Submicroscopic Structure of the Cereal Starch Grain

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SUMMARY

The submicroscopic structure of the starch grains of Zea mays and Triticum sativum was studied electron-optically from replicas made of internal, fracture surfaces. In corn starch, long cylindrical microfibrils were found, arranged in a radial direction and imbedded in an amorphous matrix. Their diameter was uniformly about 200 Å. Microfibrils were also indicated in wheat starch because of the prominence of their ends in surface view. Many microfibrils in corn starch appeared to be helically coiled. The more ordered starch substance was presumed to be localized principally in the microfibrils.

INTRODUCTION

Recent efforts to detect a submicroscopic, fine structure in the native grain of starch via electron microscopy have been notably unsuccessful. In 1952 Frey-Wyssling claimed to see visible strands in the residual sacs of gelatinized potato starch. These were said to have a diameter of about 100 Å. However, the structure was neither so uniform nor so distinct that it could be unequivocally accepted as representing any particular feature of the native starch grain. Likewise, Mühlethaler (1955), using ultra-thin sections of starch grains, was unable to detect a definite organization beyond concentric lamellation. Accordingly, Frey-Wyssling (1952, 1953) characterized the fine structure of the starch grain as amicroscopic.

Whistler et al. (1955) have also studied ultra-thin sections of starch grains and similarly have had little success in demonstrating a definite structure. The fine granulation seen by Whistler and Turner (1955) and interpreted as lamellar fine structure is not convincingly different from the ‘amicroscopic’ structure of Frey-Wyssling. Likewise, their ‘thin bead-like strands’ are difficultly distinguishable as micelles. After heating and lyophilizing suspensions of starch, Guilbot and Levavasseur (1954) have found bizarre structures with little evidence of a fibrillate or other fine texture.

On the assumption that the starch grain has not been properly treated to
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reveal its fine structure, the present writers undertook another approach to sample manipulation and preparation for electron microscopy. The results of this investigation constitute the subject of the present paper.

EXPERIMENTAL

An analysis of the procedures of the earlier studies indicated that, if there were a fine structure of electron-microscopic dimensions in the starch grain, this fine structure either had been destroyed by heating or was so constituted that differences in electron density were not to be found in adjacent structural elements. It was decided, therefore, to use a replica method to obtain the configuration of internal surfaces of the starch grain. Also, it was proposed to rupture that grain so that internal fracture surfaces should be accessible to the replica material.

Because of the recognized softness and plasticity of the substance of starch grains (cf. Lepeschkin, 1921; Sjostrom, 1936; Badenhuizen, 1937; Alsberg, 1938; &c.), because of the solubility of smaller starch molecules in water, and because of the possibility of residual amylase activity in the sample, the use of liquid water was avoided during sample rupturing. One method of preparation, used for corn starch alone, was to freeze the grains in water at $-20^\circ$ C. The block of ice, with the embedded grains, was then hammered thoroughly, while still frozen, in a cup-shaped metal dish in a room for freezing storage (cf. Scott et al., 1956). The fragments of ice were then placed in a surplus of 96 per cent. ethanol held at $-5^\circ$ C. and so stored until used.

Another method of preparation involved the preliminary dehydration of the starch grains in successive solutions of absolute ethanol and acetone. The grains were then placed in xylene (through several changes) and crushed in that medium. The manner of fracturing was varied: pressing with a flattened rod of stainless steel, rubbing the grains with a glass cover slip with a continuous rotary motion, or rolling an agate pestle back and forth over the grains.

Whatever the method of fragmentation, the grains were next placed on a glass slide and the alcohol or xylene permitted to evaporate. Replicas were made according to the platinum shadow plus carbon layer method described by Dalitz (1953) and by Roelofsen et al. (1953). Following the removal of the carbon layer with its attached starch grain fragments from the slide, the starch substance was dissolved with 30–40 per cent. chromic acid. After being washed in distilled water, the carbon films with the shadow material were mounted on specimen grids. (As indicated by Roelofsen et al., 1953, a positive replica is so produced.) The preparations were viewed with Philips EM 75 or EM 100 electron microscopes.

If frozen, hammered starch material is used, it is advisable to shadow the preparation immediately after alcohol evaporation or to keep it quite dry—otherwise the fragments gradually become liquefied. (This phenomenon also occurs if the starch grain has been ruptured in water.) The liquefaction is probably explicable in terms of the adsorption of atmospheric water by the
hygroscopic fragments and the subsequent activity of amylases in the newly formed layer of moisture. This does not occur when the fragments have been produced in xylene. Perhaps there are traces of lipophilic impurities in the xylene which form a thin hydrophobic coating over the granule surfaces.

The samples used in the present study came from specimens of the starch of *Zea mays* and *Triticum sativum* in the museum of the Laboratory for General and Technical Biology of the Technische Hogeschool of Delft. Careful microscopic examination revealed no noticeable evidence of cell-wall fragments or other contaminants in the starch. In addition, the frequency of the EM figures, as shown here, was such that it is improbable that they represent contaminants.

**RESULTS**

The characteristic appearance of the starch material which is visible after hammering is represented in Figs. 1 and 2. Occurring in elongated aggregations of various sizes, and sometimes singly, are small rodlets, which lie parallel to each other within an aggregation. The rodlets have a uniform diameter of approximately 200 Å but vary in length from 200 to 3,000 Å. (Their general appearance closely resembles that of particles produced in the hydrolysis of cellulose, Rânby, 1952.) The aggregations may occur isolated or they may be associated with amorphous masses, as shown in Figs. 1 and 2. The long axis of the rodlets is parallel to the long axis of the aggregation.

It does not seem reasonable to relate these rodlets to amylose crystals which are precipitated from solution by alcohol. In the first place, their diameter is quite uniform. Secondly, it will be recalled that there has been no opportunity for such a solution to be formed: until the addition of the excess 96 per cent. ethanol, the intact starch grain was frozen in a block of ice or existed as frozen fragments in small slivers of ice during and after the hammering. It is possible that during the evaporation of the alcohol there occurred an aggregation of the short rodlets which were already present as fragments in the ruptured starch mass. However, although it might be expected that the aggregation within a grouplet could occur in a uniform direction, a grouplet would not be expected to tend to have a constant girth along its length.

Another problem with the interpretation of these frozen, hammered fragments is that it is difficult to indicate a definite direction for the rodlets in the intact granule or even to know if one exists. Because of the polarization-optical findings of many workers (e.g. Frey-Wyssling, 1940a, b; Speich, 1942, &c.), it seems reasonable to assume that the major axes of the rodlets originally lay in a radial orientation.

The presence of the larger groups of rodlets with a preferred orientation (Fig. 2) suggests that these may have been split directly from the starch grain. The presence of longer rodlets also suggests that the short rodlets are fragments of longer ones (a viewpoint which gains support from the aspects of starch grains ruptured in xylene and from the uniform diameter and non-uniform length of the rodlets). Some of these units have an aspect very
suggestive of a helically wound, rope-like structure (see particularly the two shaded regions of Fig. 1, one region being indicated by an arrow in that figure). This aspect is also characteristic of the fracture surface of grains which have been crushed in xylene.

The method of crushing the starch grain in xylene yielded few direct views of an internal surface. Despite its being dehydrated, the starch substance was still sufficiently plastic so that the grain was often greatly deformed before being ruptured. Nevertheless, some grains (particularly those of corn starch) did present their fracture surfaces to the carbon film. The structure of such a surface is best seen in Figs. 3 and 4. These indicate that the starch substance is organized into 'microfibrils' of about 200 Å diameter and apparently indefinite length. Hence, the rodlets described above very likely represent short segments of these microfibrils.

As shown by Noda and Wyckoff (1951) for collagen reprecipitated at a low pH, many starch microfibrils appear to be helically wound. Occasionally they may combine to form a larger rope-like strand, composed of 2 (principally) to 5, or occasionally more, microfibrils. The relationship between the straight rod-like units of Figs. 1 and 2 and Fig. 4, top right, and the helical structures cannot be decided here. It appears that the convoluted forms running from the lower left to upper right in Fig. 4 are caused by shear. Although very likely not determinative for such a large body as the microfibril here, it is suggestive that various workers (Bear, 1942; Caesar and Cushing, 1941; Kreger, 1951; and others) view the starch molecule as being coiled in the crystalline micelle. Similarly, Staudinger and Eilers (1936) among others have proposed a helical form for the starch molecule in solution. To what extent the methods of preparation are responsible for differences in the size and form of the microfibrils can only be conjectured.

In the crushed grain, the microfibrils are quite close together—some being in contact while others may be as much as 50 Å apart. It is to be noted that there appears to be an amorphous ground substance in which the microfibrils are imbedded. Pit-like depressions in the centre of Fig. 4 attest to the prior presence of microfibrils on the surface of these depressions—probably pulled away in the fracturing of the starch grain. Also in Fig. 3 such amorphous regions may be seen. It is of interest to recall the blocklet-cementing substance of Hanson and Katz (1934), but more pertinent still is the idea of Lepeschkin (1921), that the soft crystallites of the starch grain are glued together by a mass of amorphous polysaccharide. (See also Katz, 1933, for a similar concept.)

The general direction of the microfibrillar strands seems to be radial (Figs. 3, 4). Thus far it has not been possible to detect differences in microfibrillar arrangement corresponding to concentric laminations in the starch grain.

Although Whistler et al. (1955) have stated that the sectioned starch grain has a smooth surface, it is quite possible that their grains were still covered by the very thin plastid. No statement is made in analysis of the surface replicas obtained by them. Probable surface views of the starch grain exterior are here shown in Figs. 5 and 6. Fig. 6 indicates how readily the starch substance flows...
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upon shearing. Fig. 5 shows, by virtue of the presence of a more or less papillary texture in the surface of the grain, that the surface tends to be organized into units of about 200 Å in diameter, which corresponds to the size of the microfibrils seen internally and to the diameter of the rodlets noted above. Possibly the very warty protuberances of Fig. 6 and of the central grain of Fig. 5 represent the ends of microfibril groups which come into prominence with relatively small stresses—by virtue of flow of the amorphous material between. These figures indicate again that the microfibrils of both wheat and corn starch are radially arranged.

**DISCUSSION**

The term, microfibril, was first advanced by Frey-Wyssling in 1937 for the as yet invisible units in which cellulose micelles of the cell wall of fibres were considered to be organized. Although the estimated diameter of the microfibril (which was indicated diagrammatically) was somewhat larger than that subsequently found, the validity of the speculation has since been abundantly verified in many electron-optical studies of the cell wall. The basis for the concept was the finding that metallic salts could be reduced within the cell wall, with the subsequent deposition of metallic crystals of a certain size range. Hence micro-capillary spaces were indicated in the body of wall substance. On the basis of other considerations, that body was conceived to be composed of elongated, rod-like units. Frey-Wyssling (1948) has also suggested that radial, submicroscopic capillary spaces exist in the starch grain.

Such a concept for starch was criticized, however, by Badenhuizen (1939), who stated, 'It has not been proved that the starch substance includes a similar system of intermicellar spaces, as found in cellulose. . . . The regular deposit of small metal crystals after reduction of the salts is an artifact, telling us nothing about a possible existence of submicroscopical spaces.' Although Meyer (1895) also believed that the starch grain had a pore system, some of his findings fit in better with the picture of the grain given here. Thus, after a 3-month treatment of potato starch with malt, fine points appeared on the surface of the grains. After a 5-year treatment with dilute sulfuric acid, although much substance was lost, the polarization figure of potato starch was brighter than that of the normal, untreated starch. Both findings point to attack of the enzyme or acid on the amorphous, inter-microfibrillar starch.

One particularly puzzling datum in starch grain behaviour is the great amount of rapid swelling which takes place in cold water at the site of an injury to the grain (Sjostrom, 1936; Alsberg, 1938; Badenhuizen, 1939). If it be assumed that the starch of the amorphous matrix and the starch of the injured microfibrils be less well ordered than the starch of the intact microfibrils, then water could be taken up more readily in this portion. In the normal grain, the microfibrillar skeleton would perhaps permit only a restricted volume change to occur. If this structure were disrupted, resistance due to the structural configuration would be diminished or lost. Thereby would be permitted an unlimited expansion of the amorphous and disrupted microfibrillar starch.
—even some dissolution—at the site of the injury and a gradually decreasing swelling toward the more intact regions, as has been observed (Alsberg, 1938).

Very likely both the microfibrils and the amorphous matrix are quite soft. When the starch grain is compressed, plastic deformation and gradual loss of birefringence are the usual consequences. Frey-Wyssling (1940a, b) has shown that, after such compression, the molecules are thrown into disorder. Consequently, he has suggested that crystalline micelles are lacking in starch grains and that the birefringence of a starch grain is due only to form effects and tensions in that structure. However, the work of many authors on X-ray diffraction patterns of native starch definitely indicates that a crystal lattice, hence micellar structure, exists. The disappearance of the X-ray pattern of starch on grinding has been ascribed by Sponsler (1922) to the plasticity of the starch crystallites.

The findings presented in this report also explain the lack of positive results in other electron-microscopic studies of starch grain organization. If the only difference between the starch substance of the microfibril and the starch substance of the amorphous matrix is in degree of organization, it is to be expected that for such a material as starch the electron density of both components will be quite similar. Hence, ultra-thin sections will show little detail of the microfibrillar structure as against that of the amorphous substance. Moreover, if the microfibrils tend to be helically wound, thin sections would show a string-of-beads organization in the radial direction, as actually so described by Whistler and Turner (1955). On the other hand, heating of the starch grain in water disperses not only the amorphous starch but also the microfibrillar starch. (The crystalline form of native starch appears to have a loose, little-resistant type of hydrogen bonding since its X-ray diagram disappears on heating, Katz, 1928). Therefore the preparations of Frey-Wyssling (1952) and Guilbot and Levavasseur (1954) cannot be expected to show the microfibrillar structure.

These experiments have indicated the existence of long, rod-like microfibrils, radially arranged and imbedded in an amorphous matrix in the normal starch grains of corn and wheat. Whether such a construction is to be found in the starch of other species of plants (and in the waxy starches) must await further studies of those materials. It is herewith suggested that the method of replicas of internal fracture surfaces should be the method of choice in the study of the organization of the starch grain and perhaps in the study of other biological materials.

**LITERATURE CITED**


—— (1939). Growth and corrosion of the starch grain in connexion with our present knowledge of the microscopical and chemical organization. Ibid. 33, 440–86.


Fig. 1. Aggregations of rodlet fragments of corn starch. Arrow at left centre points to coiled rodlets in shadow region. ×20,000.

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Fig. 2. Rodlet fragments of corn starch in more discrete elongated groups than those of Fig. 1. X 20,000.

Fig. 3. Internal fracture surface of grain of corn starch. Edge of starch grain at top of figure. Fibrillar units appear to be imbedded in amorphous ground substance. X 25,000.
Fig. 4. Internal fracture surface of grain of corn starch. Edge of starch grain at top of page. Note pit-like depressions at lower left and more or less straight, radially directed micro-fibrils at upper right. Compound, helically wound units also at lower left. $\times 25,000$. 

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FIG. 5. Surface views of portions of more or less compressed starch grains of wheat. Grains at lower left and upper right are more or less undamaged, and arrows indicate their papillate surfaces. Central grain is somewhat crushed and shows more warty protuberances at surface (at arrow). × 20,000.

FIG. 6. Portion of surface of somewhat compressed starch grain of corn. Warty protuberances are quite prominent. × 20,000.

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