A Pictorial Explanation for the Increase in Viscosity of a Heated Wheat Starch-Water Suspension[1]

B. S. MILLER, R. I. DERBY and H. B. TRIMBO, General Mills, Inc., Minneapolis, Minnesota 55427

ABSTRACT

Maximum viscosity of a wheat starch suspension heated in an excess of water occurs *after* most of the granule swelling ceases. The increase in viscosity is shown by light micrographs and scanning electron micrographs to be due mainly to the exudate (seen as a filamentous network after freeze-drying a fully heated starch suspension) released from the starch granules. The corollary is also true—when no network is seen, no viscosity develops. These same effects also appeared during limited testing of corn, waxy maize and potato starch.

The term "gelatinization" as used in this paper related to the total activity of the starch granule while a starch suspension is heated in excess water: swelling, loss of birefringence, release of exudate, and viscosity changes.

The increase in viscosity that occurs is generally ascribed to the granules imbibing more and more free water as they swell, thus increasing their chances of coming into contact with each other (1,2). Schoch (2) theorized that the increase in viscosity is a measure of the work required to move the granules past each other as they continue to expand.

Work conducted in this laboratory suggests that the exudate released from wheat starch granules may be a more appropriate cause for the large increase in viscosity of a wheat starch paste heated to high temperatures. The purpose of this paper is to present evidence relating to this concept.

MATERIALS AND METHODS

Aytex wheat starch prepared by General Mills, Inc. was the principal starch used in this study. It had an ash content of 0.21% (AACC Method 08-01), a protein content of 0.6% (AACC Method 46-11), and a starch damage of 7.0% (AACC Method 76-30) all expressed on a 14% moisture basis (3).

Gelatinization

The amylograph method was used. By removing aliquots of the starch suspension at 2-min. intervals during the heating cycle from 30° to 95°C., we could observe the visual course of starch gelatinization while recording the viscosity of the suspension in a standard amylograph bowl.

Three variations of the amylograph method used to observe the gelatinization reaction were as follows:

A. An amylograph method using a 10% starch suspension in water. This is similar to the AACC Method 22-10 (3) for determining the diastatic activity of flour using 100 g. of flour and 460 ml. of buffer.

B. An amylograph method similar to that of Crossland and Favor (4) using 5.5% starch and 0.8% CMC in 460 ml. of water.

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C. An amylograph method using 1% starch and similar to Method B above except without CMC. When samples were taken for scanning electron microscopy, 0.25% starch was used.

Micrography

Photomicrographs were made of the granule structure in each aliquot removed at intervals from the heated starch suspension. To ensure good representation, a large number of granules (approximately 400) from each aliquot was photographed on large format film.

An optical shadowing technique using oblique substage illumination and contrast process photographic emulsions made photomicrography of the heated starch granules possible during the final stage of heating in the amylograph, when they are normally difficult to observe. A cover glass was never laid directly on the granules, since the resulting physical pressure would alter the integrity of the granules, particularly since the granules had been heated above 70° C. Since the granule structure of the aliquots taken at higher temperatures did not change on cooling, all micrographs were made at room temperature.

Freeze-Drying

The sample preparation technique used for this work was to freeze the material directly in liquid nitrogen. One drop of a 1% starch suspension was placed on a No. 1 microscope cover glass, previously cleaned with chromic acid cleaning solution, and introduced quickly into liquid nitrogen. The slide was held in a vertical position for about 5 sec. until boiling activity ceased. The specimen was then placed on a massive steel plate, pre-cooled to the temperature of dry ice, and put into a vacuum chamber. A vacuum of about 50 μ of mercury was maintained until a thermometer placed on the steel plate registered room temperature. This indicated that the specimen was ready for light microscopy.

Electron Micrography

Specimens for scanning scope observation were freeze-dried on either 0.5-in. circles cut from No. 1 cover glass and mounted on Cambridge specimen stubs with silver paste, or directly on the stubs. No significant differences in the images were seen that could be ascribed to the use of these two base materials, even though the material on the stub took longer to freeze. The dried suspensions were coated with carbon and gold by the usual high vacuum evaporation procedures.

Bleaching

Starch was bleached with chlorine in a laboratory device similar to that described by Kissell and Marshall (5).

Treatment of Starch With α-Amylase

Barley malt flour was extracted with water (1:10 ratio) for 30 min. and centrifuged. A 3-ml. portion of the supernatant was added to 200 ml. of a 1% starch suspension that had been heated to 90° C. for 5 min. and cooled. The enzyme was allowed to act on the suspension while it was reheated at 0.75° C. per min. to 95° C.

RESULTS AND DISCUSSION

Two separate means (hot stage and viscosity measurements) have sometimes been employed together in observations concerning the extent of starch gelatinization as indicated by swelling, loss of birefringence,and increase of paste viscosity. In our experience, hot stage methods are deficient—even though the thermocouples are the size of one wheat starch granule (diameter of 20 μ)—because the temperature recorded by the thermocouple is only an approximation of the temperature of the total environment of the hot stage. This condition is the result of convection currents, limited sample volume, and evaporation at high temperatures. Thus, granule size determined with a hot stage cannot be related satisfactorily to sample viscosity determined with an amylograph.

The well-known viscosity curve obtained with amylograph Method B (curve 1, Fig. 1) was not observed when Method C was used, since an insufficient amount of material was present to register an increase in viscosity. However, microscopic observation of starch granule structures at corresponding sampling times throughout each of the three methods showed that they were identical. This being the case, the 1% starch suspension (Method C) was used to obtain samples for microscopic study, since the diluted materials yielded more satisfactory images than the more concentrated solutions of Methods A or B where the aliquots required dilution for good photography. Samples taken at high temperatures
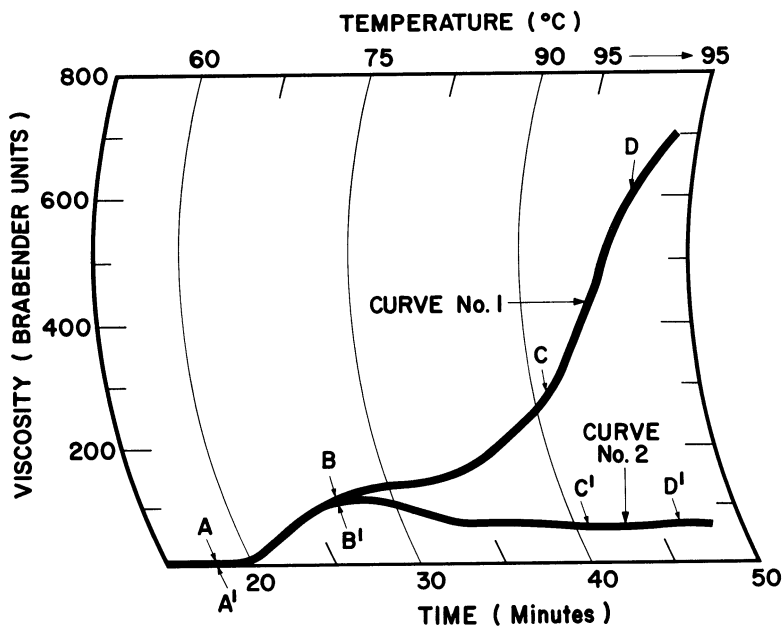

Fig. 1. Amylograms for wheat starch. Curve 1: Method similar to that of Crossland and Favor (4) using 5.5% starch and 0.8% CMC in 460 ml. of water. Curve 2: Same as for Curve 1 except 0.1% sodium stearyl fumarate (based on the starch) was added.

using Methods A and B do not dilute satisfactorily, since the granules tend to agglomerate. Thus, the methods employed in this work were to use a 5.5% starch suspension with 0.8% CMC (Method B) to obtain a viscosity curve revealing initial swelling of the granules, and a 1% starch suspension without CMC (Method C) to obtain micrographs.

By visually monitoring the starch suspension during heating in the amylograph using both polarized and plain oblique illumination, the entire visual course of the gelatinization could be observed. Monitoring included initial swelling and loss of birefringence, swelling, folding and the final structure of starch at the highest obtainable instrument temperature. This permitted the visual granule structure, as it developed during gelatinization, to be correlated with the instrument viscosity at any desired temperature. A brief summary of what occurs is as follows:

Using Method A one observes microscopically a very slight swelling and concomitant loss of birefringence of the granules before any instrument viscosity is recorded. The loss of birefringence occurs over a range of about 5° C. from 55° to 60° C. The percentage of birefringent granules is reduced from 90 to 1% during this period.

During the temperature range of 70° to 80° C. slight granule swelling causes the plateau of viscosity to appear when Method B is used (point B, curve 1, Fig. 1). At the beginning of the final steep rise in viscosity (point C, curve 1, Fig. 1), the granules first continue swelling and then start to fold. The folding becomes more pronounced as the viscosity rises. Final folding occurs as peak viscosity is developed.

In studying the course of gelatinization by microscopy (using amylograph Methods B and C) some observations attracted our attention because they were different than were expected from the literature. The visual starch granules did not disintegrate or become unrecognizable even at the highest amylograph temperatures. After initial granule swelling, folding followed during which the visual granules became relatively small with increasing temperature near the end of the heating cycle, even though the instrument viscosity was increasing rapidly. Obviously something occurred, but not at the expense of a recognizable granule. Although it is a common belief that the sharp increase in the viscosity of a cooked starch paste occurs "only when the granules are sufficiently swollen to crowd one another" (1), the visual observations and changes in viscosity recorded for this study suggested that this concept was not valid for wheat starch.

A representation of the granule structures generated in the amylograph is shown in Fig. 2. The relationships between the size of the granules as shown in Fig. 2 and the viscosity of the starch suspensions at points C and D on curve 1, Fig. 1, strongly suggest that granule swelling cannot account for the sharp rise in amylograph viscosity during gelatinization.

Further evidence that granule swelling alone cannot account for the observed changes in starch viscosity during gelatinization was obtained when sodium stearyl fumarate[2] (0.1% based on the starch) was added to the starch suspension before heating in the amylograph (curve 2, Fig. 1). The viscosity plateau at 70° C. developed as in a normal curve and the starch granules swelled normally up to

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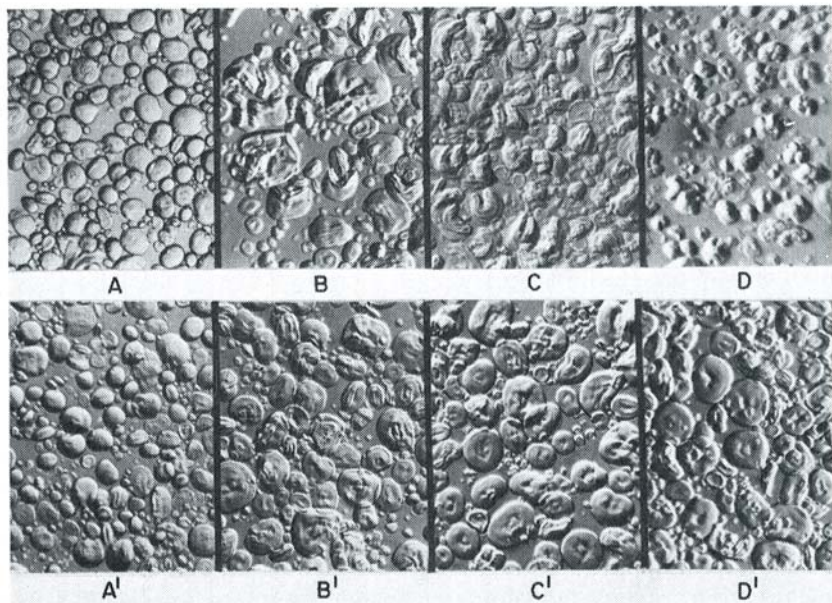[2]Chas. Pfizer & Co., New York, N.Y. 10017.

Fig. 2. Granule structure (light micrographs, 250X) of untreated wheat starch taken from the amylograph at temperatures indicated by the points A-D, Fig. 1. Granule structure (light micrographs, 250X) of sodium stearyl fumarate-treated wheat starch taken from the amylograph at temperatures indicated by the points A'-D', Fig. 1.

this point (70° C.). However, in this case, both viscosity and swelling reached a maximum at the plateau (point B') as shown in Fig. 1 and 2. At 95° C., the granule size (point D', Fig. 2) was larger than that of normal starch (point D, Fig. 2), but the viscosity was much less for the starch suspension containing the sodium stearyl fumarate (compare points D and D', Fig. 1). This is also indicates that a correlation between starch paste viscosity and granule size is virtually non-existent for wheat starch. Another polar surfactant, sodium stearoyl-2-lactylate[3], gave similar results with somewhat more network showing.

Since granule swelling does not account for the final rapid rise in viscosity, there must be another explanation. It is well known that an exudate is released as untreated starch is heated in sufficient water so that gelatinization is a function of temperature only. What is not so well known is that this material is released in relatively large amounts as the temperature approaches 90° C. (6) and in the present work forms, upon freeze-drying, a continuous and complex filamentous network throughout the suspension. This structure has a high positive correlation with paste viscosity, and we postulate that the exudate is the principal reason for the large increase in viscosity at high temperatures.

The exudate in samples heated at the higher temperatures in the amylograph is

difficult to observe and even more difficult to record pictorially without special techniques. As taken directly from the heated starch suspension, the exudate appears to be a mass of connected structure associated with the starch granules, but the fine structure cannot be resolved. Diluted iodine stain (0.8 g. of iodine and no excess potassium iodide/liter) will precipitate and color the exudate blue, revealing a network structure, while the starch granules stain red or magenta. However, the exudate plus true solubles can be best prepared for examination in detail by freeze-drying the suspension followed by careful optical shadowing. Admittedly, the filamentous network formed during the relatively mild process of freeze-drying may be an artifact due to retrogradation of the exudate.

The freeze-dried network from wheat starch, produced when the viscosity of the starch suspension is high, is shown by a light micrograph at 100× (photo D, Fig. 3) and by a scanning electron micrograph at 1,000× magnification (Fig. 4). The network in Fig. 4 represents only a small part (approximately one thousandth) of that found in one drop of the starch suspension taken at a point comparable to point D (curve 1, Fig. 1) using 0.25% starch. Freeze-drying and microscopic examination also proved useful for studying a starch suspension treated with sodium stearyl fumarate prior to heating. At point B′ (Fig. 1) the granules swell normally and the very slight but normal amount of exudate expected even from untreated starch is released. However, on further heating a unique situation develops in that no more exudate is released so that there is no network in a freeze-dried sample of the final heated suspension. The related micrograph is photo D′ in Fig. 3.

When a network (as seen after freeze-drying) and viscosity do not develop because of pretreatment of the starch suspension with a chemical agent such as
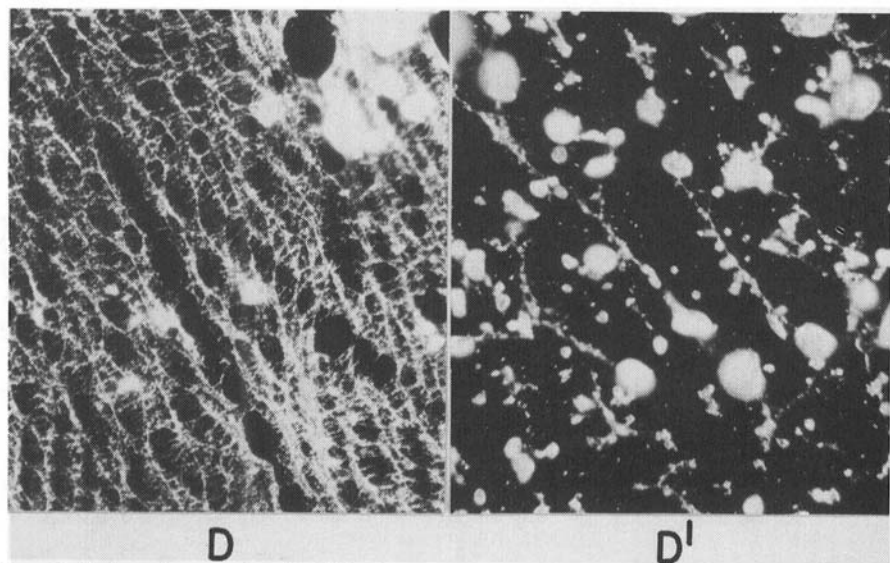

D   D′

Fig. 3. Light micrographs (200X) of the exudate from wheat starch taken from the amylograph at point D compared with that for wheat starch treated with sodium stearyl fumarate and taken at point D′, Fig. 1.
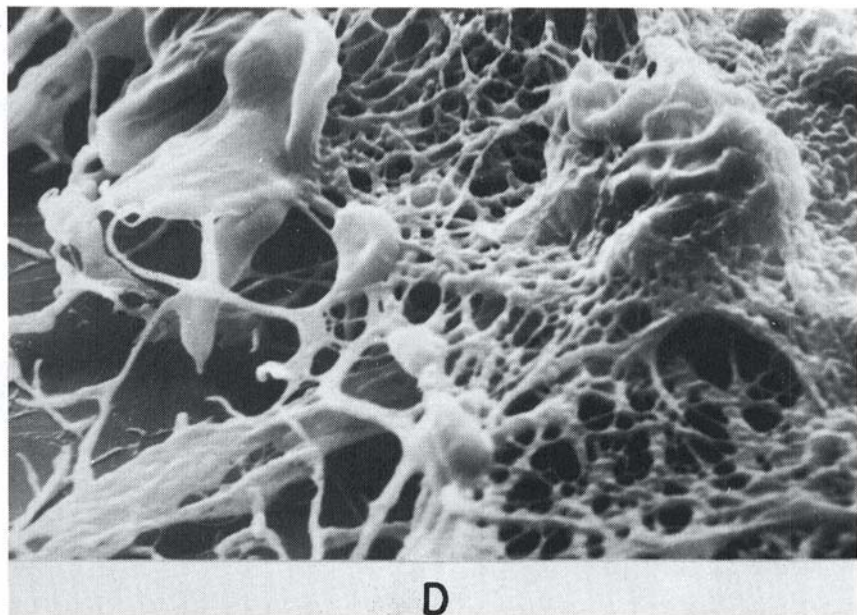
Fig. 4. Scanning electron micrograph (1,000X) of the exudate from wheat starch taken from the amylograph at a temperature equal to that at point D, Fig. 1.

sodium stearyl fumarate, it must be assumed that the fumarate enters the granule after the first slight swelling and acts within the granule structure to prevent release of the exudate. In subsequent freeze-drying, no substantial residue was seen, aside from the starch granules themselves, in the aliquots taken during the heating cycle. This indicates that there was little or no exudate.

Further evidence concerning the role of the exudate in determining starch viscosity is provided by the fact that a heated water suspension of wheat starch bleached with chlorine (pH 2.5-2.9) exhibits no network after freeze-drying and a viscosity similar to that shown by curve 2, Fig. 1. The granules swell in a fashion similar to the starch shown in photos A'-D', Fig. 2.

Starch granules reacted in solution with α-amylase during heating in an amylograph also exhibit no network after freeze-drying and their viscosity is similar to that shown by curve 2, Fig. 1. A picture of granules taken at a temperature comparable to point D' in Fig. 1 is shown in Fig. 5.

Still further evidence that the exudate from heated starch granules contributes to viscosity was provided by heating a 1% wheat starch suspension in an amylograph and centrifuging the cooled mass. Microscopic examination of a freeze-dried sample revealed the network, but no granules, in the supernatant. However, the viscosity of the suspension was 25% greater than that of distilled water even though most of the exudate centrifuged out together with the starch granules and thus did not contribute to the viscosity of the supernatant. It was not possible to obtain a suspension of granules without the exudate network being present as revealed by freeze-drying. Therefore, the contribution of the granules themselves to viscosity is not known.
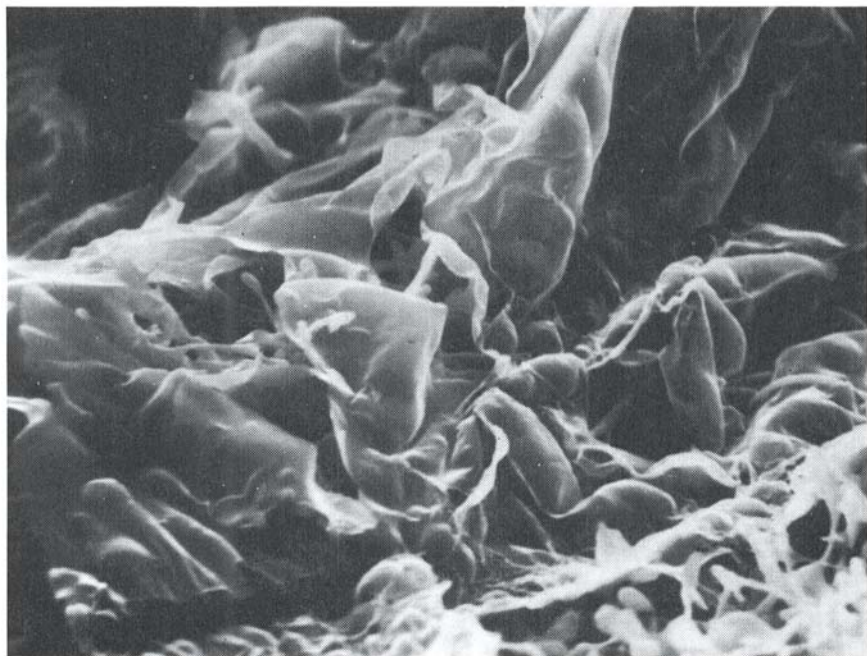
Fig. 5. Scanning electron micrograph (1,000X) of starch digested with malt α-amylase during heating in the amylograph. Sample taken at point D', Fig. 1.
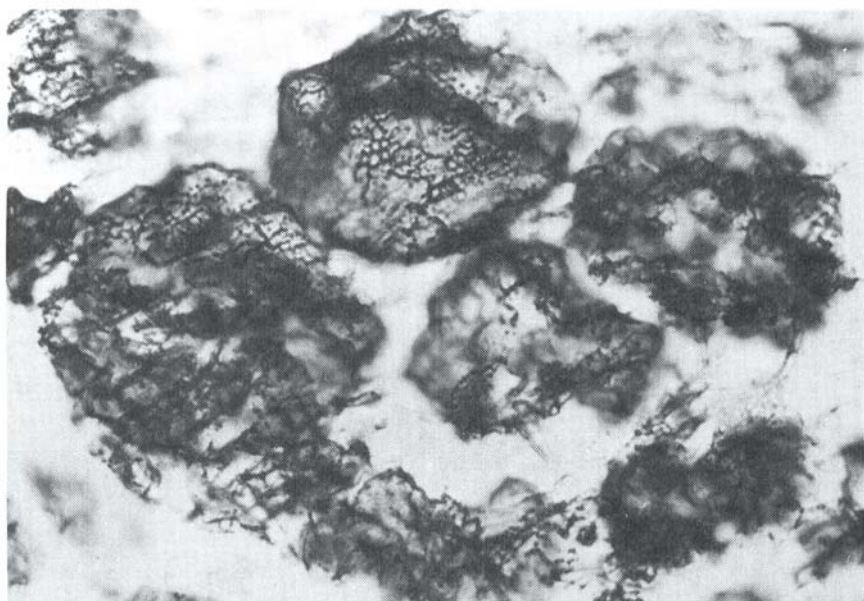

Fig. 6. Light micrograph (300X) of the exudate from waxy maize starch taken from the amylograph at a temperature equal to that at point D, Fig. 1.
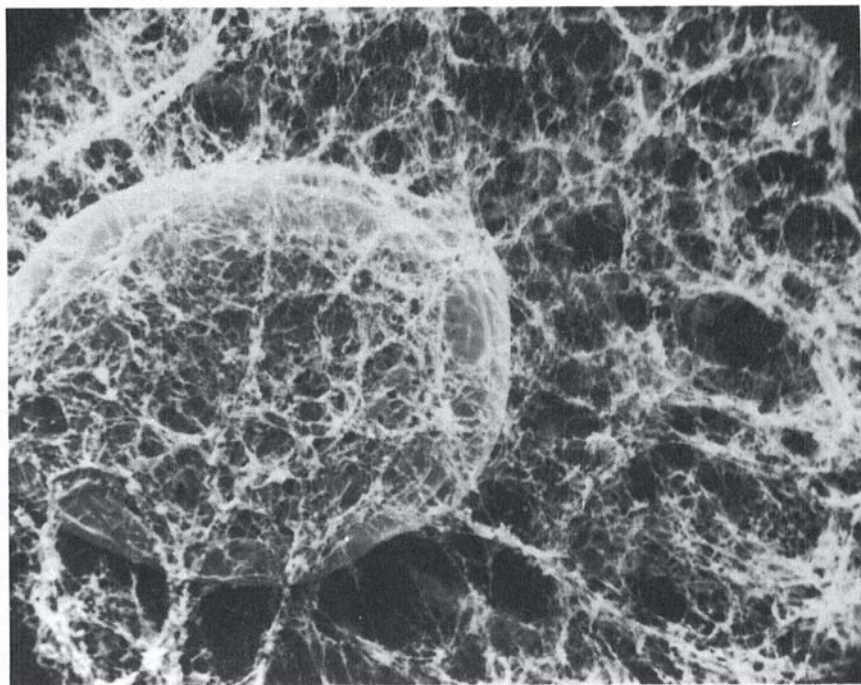
Fig. 7. Scanning electron micrograph (1,000X) of the exudate from potato starch taken from the amylograph at a temperature equal to that at point D, Fig. 1.

Some of the properties of the exudate network have been studied in the scanning electron microscope. The main visual form is a very dense interlaced and interconnected mass of fibers ranging from 0.05 to 2 μ in diameter and with great length in relation to diameter. Thus, the exudate (shown in a freeze-dried sample with a three-dimensional structure) probably accounts for much more water absorption than the visual granules themselves.

Not all of the exudate originates from within the granule. This is especially true of waxy maize (Fig. 6) and potato starch (Fig. 7) granules where it can be seen that the exudate originates from all parts of the granule which becomes itself a very open, filamentous structure after freeze-drying. However, the visual integrity of most of the granules, as seen by both the electron and the light microscopes, remains identifiable even when the starch suspension is heated to 95° C. Figure 8 shows the voids developing in wheat starch as the exudate leaves the starch granule. This is evidence that many of the granules have a structure that could be considered approximately 50% network.

During many observations of the common industrial starches (wheat, potato, corn, and waxy maize) heated in an amylograph in order to relate granule structure to viscosity, the viscosity always increased sharply *after* most of the granule swelling ceased. This relationship was especially noticeable with wheat starch. It was not observable, however, when the starch was heated on a
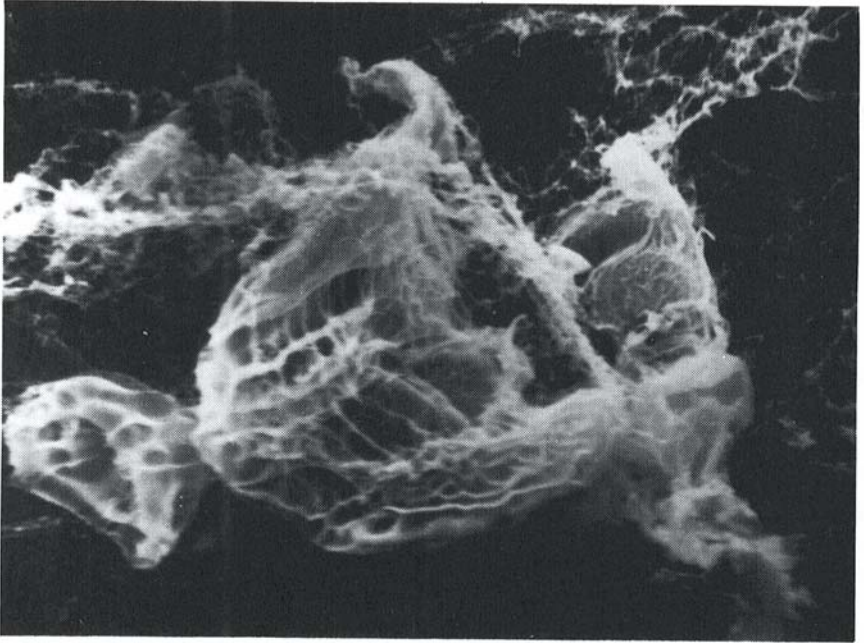
Fig. 8. Scanning electron micrograph (1,000X) of the void in a wheat starch granule taken from the amylograph at a temperature equal to that at point D, Fig. 1.

microscope stage where some might assume that the viscosity increase ceases when the granules become fully expanded.

When a fully heated starch suspension exhibits viscosity, an exudate network is seen after freeze-drying an aliquot of the heated starch suspension. The corollary is also true. When a fully heated starch suspension exhibits no viscosity, no exudate network is seen after freeze-drying an aliquot of the heated starch suspension. Thus, we conclude that the increase in viscosity of a wheat starch suspension heated in an excess of water is due mainly to the release of exudate from the granules rather than to the swelling of the granules.

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