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PREPARATION OF CEREALS AND GRAIN PRODUCTS FOR TRANSMISSION ELECTRON MICROSCOPY

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Abstract

This tutorial specifically addresses the techniques used in the processing of cereals and grain products for various aspects of transmission electron microscopy. Methods covered include sample treatment, chemical and physical fixation, dehydration, embedding, sectioning techniques, immunocytochemistry, enzymatic digestions, carbohydrate localization, and lectin binding. The primary goal is to provide information on the preparation of cereals and cereal-based products for microscopic analysis and to assist the reader in solving technical problems associated with studying cereals or other difficult-to-prepare samples.

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Introduction

This tutorial addresses the techniques used in the processing of cereal grains and cereal products for various aspects of transmission electron microscopy (TEM). While much of the information presented will deal specifically with TEM, many of the techniques could be adapted to other microscopic methods such as light and scanning electron microscopy. The specimen preparations for these various techniques so often closely parallel each other that one can utilize a single tissue preparation for several different microscopic techniques.

Not presented is a review of the history of cereal structure. The reader is referred to the review by O'Brien (1983) for such information. Indeed, much of this paper will deal with research presented following the period I designate as the post-paraffin era. The paraffin era of light microscopy concluded with two beautifully conducted studies; the first a series of papers on maize (Wolf et al., 1952a, b, c, d) followed by a similar study on the wheat caryopsis (Bradbury et al., 1956a, b, c, d). These two studies, in my eyes, were the final word on cereal structure until the adaptation of techniques for electron microscopy.

This review will also not deal with generalized descriptions of microscopy techniques as they have been covered numerous times in volumes such as: The Study of Plant Structure; Principles and Selected Methods by O'Brien and McCully (1981); Botanical Histochemistry by Jensen (1962); Botanical Microtechnique and Cytochemistry by Berlyn and Miksche (1976); Botanical Microtechnique by Sass (1958); as well as many other papers and books.

Many of the comments and descriptions concerning the preparation of cereals for TEM have not yet been brought together in a volume and reflect answers to numerous questions received over the years. These include the whole range of procedures from fixation, dehydration, embedding, and sectioning, to understanding what the limitations are with the techniques used. Hopefully, the reader will find information that will help in solving technical problems associated with studying cereals, seeds, or other difficult-to-prepare samples. Little transmission electron microscopy has been published on cereal-based products such as

bread and crackers resulting in a small data base of techniques for this area.

Obtaining Samples

The way in which samples are obtained can be extremely important and is often glossed over or totally ignored in materials and methods sections of papers. If the samples of grain are developing out in the field, they either need to be brought back into the laboratory for processing or initially processed in the field. Samples brought back to the laboratory for processing can not be expected to resemble the "field" condition if they are treated harshly with excessive heat or cold. It is well known that both of these temperature extremes affect the biochemical machinery of plants. Still, many samples are transported back to the laboratory close to freezing. Plants also can not be allowed to become dehydrated prior to preparation. The technique I prefer is to cut off the cereal head and 15 to 20 cm of stem, place the cut end immediately into a flask of water and transport the sample back to the laboratory without exposure to temperature extremes or wind. Once in the laboratory, individual caryopses can be dissected from the head and processed.

I have found that developing grains, especially the young stages, tend to be very susceptible to mechanical damage which necessitates handling them very gently. Since entire caryopses are too large to fix whole, even at early stages of development, they need to be cut into smaller pieces without causing damage. One method found to reduce damage is to place the grain on a piece of dental wax and use a clean, new, sharp stainless steel razor blade to slice the caryopsis by drawing the blade in one direction while gently pressing down. This method has reduced much of the damage caused by cutting. Even with gentle handling some damage will occur and recognizing it is important to avoid reporting artifacts (Figs. 1 and 2). The aleurone layer seems to be particularly susceptible to mechanical stress (Fig. 1), even at maturity (Bechtel and Pomeranz, 1977; 1978c).

The presence of waxy cuticles on and in the caryopsis coats (Morrison, 1975; Bechtel and Pomeranz, 1977) makes it important to have two cut ends on each sample and to keep thickness of samples to 1 mm or less in order to allow fixative penetration. Sample thickness depends upon stage of development and size of the cells: the larger the cells the larger the blocks of tissue have to be. The larger block size can result in poor overall fixation quality, however. A certain amount of compromise has to be accepted.

Similar care must also be taken when sampling grain products such as bread doughs and baked goods. While these systems are inanimate (except for the yeast in yeast-leavened products), they are also quite dynamic and require careful handling to avoid mechanical artifacts. Doughs in particular are susceptible to "relaxation" following optimal mixing, therefore, sampling must occur immediately after the mixer has stopped (Bechtel et al. 1978). Another problem one has to contend with involving bakery products is deformation of the product during sampling. Some problems just do not have any easy solutions; one must make the best of a difficult situation and keep these problems in mind when making interpretations.

Fixation Procedures

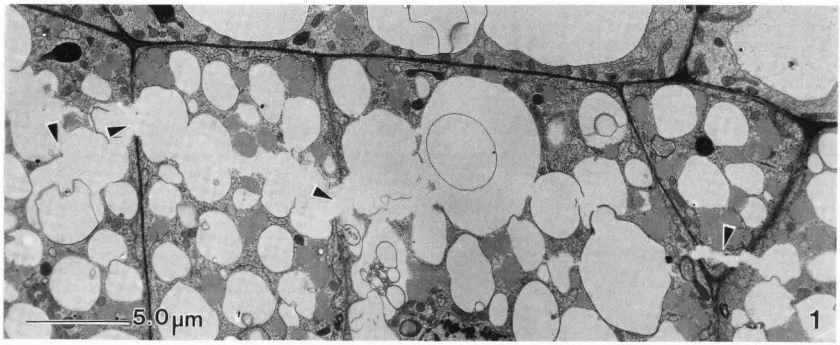
Fixation is the process of stabilizing the cell's structure and contents in as near a life-like condition as possible, or in the case of grain products, in a condition close to the real state at the time of sampling. There are two general classes of fixation techniques, chemical and physical. Chemical fixatives can be further divided into coagulant and non-coagulant types based on their ability to coagulate albumin proteins. Very early in electron microscopy, it was found that the coagulant type fixatives were not applicable. The non-coagulant types have, therefore, been used nearly exclusively for electron microscopy, primarily because many are able to cross-link various cellular components without large changes in cellular appearance.

Chemical Fixation

Early studies on cereal ultrastructure relied on potassium permanganate ($KMnO_4$), osmium tetroxide (OsO_4) and formaldehyde by themselves or in various combinations. Buttrose (1960, 1963) used $KMnO_4$ or OsO_4 in buffered veronal-acetate to fix developing wheat endosperm. MacLeod and coworkers (1964) used buffered $KMnO_4$ to study the endosperm of barley and *Bromus*. In a series of studies on wheat endosperm, Morton and coworkers utilized various combinations of formaldehyde and $KMnO_4$, as well as OsO_4 and $KMnO_4$ individually (Morton and Raison, 1963; Morton et al., 1964; Jennings et al., 1963; Graham et al., 1962). Rice endosperm development was studied by Hoshikawa (1968; 1970) using only $KMnO_4$ as a fixative.

Glutaraldehyde was introduced as a fixative by Sabatini et al., in 1963, and the quality of cellular preservation improved dramatically as its use spread in the study of cereal structure (O'Brien, 1967; O'Brien and Thimann, 1967a, b; Jones, 1969a, b, c). The concentration of glutaraldehyde, the type of buffering system, length of fixation, temperature of fixatives and use of various additives are all variables that must be established for each system. There are no set rules as to which combination of fixatives and procedures are best. Generally, one will test a variety of fixation schemes and select the one that gives the best overall fixation quality (see Parker, 1980 as an example). Concentration of

Figs. 1-5. Fixation of cereal endosperm. 1. Aleurone region of 12 DAF (days after flowering) developing barley endosperm showing damage caused by cutting caryopsis during tissue preparation (arrows). 2. Probable mechanical damage in 12 DAF barley endosperm revealed by broken tonoplast (arrow) and washed out cytoplasm (*). 3. Freeze fracture micrograph of bread dough showing portion of yeast cell (Y), gluten strand (G), and starch granule (S). Note lack of ice crystal formation. 4. Freeze-fracture of freshly frozen 21 DAF wheat endosperm showing large ice crystals (I) that are located between cell wall (CW) and vacuole (V). 5. Freeze-substitution of developing wheat endosperm showing freezing damage (arrows) in the cytoplasm and protein bodies (PB).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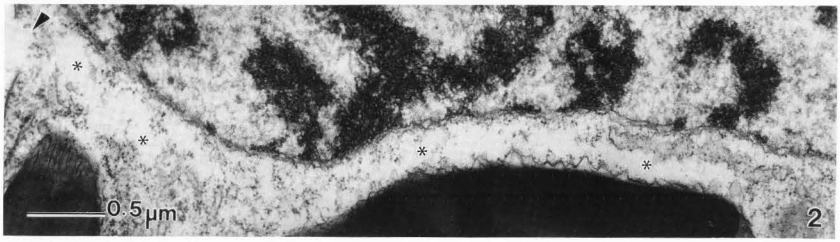
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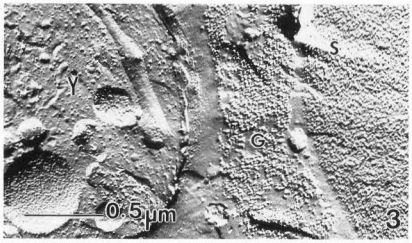
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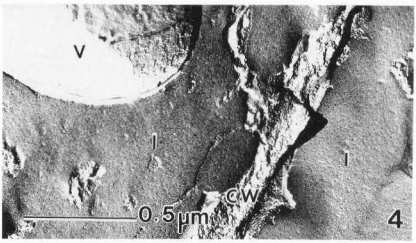
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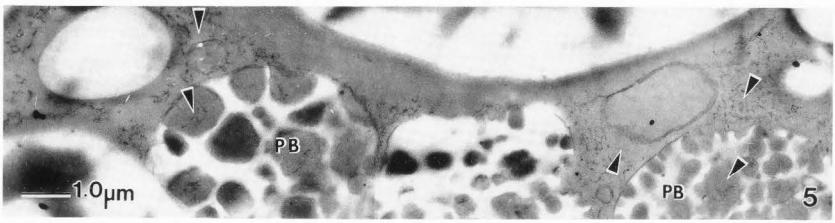
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glutaraldehyde has varied from lows of 2% (Cameron-Mills and von Wettstein, 1980) and 2.5% (Briarty et al., 1979; Oparka and Harris, 1982) to a high of 6% (Swift and O'Brien, 1972; Hallam, 1972; Hallam et al., 1972; 1973; Morrison, 1975; Morrison et al., 1975; Cameron-Mills and von Wettstein, 1980). Percentages of glutaraldehyde that were in between these values have also been commonly used (Jacobsen et al., 1971; Parker and Hawes, 1982; Hinchman, 1972; Simmonds, 1972a; Barlow et al., 1974; Parker, 1980; 1981b; 1982; Campbell et al., 1981; Morrison and O'Brien, 1976). Some investigators have used combinations of glutaraldehyde and paraformaldehyde after the Karnovsky (1965) method to improve fixative penetration of the caryopsis, especially in more mature stages of development (Fineran et al., 1982; Parker, 1980; 1981a; Bechtel and Pomeranz, 1977; 1978a; b; 1979; 1981; Bechtel and Juliano, 1980; Bechtel et al., 1982a; b; Bechtel and Gaines, 1982). The use of a third aldehyde (acrolein) added to the above fixation medium has also been tried (Buckhout et al., 1981; Sraon, 1972). The quality of fixation, in my opinion, is not as good with the combination type fixatives as with glutaraldehyde alone. When penetration of a mature caryopsis at 12% moisture is needed, the combination type fixatives provide satisfactory tissue preservation that would otherwise not be obtainable with glutaraldehyde alone. A phosphate buffering system is most commonly used, followed by cacodylate. Certain cytochemical procedures, particularly involving enzyme localizations, require specific buffering systems to avoid interference. For example, localization of acid phosphatase (Bechtel et al., 1990) requires the use of sodium cacodylate buffer because phosphate buffer would cause heavy metal precipitation during the procedure.

Few studies have been conducted on doughs and bread (Simmonds, 1972b; Khoo et al., 1975; Bechtel et al., 1978). Khoo et al. (1975) used a variety of fixative methods including OsO_4 vapor and acrolein vapor followed by OsO_4 vapor. These two methods were found to be unsatisfactory. Best results were obtained with 3% glutaraldehyde followed by OsO_4. Similar fixation procedures were employed by Simmonds (1972b) and Bechtel et al. (1978).

Post-fixation in 1-2% osmium tetroxide in the same buffer as the primary fixative is routinely used to further stabilize and stain cellular components. Nearly all cytochemical procedures are interfered with by OsO_4 and post-fixation should be omitted from these samples. A post-fixation procedure used in conjunction with high voltage electron microscopy (HVEM) but used infrequently in the study of cereals is fixation with an aldehyde followed by a zinc iodide-osmium tetroxide impregnation (Parker and Hawes, 1982). This method impregnates the double membrane bounded organelles such as RER, Golgi bodies, mitochondria and nuclear membranes with an electron dense precipitate. Examination of sections up to 1 µm thick in the HVEM allows stereo pairs to be taken and three-dimensional views of organelles established.

As fixatives are extremely reactive chemicals, protective equipment such as a fume hood, gloves and protective glasses must be used. Many of the chemicals for microscopy are dangerous. As more studies are conducted on the toxicology of these chemicals, microscopists should keep up-to-date on the results. A recent review covers the hazards of handling microscopy chemicals (Ellis, 1989).

Physical Fixation

Physical methods of fixation are limited to freezing the samples. Several different methods are available to visualize the samples after they have been frozen. One is to replace the water while the sample is still frozen using a process called freeze-substitution. Another is to fracture the frozen sample and then make a replica of the fractured surface using the freeze-fracture, freeze-etch technique. Frozen samples can also be sectioned while in the frozen state and the frozen sections viewed. The final freezing method involves sublimation of ice from the frozen sample in order to render it dry. Only the freeze-fracture, freeze-etch and freeze-substitution methods have been applied to the study of cereal caryopses to my knowledge. Regardless of the technique, samples are quickly (snap) frozen using a variety of methods to preserve the cell ultrastructure in near life-like conditions without detrimental ice damage. Some of these methods include: quenching (immersion in a variety of freezing fluids such as propane or one of the freons), freezing against a cold metal block, jet freezers which utilize a high pressure jet of freezing fluid to freeze the sample, or high pressure freezing (Moor, 1987). Freeze-etching of ungerminated and germinated barley aleurone cells was successfully conducted (Buttrose, 1971), primarily due to low water concentrations in the dormant or near dormant tissue. Similar success was obtained with ungerminated rice coleoptile cells (Buttrose and Soeffky, 1973). Fretzdorff and coworkers (1982; 1983) found that freeze-fracture could be conducted on bread dough samples without ice damage even though water concentration was about 65% (Fig. 3). When similar studies were conducted on developing wheat caryopses, however, ice damage became a problem (Bechtel and Barnett, 1986a, b). Even samples that contained less than 50% moisture exhibited ice crystal damage (Fig. 4). Apparently the water is selectively segregated in the endosperm cells so cell functions can be maintained even while the overall moisture content of the caryopsis has dropped below 50%. This presented the difficult problem of trying to freeze a relatively large piece of endosperm tissue fast enough not to cause ice crystal damage. A variety of cryoprotectants have been used, but glycerol worked well with wheat grains (Bechtel and Barnett, 1986a). Freezing developing cereals without cryoprotectants has not produced very promising results for either freeze-fracture (Bechtel and Barnett, 1986a, b) or freeze-substitution (Unpublished results; Fig. 5). Whichever system is used, appropriate controls must be conducted to determine the effects of the cryoprotectant agent on cellular ultrastructure.

Dehydration

Following chemical fixation, samples are typically washed in water or buffer to remove unreacted OsO_4 and are dehydrated in a graded solvent system that is compatible with the

embedding medium. If the solvent is not compatible, then a transitional solvent must be used that is compatible with both the dehydration solvent and embedding medium. An example of this would be using propylene oxide as the transition between ethanol and Epon 812. Chemical methods of dehydration such as 2,2-dimethoxypropane can be useful in some situations. This chemical reacts with water to form acetone and methanol; once conversion of the water to organic solvents is complete, the mixture is replaced by acetone. The 2,2-dimethoxypropane has not worked well for cereal grains in my hands, I believe primarily due to poor penetration. The importance of excellent dehydration techniques can not be overstated for cereal grains. I have found that it is poor dehydration that causes poor infiltration and polymerization of the embedding resin. Maturing cereal grains require additional time to complete dehydration in comparison to many other tissues. It may take as long as a day to fully dehydrate nearly mature samples. The tell-tale signs of poor dehydration are samples that are sticky or gummy on the bottom and/or have little embedding medium present in the interior of the block of tissue. Dehydration fluids that are typically used are acetone and ethanol. Acetone may act as a radical scavenger and can interfere with polymerization of some acrylic resins. Similarly, use of 2,2-dimethoxypropane must be avoided when embedding in acrylic resins.

Embedding Samples

Numerous resins for electron microscopy are on the market. They need to be selected on the basis of type of information sought and type of procedures to be conducted, as well as their compatibility with the system. For cereals, a low viscosity type resin is needed for routine work, such as Spurr (1969) resin or Effapoxy (Ernest F. Fullam, Inc.) or an ultra-low viscosity formulation such as that used by Mascorro et al. (1976). Many of the epoxy resins such as Epon 812 (now replaced by resins such as Poly/Bed 812, LX-112, EMBed 812, Eponate 12, Epox 812) tend to be very viscous and do not penetrate well enough to obtain satisfactory sections. Even with the lower viscosity resins, long infiltration times from 2 to 6 wks may be necessary to obtain satisfactory sections (Parker, 1980). Several of the newer acrylic resins will probably be finding their way into cereal structure research. Once a freezing system free of ice artifacts has been worked out, the acrylic Lowicryl embedding kits such as K4M and HM20 will be very useful in low temperature studies. Similarly, the LR Gold and White resins should be tried. LR White has several advantages: it is low in viscosity, will accept samples from 70% ethanol, and can be heat cured. Aside from the advantages listed above for LR White, both LR White and Gold consist of a single resin system and both can be photopolymerized.

One aspect of embedding that has not received much attention in this country is the toxicity of the unpolymerized components as well as the polymerized resins. The toxicological properties of most of the resins and resin components have not been studied in any great detail (see Ellis, 1989). Great care should be used in their handling, especially when blocks are being cut with saws, files or grinders. Even polymerized blocks contain unpolymerized components. Fume hoods, gloves and dust masks must be used and the dust created picked up and disposed of properly. One foreign company that markets Spurr resin lists the resin components and the polymerized plastic as suspected carcinogenic compounds. Safety can not be over-emphasized when using these chemicals.

Sectioning

Sectioning hard materials is probably the most difficult and frustrating experience one can encounter. There are several factors why cereals are so difficult to thin section. One is the grain density and large amount of material present. Mature cereals at 10-14% moisture are much more dense than leaf or stem tissue at 70-90% moisture. A caryopsis also contains fewer air spaces into which the plastic has an opportunity to infiltrate. Cereals also tend to be very hard and some possess inorganic crystals in various portions of the grain. These factors contribute to cutting down the amount of plastic that can be present in the tissue that acts as a support for sectioning. Couple this lack of plastic support with difficult-to-section components such as starch and protein bodies and it is no wonder one has difficulty in obtaining thin sections. Fortunately, there are several partial solutions to this sectioning problem. Several have already been alluded to such as complete dehydration, long-term infiltration of plastic, and the use of low viscosity resins. One sectioning technique that works for rice endosperm tissue involves sectioning for long periods of time (30-60 min) at very slow speeds (0.2 mm/sec; Bechtel and Pomeranz, 1978b). It seemed to take a long time for the block, knife, and microtome to equilibrate with each other in order for sections to be cut. Other suggestions include choosing small samples, trimming them so that plastic completely surrounds the tissue, and using low water levels in the boat of the diamond knife. We have since found that sectioning quality can be improved if the sample block is re-embedded by first trimming, thick sectioning, and retrimming for thin sectioning. Then the block is placed in a Beem or gelatin capsule, fresh plastic poured around the block, the capsule placed in a vacuum desiccator, a vacuum drawn until bubbles stop emitting from the trimmed surface, and the capsule is then polymerized in the usual fashion. The reinfiltrated block is carefully retrimmed following polymerization to avoid cutting into the tissue. Thin sections are cut from the new reinfiltrated block face. Many quality sections can be obtained in this manner.

Specialized Techniques

Immunocytochemistry

Immunocytochemistry is a very powerful technique that can be applied to cereals for localizing proteins. Three methods are commonly used. The first is direct labelling in which the specific primary antibodies are complexed with colloidal gold. The labelled antibodies are then

immunospecifically bound to TEM thin sections. A second method, indirect labelling, involves reacting primary antibodies with the thin sections and then localizing the bound antibodies with a colloidal gold-Protein A complex. The third method is also an indirect method and uses secondary antibodies made against the primary antibodies and is complexed with colloidal gold.

There are a multitude of ways for conducting the technique and fine tuning the procedures can require considerable time. Almost every step of the specimen preparation can influence the results. The type of fixative used can block or alter the antigenic determinants so as not to be recognized by the antibody. Osmium tetroxide should be avoided because of its interference, but, if it is used, a treatment with 3% hydrogen peroxide (Bechtel and Gaines, 1982) for 30 min or 1% periodic acid for 10 min (Parker, 1982) may be helpful in removing the osmium. Embedding resins can similarly prevent the procedure from working properly. Spurr's resin has been shown to have a pronounced detrimental effect on the localization of antigens, whereas the acrylic resins appear to give much more labelling (Craig and Goodchild, 1982). Our work with wheat endosperm has revealed that immunocytochemistry can be highly varied and each antiserum should be tested separately (Figs. 6-8).

The procedure used in my laboratory for wheat endosperm sections is as follows: grids are first rinsed on a drop of distilled water for 10 min, 30 min on sodium metaperiodate, 5 min on distilled water, 15 min on 3% bovine serum albumin (BSA), 1.5 h on primary antibodies (dilution varied from 1:50 to 1:1000 depending upon antibody concentration and specificity), 2, 5-min rinses on buffer (0.5% BSA, 0.05% Tween 20, 0.5 M NaCl, 0.01 M sodium dibasic phosphate buffered to pH 7.2 with 0.01 M sodium monobasic phosphate), 1.5 h on gold-conjugated secondary antibodies, 2, 5-min rinses on buffer, 2, 5-min rinses on phosphate buffered saline (0.01 M phosphate buffer in 0.15 M NaCl at pH 7.2), and finally 2, 5-min rinses on water. Grids are dried with filter paper and the sections checked in the electron microscope unstained or stained with aqueous uranyl acetate and lead citrate. Controls should also be conducted: preimmune serum and buffer should be substituted for the primary antibodies. One of the most important factors regarding immunocytochemistry is the dilution of the primary antibodies, secondary antibodies, and Protein A solutions. Extremely dilute antisera yield much more specific labelling than concentrated ones. We have found that the primary antisera can be diluted as much as 1000x and that the gold-labelled secondary antibodies can be diluted 50-100x to yield high specific labelling. In our hands, Protein A yields a great deal of nonselective but specific binding of wheat storage proteins. As a result, we have used the secondary antibody indirect method exclusively. Protein A also seems to give much more background labelling and tends to label less when compared to use of a secondary antibody (Fig. 9 and 10).

Enzymatic Digestions

Enzymatic digestions can provide useful information regarding the composition of cellular components. Bulk digestions of rice caryopses with either pepsin or Pronase revealed that the core of the large spherical protein body in the starchy endosperm was resistant to digestion while the other two types of protein bodies were susceptible (Bechtel and Pomeranz, 1978b). Wheat was also shown to possess portions of protein bodies, the densly-stained inclusions, that were resistant to proteases when thin sections were digested (Parker, 1980; 1982; Bechtel et al., 1982b). More recent preliminary data suggests that the embedding resin has a substantial effect on the rate at which the substrate can be digested. We have found that the matrix of wheat protein bodies are digested much faster (1 hr rather than 24 hr) when embedded in LR White instead of Spurr resin (Figs. 11-15). More importantly, the dense inclusions that were resistant to proteases when embedded in Spurr resin were completely digested after only 4 hr if embedded in LR White (Figs. 13-15). Fixation can also have a detrimental effect on the digestibility of components (Bechtel and Gaines, 1982). As with any other technique, the limitations must be fully understood before conclusions can be drawn from the results.

Carbohydrate Localization

Carbohydrates can be localized utilizing the periodic acid-thiocarbohydrazide-silver protein technique, the cytochemical equivalent of the periodic acid-Schiff's reaction used for histochemistry. This cytochemical procedure is dependent upon the detection of adjacent hydroxyl or amino groups of sugar residues. These groups are first oxidized with periodic acid, detected by thiocarbohydrazide or thiosemicarbazide, and visualized for electron microscopy with silver proteinate. Osmium tetroxide should not be used in the fixation scheme if this procedure is to be used as it results in nonspecific silver deposition. Even fixatives such as glutaraldehyde and acrolein can induce free aldehyde groups and cause interference. Formaldehyde fixation seems to be the best choice, but lack of quality fixation usually forces the use of glutaraldehyde coupled to stringent controls. Free aldehydes induced by fixation can be blocked using compounds such as

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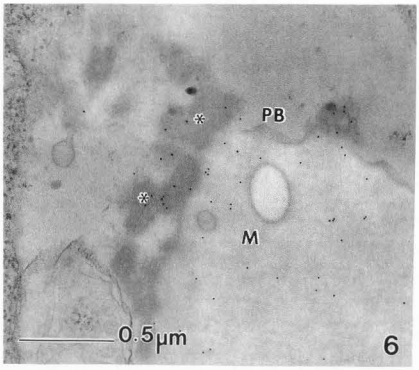
Figs. 6-10. Immunocytochemistry of developing wheat starchy endosperm. 6. Wheat endosperm fixed in glutaraldehyde and paraformaldehyde, post-fixed in OsO_4, embedded in Spurr resin, and labelled with anti-triticin/gold-goat anti-rabbit. Note limited amount of label on both the matrix protein (M) and inclusions (*) of protein body (PB). 7. Developing wheat endosperm fixed with paraformaldehyde only, embedded in LR White, and labelled with anti-triticin/gold-goat anti-rabbit. Note heavy label on both matrix and dense inclusions (*) of protein body (PB). 8. Developing wheat endosperm fixed in glutaraldehyde only, embedded in LR White, and labelled with anti-triticin/gold goat anti-rabbit. Labelling is heavy on both the matrix and inclusion (*) of protein body (PB). 9. Protein body (PB) with inclusion (*) of developing wheat labelled with anti-C hordein/gold protein A. Note background labelling (arrows). 10. Wheat protein bodies (PB) labelled with anti-high molecular weight subunit glutenin/gold goat anti-rabbit. Note large amount of label and lack of background.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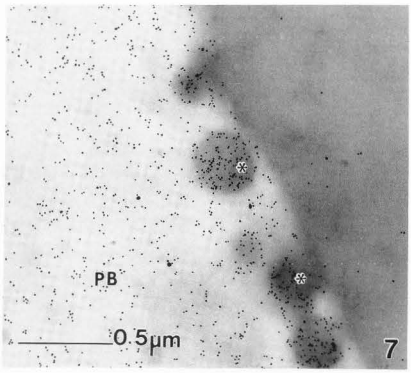
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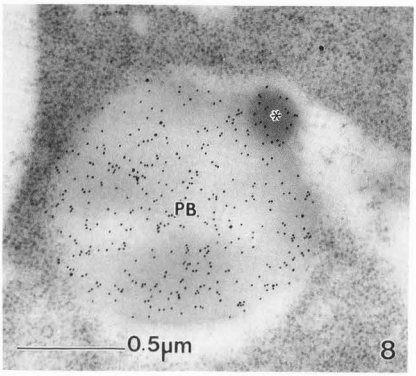
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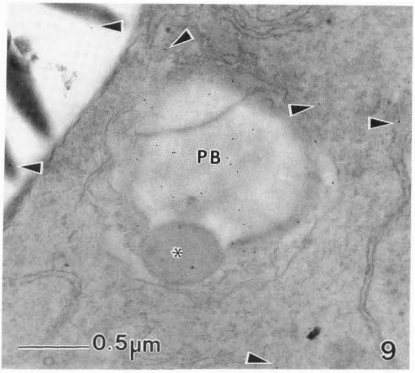
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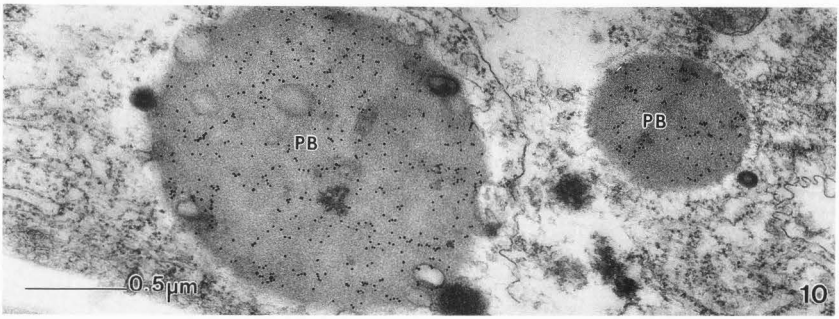
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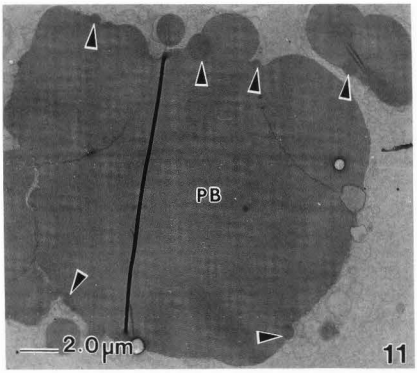
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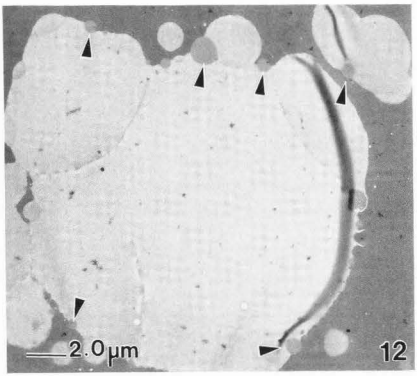
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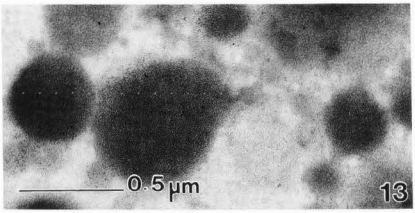
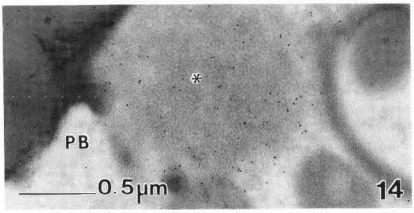

0.5 µm
13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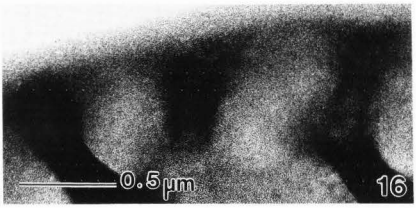
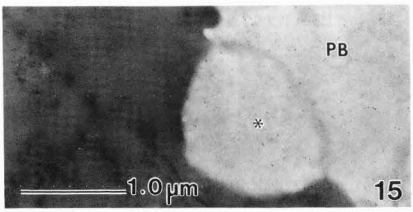
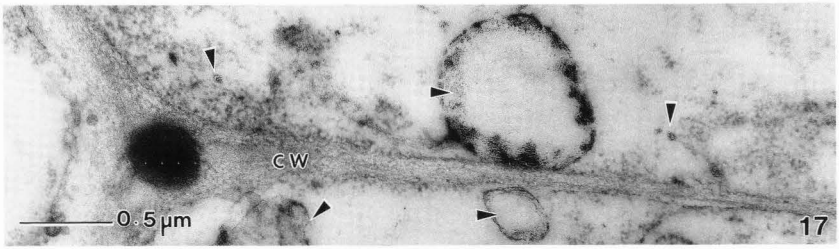
0.5 µm
14


0.5 µm
16


PB
1.0 µm
15


CW
0.5 µm
17

dimedone or sodium borohydride and interferrence of sulfhydryl groups can be eliminated by treatment with β-mercaptoethanol and iodoacetate. This method was used to investigate the presence of carbohydrates in protein bodies of mature oat endosperm (Bechtel and Pomeranz, 1981). Although some staining of protein bodies was initially obtained, control tests revealed the staining was due to the glutaraldehyde fixation. Starch is the most abundant component in cereal caryopses and is easily identified using the thiocarbohydrazide method (Fig. 16). The major source of non-starch polysaccharides in cereal endosperm is the cell walls. The periodic acid-thiocarbohydrazide-silver protein method can not only demonstrate the presence of polysaccharides in the cell walls, but also show how they are transported through the cytoplasm (Fig. 17).

Lectin Cytochemistry

Lectins are carbohydrate-binding proteins with binding sites that recognize specific sugar sequences. Several of these lectins have been used to label protein bodies and cell walls of wheat grain (Baldo and Lee, 1987). The nucellar epidermal cell wall and starchy endosperm protein bodies stained when treated with gold-labelled wheat germ and peanut lectins. Samples were fixed in 4% buffered paraformaldehyde and embedded in Lowicryl K4M resin. Sections were treated with test solutions for 40 min and washed with 500 mM sodium chloride in 0.5% Tween 20 and then washed in water. The use of the large number of lectins available should provide an interesting approach to the study of lectin binding in cereal caryopsis tissues.

Conclusions

The study of the microscopic structure of cereals and cereal products is fraught with numerous technical problems. Many of these difficulties lack specific solutions and one must experiment to obtain the best results. The area of food structure related to cereals and cereal based products is wide open for investigation. Techniques such as those described here can be adapted to investigate a very wide range of problems now facing the cereal industry.

Figs. 11-17. Cytochemistry of developing cereal endosperm. 11. Large protein body (PB) in developing wheat endosperm which was embedded in Spurr resin. Section was unstained. Note dense inclusions (arrows). 12. Unstained serial section adjacent to the one in Fig. 11 treated for 24 hr with pepsin. Note undigested inclusions (arrows). 13. LR White-embedded wheat endosperm digested for 30 min in pepsin leaving only the dense inclusions undigested. 14. LR White-embedded wheat sample digested for 30 min with Protease VI and labelled with anti triticin/gold goat anti rabbit. Matrix of protein body (PB) was completely digested, but label indicates that triticin still present in inclusion (*). 15. LR White embedded wheat endosperm digested for 4 hr with pepsin. Entire protein body (PB) including inclusion (*) is digested. 16. Portion of starch granule stained with the periodic acid thiocarbohydrazide-silver protein technique showing dense granular silver deposition. 17. Periodic acid-thiocarbohydrazide-silver protein staining of developing wheat endosperm showing localization of carbohydrate in cell wall (CW) and in various vesicles (arrows) associated with cell wall.

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Discussion with Reviewers

F.Felker: Since bread doughs contain live yeast, aren't they animate?

Author: Some dough systems are chemically leavened while others are yeast leavened. Bread doughs do contain live yeast cells, but these cells do not contribute substantially to the inanimate dough structure. Many of our studies were conducted on water flour doughs. We typically use the fixation quality of the yeast cells as an internal guide as to the quality of the overall fixation of the dough system when a complete formulation is used.

F. Felker: Don't the starch granules swell when sections are cut and floated out on the water filled trough?

Author: Starch granules most certainly swell during sectioning and in the process tend to cause a wetting of the block face that can result in sectioning difficulties. There are only partial solutions to this problem: re-embed the sample, low water levels in the trough, and have plastic surround the sample completely. The wetting of the starch also leads to artifacts such as the folding described by Gallant D, Guilbot A. (1971) Artefacts during the preparation of sections of starch granules. Studies under light and electron microscope. Starch 23:244-250. The use of nonwater-containing solutions in the knife boat, to my knowledge, have not been successful.