltlike linkages. Figure 11 is a hypothetical scheme showing a possible structure for a gluten sheet made up of protein platelets and lipoproteins in the appropriate concentrations.

To understand the elastoplastic properties of such a configuration it is necessary to consider the relative bond strengths involved. The hydrogen and saltlike linkages are much stronger than the pseudo bonds existing between the phospholipid bimolecular leaflets, either at the hydrophobic or hydrophilic ends. Moreover, the nature of the two bonds is entirely different. The former are short range and, if broken, unlikely to rebond until the proper chemical groups are brought into close contact again. On the other hand, the bimolecular leaflet configuration results from the dielectric properties of the phospholipid molecule and the pseudo bonds between hydrophobic and hydrophilic ends are fairly long range. Especially is it possible to displace one leaflet with respect to the other in shear as shown in Fig. 11, without disrupting the over-all orientation. Thus, because of the nature and relative strengths of the bonds involved in Fig. 11, an applied stress will result in slip along the interfaces of the phospholipid layers before the rupture strength of the interprotein bonds is reached. This is not to say that rupture will never occur; indeed, the evidence of Fig. 4 shows that rupture does occur frequently. However, on the proposed model, such rupture will occur either in regions poor in lipoproteins or when the plastic strain in the lipoprotein exceeds even the range of the dielectric orienting forces. If the lipoprotein is extracted from gluten the ability to deform plastically (slip) is removed

and the application of stress can result only in widespread rupture of the gluten sheets, once the elastic limit has been reached. It is also easy to see on such a model that too much phospholipid would make for a runny or very plastic gluten, while too little of this component could give rise to a nonplastic gluten easily ruptured under large stress.

Several attempts were made to confirm this model by electron microscopy, using the techniques employed by Stoeckenius (9). Samples of stretched gluten were fixed and stained with osmium tetroxide and then dried in successive ethyl alcohol washes. Since the osmium tetroxide reacts at unsaturated double bonds, it will be distributed more or less uniformly along the protein chain (wherever unsaturated amino acids are found). On the other hand, Stoeckenius has shown that it will deposit preferentially along the interior of the phospholipid bimolecular leaflet, i.e., at the hydrophobic ends. Because the osmium is highly opaque to electrons, the bimolecular leaflets should then appear as alternating light and dark lines in the microscope, separated by approximately 40 to 50 angstrom units. The attempts which we have made to detect these striations have not been successful. Because we expect at most only 5% of a field of view, say, to consist of these lines, it is very easy for artifacts to be misinterpreted as the true structure. Secondly, 40A is near the limit of resolution of the electron microscope used in these studies. Although striations of the correct spacing have been observed on occasion, the difficulties just mentioned do not allow one to interpret this as an unqualified confirmation of the proposed structure.

Literature Cited

1. BALLS, A. K., HALE, W. S., and HARRIS, T. H. A crystalline protein obtained from a lipoprotein of wheat flour. *Cereal Chem.* **19**: 279-288 (1942).
2. GEDDES, W. F., and LARMOUR, R. K. Some aspects of the bromate baking test. *Cereal Chem.* **10**: 30-72 (1933).
3. GROSSKREUTZ, J. C. The physical structure of wheat protein. *Biochim. et Biophys. Acta* **38**: 400-409 (1960).
4. HESS, K. Protein, Kleber, und Lipoid in Weizenkorn und Mehl. *Kolloid. Z.* **136**: 84-99 (1954).
5. JONES, R. W., TAYLOR, N. W., and SENTI, F. R., Electrophoresis and fraction of wheat gluten. *Arch. Biochem and Biophys.* **84**: 363-376 (1959).
6. MECHAM, D. K., and MOHAMMAD, A. Extraction of lipids from wheat products. *Cereal Chem.* **32**: 405-415 (1955).
7. OLCOTT, H. S., and MECHAM, D. K. Characterization of wheat gluten. I. Protein-lipid complex formation during doughing of flours. Lipoprotein nature of the glutenin fraction. *Cereal Chem.* **24**: 407-414 (1947).
8. SCHMIDT, P. W. The small-angle X-ray scattering from polydisperse solutions of ellipsoidal particles. *Acta Cryst.* **11**: 674-676 (1958).
9. STOECKENIUS, W. An electron microscope study of myelin figures. *J. Biophys. Biochem. Cytol.* **5**: 491-500 (1959).
10. SULLIVAN, BETTY, and NEAR, CLEO. Lipoid phosphorus of wheat and its distribution. *Cereal Chem.* **5**: 163-168 (1928).
11. TRAUB, W., HUTCHINSON, J. B., and DANIELS, D. G. H. X-ray studies of the wheat

- protein complex. *Nature* **179**: 769-770 (1957).
12. WORKING, E. B. Lipoids, a factor influencing gluten quality. *Cereal Chem.* **1**: 153-158 (1924).
 13. WORKING, E. B. The action of phosphatides in bread dough. *Cereal Chem.* **5**: 223-234 (1928).

