EFFECT OF HEATING ON THE FREEZE-ETCH ULTRASTRUCTURE OF HYDROXYPROPYL DISTARCH PHOSPHATE AND UNMODIFIED TAPIOCA STARCHES[1,2]

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ABSTRACT   Cereal Chem. 54(4): 783—793

The freeze-etching technique was used to study ultrastructural changes that occur during heating of hydroxypropyl distarch phosphate (modified) and unmodified tapioca starch granules in a Brabender Visco-amylograph®. Prior to heating, the fracture faces of both the unmodified and modified granules had similar particulate appearances. As heating progressed, water infiltrated into the granules from the truncated end, and a starch-water reticulum formed in the central regions and subsequently enlarged throughout the granules. Swelling took place after reticulate bands were formed in the peripheral regions of the granules. These outer bands remained until the integrity of the granules was lost near peak viscosity and the gel matrix was formed. Chemical modification of the granules stabilized the outer reticulate bands and reduced the size of the interstices within the matrix.

Many cooked foods rely upon starch for their structural and textural properties. Chemically modified starches are used extensively in products that must withstand modern processing and storage techniques (1).

The effect of modification upon granule structure has been studied. Srivastava and Patel (2) confirmed that cross-linking of tapioca starch granules inhibited viscosity development and at high levels prevented gelatinization. Earlier, Schoch and Maywald (3) developed a light microscopic method to identify the extent and type of modification. Electron microscopy has been used for the

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examination of acid-treated starch granules (4,5). However, the ultrastructure of gelatinized granules has been difficult to describe because of artifacts resulting from shrinkage of swollen granules during dehydration and the folding of granule thin-sections (5–7).

The scanning electron microscope has been used to study the extragranular starch network or exudate which develops during heating (8,9). Chabot *et al.* (10) examined the structure of various cooked and uncooked chemically modified starch granules. The scanning electron microscope provides a good three-dimensional image but requires specimens to be dehydrated or freeze-dried and coated with a conducting metal layer. These steps may produce artifacts or mask surface detail. In addition, resolution is limited to approximately 20 nm.

Freeze-etching has been used successfully to study isolated starch granules (11,12). The principle of this technique is to obtain a replica from a fractured surface of a frozen sample. The sample must be frozen quickly to cause vitrification and to avoid artifacts, and then fractured under high vacuum to avoid contamination of the fractured surface. The replica formed by evaporating platinum and carbon onto the fracture surface reveals the relief pattern of the fracture face.

In the work reported here, the freeze-etching technique was applied to study ultrastructural changes which take place in the granules during heating of starch/water slurries. Differences in the ultrastructure of an unmodified and a chemically modified tapioca starch were related to the viscosity measurements obtained by a Brabender Viscoamylograph®.

MATERIALS AND METHODS

An unmodified tapioca starch and a chemically modified tapioca starch (hydroxypropyl distarch phosphate, MS = 0.045; Stein, Hall Co., Inc., New York, N.Y.) were used.

Eight per cent (w/v) starch slurries in distilled-deionized water were heated at 1.5° C/min in a model VA-VE Brabender Viscoamylograph (C. W. Brabender Instruments Inc., Hackensack, N.J.) fitted with a 700-cm-g head. The temperature range was from 35° C to a maximum temperature of 95° C with the viscometer bowl rotating at 75 rpm. Samples for microscopy were taken at intervals (Fig. 1) from a point just below the center of the slurry in the viscometer bowl, to avoid variations caused by the presence of the viscometer pins or sedimentation. The bore size of the sampling tubes was increased from 2 to 4 mm as the viscosity of the slurry increased, to minimize mechanical damage to the starch granules. Droplets of the starch slurry were transferred onto gold sample cups and immediately frozen in liquid Freon '12,' then stored for a short time in liquid nitrogen.

Freeze-etching was carried out in a Balzers BA360M freeze-etching instrument (Balzers A.G., Balzers, Furstentum, Lichtenstein) according to the method of Moor and Mühlethaler (13). The specimens were fractured under high vacuum at a temperature of −102° C and etched for 10 sec. Etching involves the vacuum sublimation of water from the frozen fractured surface to reveal the fine surface details of the unfractured material. Replicas were formed by evaporating platinum-carbon at an angle of 45° onto the fractured and etched specimen surface, and they were reinforced with carbon.

The platinum-carbon replicas were cleaned with 70% sulfuric acid overnight, rinsed twice with distilled water, cleaned again for 3 hr with 5% sodium hypochlorite solution, and rinsed three times with distilled water. The replicas were mounted on Formvar-coated 75 × 300-mesh copper grids and viewed with a Philips EM201 electron microscope at 60 or 80 kV. Micrographs included in this paper illustrate typical granule structures at important transition points on the amylograph curve.

RESULTS

Unmodified Tapioca Granules

Tapioca starch granules were nearly spherical with a truncated end. Figures 2–4 are micrographs of samples of unmodified tapioca starch taken at 50°C. Figure 2 shows the smooth outer surface of one granule revealed by etching and the fracture face through the central region of a second granule. Water had infiltrated into the central region, changing the fracture face into a pattern of large depressions as a result of etching, and starch material had leached out of the
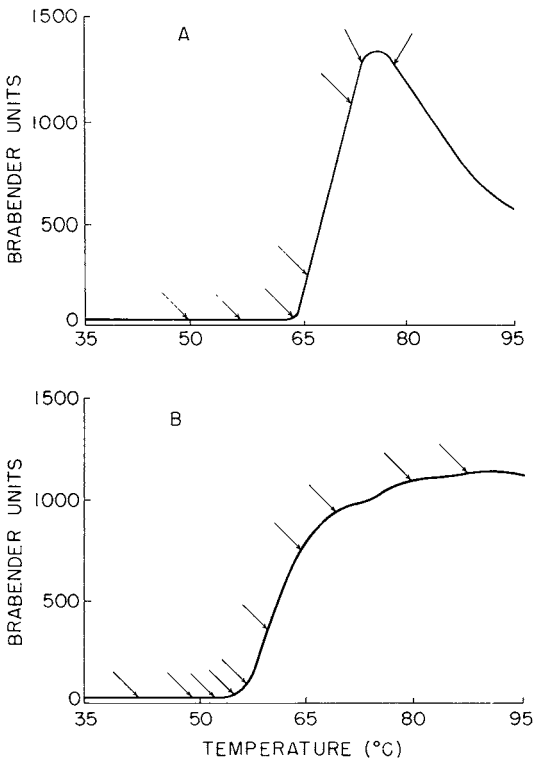

Fig. 1. Amylographs of 8% (w/v) starch in distilled-deionized water. The arrows indicate the sampling points. A) Unmodified tapioca starch; B) hydroxypropyl distarch phosphate.
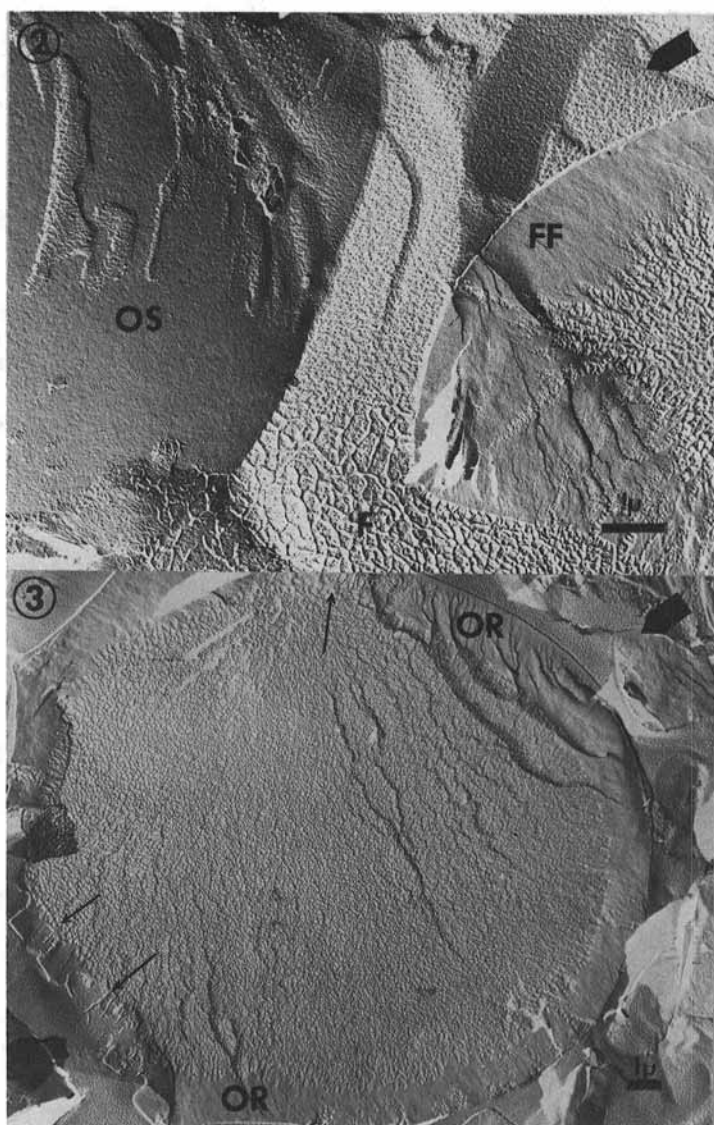
Fig. 2. Outer surface (OS) and fracture face (FF) of unmodified starch granules after heating to 50° C. Extragranular material (F) is evident between granules and is interacting with the flat side of the cross-fractured granule. The marker in the top right corner indicates the shadowing direction. 7,840×. Fig. 3. Fracture face of an unmodified starch granule after heating to 50° C. The outer regions (OR) of the granule are intact except in a few places (arrow). The marker in the top right corner indicates the shadowing direction. 3,920×.

granule, forming a reticulum along the truncated end. It appears that the truncated end of the granule was most susceptible to this heat treatment, as the outer spherical region remained relatively unaffected until the granule center was markedly altered (Fig. 3). The diameter of the granule was about 20 μm, suggesting that little swelling had taken place.

The undisrupted outer regions of the granules in the initial stages of infiltration (Fig. 4) had the same particulate fracture face as the unheated granules (14). A number of small ridges were present on the fracture face. The water had infiltrated into the central regions of the granules and formed pockets enclosed by starch. Etching removed some of the water from the fracture surfaces and revealed the smooth inner surfaces of the starch fibrils. Cross-fractured fibrils had the same particulate fracture pattern as did unheated starch granules.

As heating progressed, water continued to infiltrate into the granules. The water-filled pockets of the central reticula enlarged and the width of the unaffected outer regions decreased. Figures 5 and 6 are of samples taken at 72° C just before peak viscosity. Granule swelling occurred as the outer regions of the granules were converted into reticulate bands (Fig. 5). The outer diameter of the granule increased as the reticulate bands expanded and the characteristic uncooked tapioca granule shape was lost. The width of these bands was usually about 4 μm, with occasionally wider areas. The inner edges of the bands were connected to the remaining central reticula (Fig. 6). The interstices of the reticulate bands varied in size and shape. A prominent feature of the bands was the 'lobe-like' structures projecting from the outer edge (Fig. 6).

Near peak viscosity at 74° C, the outer reticula of the swollen granules and the extragranular starch material formed a final matrix structure (Fig. 7). A relatively homogeneous gel structure was found at peak viscosity with few definitive granules remaining. The final starch network (Fig. 7) had the same particulate fracture face and smooth inner surface as the starch in the partially infiltrated granules (Fig. 4). The size of the interstices was 0.63 to 2.11 μm.

Modified Tapioca Granules

Modified granules were similar in appearance to the unmodified granules. The general pattern of early infiltration by water into the granule centers and the leaching of starch from the truncated ends while the spherical region remained intact was also similar. However, several differences were noted.

Infiltration of some granules started at a lower temperature, but complete infiltration progressed at a slower rate. Figure 8 shows a modified granule with only a narrow strip of uninfiltrated structure preventing swelling. Small regions of solid starch may also be found in the interior. There was less extragranular material leached from the modified granules.

Granule swelling coincided with the formation of the outer reticulate bands. However, in contrast to the unmodified granules, the outer reticulate bands did not have a uniform width. The interstices in the final gel network at peak viscosity (Fig. 9) were smaller (0.12 to 0.36 μm) than those formed in the unmodified starch (Fig. 7).

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DISCUSSION

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The fracture face of the starch granules in this study had the same particulate appearance as has been observed by others for freeze-etched starch granules
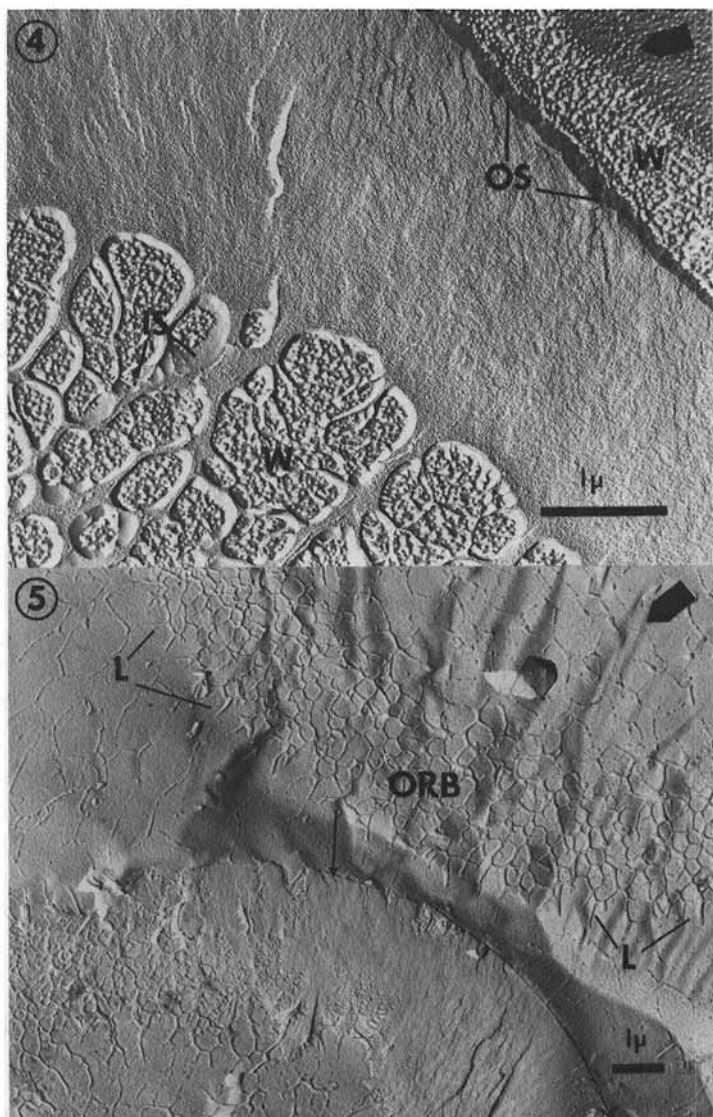
Fig. 4. Fracture face of an unmodified starch granule after heating to 50° C. Water (W) is present outside the granule and in the enclosures of the matrix within the granule. Etching has revealed the outer surface of the granule (OS) and the inner surface (IS) of the matrix enclosures. The marker in the top right corner indicates the shadowing direction. 16,000×.

Fig. 5. Fracture face of two unmodified starch granules after heating to 72° C. The lower granule shows the outer reticulate band starting from the uninfiltrated outer regions (arrow). The upper swollen granule shows a completed outer reticulate band (ORB) with fibrils connecting the inner regions of the granule. There are lobes (L) on the outer edge of the reticulate band which are connected to some extragranular starch fibrils between the edges of both granules. The marker in the top right corner indicates the shadowing direction. 6,520×.
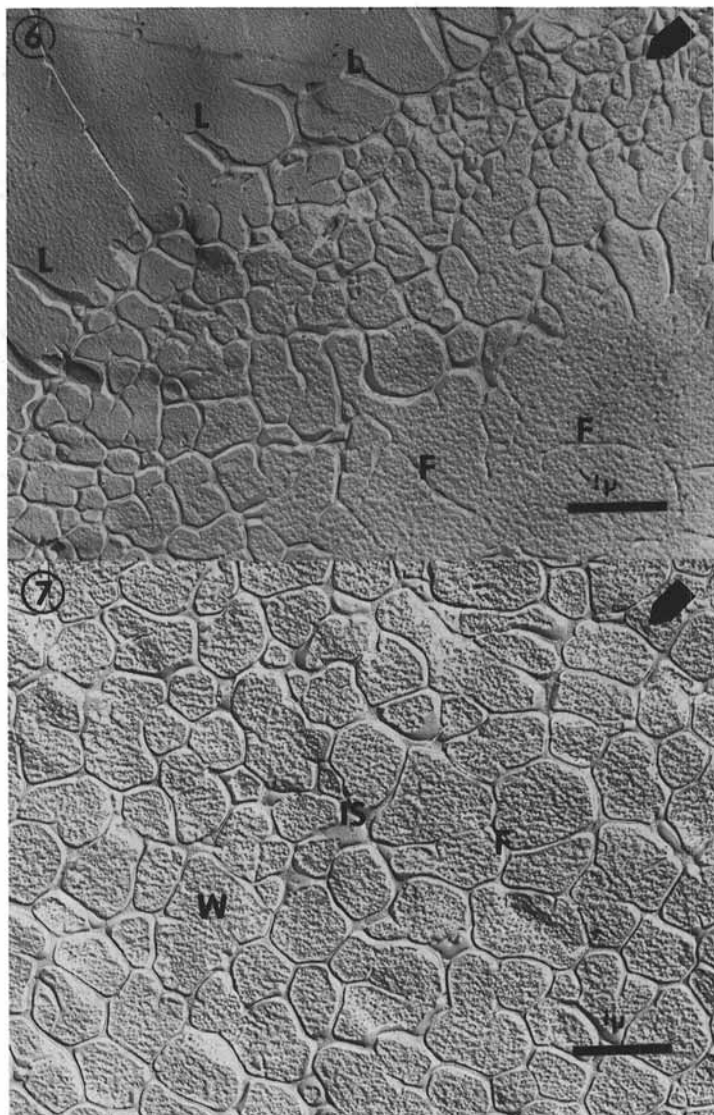
Fig. 6. A high magnification view of the outer reticulate band of the swollen unmodified starch granule shown in Fig. 5. The lobes (L) are on the outer edge of the swollen granule and there are thin fibrils (F) passing into the center of the granule. The marker in the top right corner indicates the shadowing direction. 12,800×. Fig. 7. Fracture face of the final matrix of an unmodified starch granule after heating to 74° C showing the fibril network (F) forming the water-filled enclosures (W). Etching has revealed the inner surface (IS) of the starch matrix. The marker in the top right corner indicates the shadowing direction. 12,320×.
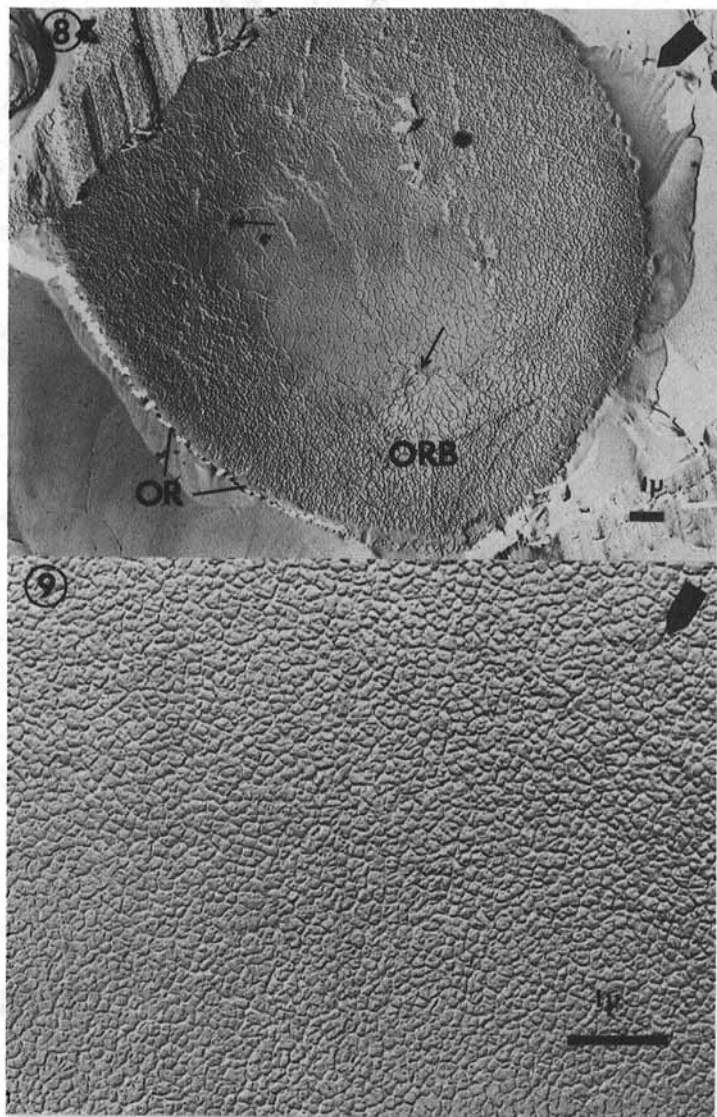
Fig. 8. Fracture face of a modified starch granule heated to 50° C. The outer region of the granule (OR) remains as a thin ring around the developing reticulate band (ORB). Some uninfiltrated granular material remains within the granule (arrow). The marker in the top right corner indicates the shadowing direction. 4,000×. Fig. 9. Fracture face of the final matrix structure of modified starch granules. The marker in the top right corner indicates the shadowing direction. 12,320×.

(11,15,16). However, the inner surface of the starch network was smooth, with no indication of particles or microfibrils. The outer surface of the granule was also smooth, with a faint fibrillar pattern, and appeared relatively resistant to the entry of water. Perhaps processing conditions had altered the granule properties, especially on the outer surface. Whistler et al. (17) reported that air drying of granules may induce a case-hardening effect. Others have observed the relative resistance of the outer regions of the granule to enzymatic attack (18, 19) and the greater sensitivity of the sharper edges.

In the early stages of water infiltration and solubilization of starch, there was little size change in the granules, indicating some degree of flexibility to the effect of heat and water. The freeze-etching technique may not reveal water entry until large interstitial channels have formed. Sufficient water must be present between the starch molecules to allow for some sublimation to occur. Water entered the center of the granule and migrated toward the outer curved surface, forming a network with the starch. This network structure closely resembles the diagrams of Meyer (20) and Schoch (21,22) for the swelling of starch granules, and could correspond to the three-dimensional network of amylose and amylopectin. However, the thickness of the strands of starch network correspond to macromolecular dimensions. Granule swelling occurred when the compact granule material in the outer region was disrupted, and a reticulate structure formed corresponding to the amylopectin envelopes or sacs observed with the light microscope (8,23). This could provide the necessary flexibility to maintain the integrity of the swollen granules.

Leach (24) has described the critical concentration as "that concentration of starch which forms a paste at 95°C in which the swollen granules occupy the entire volume with essentially no 'free' water between them." The concentration of starch used in the amylograph procedure was higher than the reported critical concentration for tapioca (24). As the granules imbibe water, some starch material is leached from the granule structure. In the initial stages of heating, this extragranular starch can remain fully hydrated and in solution. It is possible that, at some stage, there are insufficient water molecules for complete hydration of this solubilized starch. Some reassociation of the starch molecules would then take place, forming a starch network which can be visualized by the freeze-etching procedure. A similar phenomenon is observed in polymer solutions in which, above certain combinations of temperature and polymer concentrations, both dissolved phase and crystalline polymer phase exist in the solvent (25). More granules will be infiltrated by the water and become swollen as the amylograph temperature increases. At starch concentrations above the critical concentration, the swollen granules and extragranular starch will at some point imbibe all the free water in the system and a gel will form (20,24). Schoch (22) proposed the theory that viscosity developed as a result of the swollen granules restricting the flow of the suspension. However, this does not account for the extensive extragranular starch network which we observed developing during the increase in viscosity. Recently, Miller et al. (8) demonstrated the extent of this extragranular network at low starch concentrations and postulated that it was responsible for viscosity development in low starch concentrations. Neither of the latter two theories is sufficient to explain the situation in higher starch concentrations. As the viscosity increases, so also does the mechanical stress on the swollen granules in the viscometer system (24). This will cause a breakdown

of the swollen granules, and the final image of the gel at peak viscosity (Fig. 7) contains no identifiable granules. The development of viscosity that we have observed as a result of the interaction of the swollen granules and the extragranular reticulum closely follows that discussed by Leach (24).

In contrast, modified granules lost less starch material than unmodified granules during heating and, consequently, there was less extragranular reticulum. The modified granules also had wider outer reticulate bands. This may have been due to the phosphate cross-linking of the starch, which could have prevented the leaching of starch material and reinforced the outer network of the swollen granules. These findings confirm those observations made with the scanning electron microscope (10). The final gel structure of the modified starch (Fig. 9) did differ from that of the unmodified starch (Fig. 7). It was possible in some areas to identify the outline of the swollen modified granules, but most notable were the reduction in size of the enclosures and the width of the starch network surrounding them. Etherification of the starch chains with hydroxypropyl groups is known to prevent chain association (1); it is possible that this phenomenon has resulted in less chain reassociation and narrower starch fibrils in the final modified gel network.

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