STRUCTURE OF THE STARCH GRANULE

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Introduction

The purpose of this paper is to present an hypothesis regarding the anatomical¹ structure of natural starch granules. The hypothesis itself is not new; it is a combination of parts of older hypotheses, but with the introduction of some concepts new for starch, though not new in themselves. The hypothesis, reduced to its simplest terms, will first be stated without proofs at the risk of biasing the reader against it. The physical properties of granules, so far as pertinent, will be examined to determine whether the hypothesis is useful for understanding them.

The hypothesis is based on certain observations recorded in the literature or newly presented here. These observations are:

1. The starch granule may be made to swell and shrink by varying its water content.
2. At ordinary temperatures, swelling in water is limited; at higher temperatures, it is great and may lead to ultimate dispersion of most of the granule substance (gelatinization).
3. The granule, if mechanically injured, i.e., if it is chipped or cracked, swells greatly in cool water at the site of injury, and only there.
4. The granule is insoluble in cold water.
5. The granule, if mechanically injured, is more or less soluble in cool water by partial dispersion of the swollen portion.
6. The natural granule consists—at least in large measure—of material in orderly arrangement and exhibits a characteristic x-ray diagram. The diagram may be changed by causing the granule to swell, and under some circumstances the process upon which the change in diagram depends is reversible.
7. Completely anhydrous starch exhibits no x-ray diagram.
8. Thoroughly ground-up and mechanically disintegrated starch exhibits no x-ray diagram.

The Hypothesis

The peculiar anatomical organization of the intact granule restrains its swelling beyond a certain point in cool water. This restraining anatomical structure is the parallel arrangement of the long-chain macromolecules of which the starch granules' substance in large part consists. They are tied together in crystallites which are oriented radially in the granule. More-

¹ This word is used here to indicate that this paper does not deal with the finer molecular structure nor with the arrangement of the atoms in the molecule.
over, the starch-chain molecules possess an inherent tendency, probably due to intramolecular attraction of side groups for one another, to crumple or fold so as to reduce their own length. In crumpling, the macromolecule increases its diameter laterally; it thickens. In the natural granule containing adsorbed moisture, the macromolecules are not crumpled or shortened to their utmost because, being surrounded on all sides by other molecules which are also trying to thicken, room for complete contraction is lacking. The molecules at the same time endeavor to adsorb water, whereby they are also thickened. Thus, in swelling, a lateral pressure is exerted on the chains or bundles of chains, the micelles, which not merely prevents them from contracting but may even lengthen them so that the granule enlarges its diameter. In a sense, therefore, the macromolecules of a granule containing adsorbed moisture may be said to be stretched. They differ from the macromolecules in a mechanically stretched wool fiber in that the stretching force is a lateral pressure applied from all sides instead of a pull in the direction of the long axis of the fiber. The starch macromolecule is lengthened by squeezing, as one flattens a sheet of corrugated-iron roofing by lateral pressure; wool macromolecules are lengthened by pulling, as one straightens a coil of soft wire. The micelles are prevented from swelling to capacity, because there is not room for them to thicken as much as would be necessary if they sorbed or associated water to the maximum possible for them in a mechanically free state. Therefore, the swelling of granules in cool water is limited. Conversely, as a granule is dried, the transverse diameters of the macromolecules become smaller; the molecule shrinks laterally. The macromolecules crowd against one another with less force; space between them or between the micelles is made available to the extent that the water leaves. The granule shrinks until, in the completely anhydrous state, the chains approach a state of complete contraction.

So much for the hypothesis. We have now to present the evidence upon which it is based, and to test its usefulness in interpreting the physical properties of starch.

Supporting evidence

Swelling

It has been established conclusively by actual measurement that the starch granule shrinks when dried, and swells again when moistened (3, 64, p. 18; 77). MEYER (64) found that drying at 20° C. for only 2 hours shortened the longest diameter of a potato-starch granule, freshly prepared and washed in water, by 15 per cent., and this observation has been confirmed in the writer’s laboratory. Further drying at 90° C. for half an hour reduced its diameter by only 4 per cent. more. Additional final drying at 110° C. failed to produce further shortening. Total shrinkage is clearly very co-
siderable and therefore difficult to explain, as we shall see, if starch is composed of chain macromolecules that behave like those of fibers.

As one would expect from the capacity of the granule to swell in cool water, granules saturated with moisture are quite elastic. They can be deformed by mechanical pressure; but their greatest possible linear increase is only 3 to 6 per cent. Though very elastic, they do not stretch much (70, p. 39).

Like textile fibers (8), starch seems to exhibit hysteresis with respect to moisture sorption. Only moderate shrinking is promptly reversible. Sharply dried granules, if exposed to water vapor, do not swell rapidly back again to their original volume (3, 107).

Moreover, even moderate drying diminishes the power of starch granules to swell when boiled. They do not—at least for some time—swell to the same size that they would have reached had they not been dried (3).

Drying raises the gelatinization temperature (56, 73, 76) and affects other properties as well. The length of time starch has been wet also seems to be of influence upon the gelatinization temperature (81). If drying is carried to the extreme, but only then, the x-ray diagram of the granule disappears;

2 The effect of treatment with alcohol and ether is probably also to be attributed to dehydration. Wheat starch treated only with ether yields pastes of much greater mobility than the same starch sample treated with both alcohol and ether (55).

Not merely does thorough drying alter the swelling and water-sorption capacity, but starch thoroughly dried is said to be more easily hydrolyzed than it is before drying (79). Possibly this is because drying tends to produce rifts and cracks. Injuries tend to cause some of the starch substance to disperse (see pp. 298, 318). This interpretation finds support in the observation of Nägele (70, pp. 52, 63, 107) that the rifts and cavities grow larger in dry potato starch when it is placed in water. Meyer (64, pp. 96-99) and Baranetzky (13) have observed that dry grains are more quickly affected by diastase than freshly prepared ones, because fissures in them facilitate the entrance of the enzyme. Meyer also observed that potato starch, which is slowly attacked by diastase, is attacked more readily if the outer layer is broken.

Brown and Heron (17) and Maquenne (59) have shown that mechanically injured granules are attacked with great rapidity by diastase, observations which have been confirmed by Griffing (cf. 2, 76).

These effects of drying are therefore probably due to rifting of some of the granules which renders them partially soluble (see p. 309, and cf. also 87, 88).

Dehydration may also account for some of the differences between root and seed starches. In roots, the starches are never dry; in many seeds, for example the small grains, the starch may be quite dry, for the moisture content may sink as low as about 6 per cent. Indeed, the condition of the starch may perhaps account for some of the peculiar properties of flour made from such very dry wheat. Clearly, the starch of wheat harvested with a moisture content of 18 per cent. is not in a condition identical with that of wheat harvested with a low moisture content, say 8 per cent. It may be that some of the granules of very dry wheat, such as is harvested in semi-arid countries like California and British India, are rifted and thus influence the diastatic power of flour made from them.
incompletely dried starch retains its x-ray diagram (47, 48, 65, p. 1111; 66), some starches losing their x-ray diagram more readily than others (47, 48).

It has long been known that the temperature at which the granules of a given starch species swell is distinctly different for granules of different size. The phenomenon has recently been studied in some detail by Katz and Hanson (49). They concluded that each individual starch granule has a growth organization (gewachsene Struktur) which resists swelling and which must be overcome if gelatinization is to take place. No doubt there must be some such organization, but it may well be substantially the same in all granules of the same species and yet permit granules of different sizes to swell at different temperatures. Some years ago, the writer (3) pointed out that the relation of the mass of swelling material to the surface area of granules must in itself be a factor in determining the fact that in the same sample large granules tend to gelatinize more easily than small ones. The volume of a sphere or its mass increases as the diameter increases much faster than does its surface area. Therefore, given structure of identical rigidity in two granules of different diameter, the larger one should swell more readily than the smaller. It should swell sooner as the temperature is raised, because it will begin to exert enough intragranular pressure to distend the restraining structures of the granule before these have been softened to such a degree as they must be to permit the smaller granules to swell at all. This explanation is supported by the fact apparently first pointed out by Nügeli (70, p. 68) that in granules with very eccentric lamination swelling begins sooner on their eccentrically larger side, while the smaller side is still unchanged. Indeed in very eccentric granules swollen in dilute potassium hydroxide the eccentrically larger side may swell around the still unswollen hilum end so that the latter forms a depression until it, too, swells and the whole granule becomes more or less uniformly inflated (70, p. 77). The pressure exerted in swelling, however, must be unequal in such granules on the two sides, for the reason given above. It must be greater on the larger side and swelling must be greater there than on the smaller side.

Mechanical injury

That mechanically injured granules swell in a different manner was first observed by Schleiden (84), and has often been confirmed. A cracked or chipped granule swells at the site of injury and only there. Harrison (36) has reproduced a photomicrograph of such a granule. Injured granules,

Katz and Hanson (49), however, report that in every sample there are a few granules which partially resist gelatinization when heated to the threshold temperature at which gelatinization begins. Only a part of them becomes isotropic and stains with Congo red. This is commonest for eccentric granules. The eccentrically larger side may remain ungelatinized even when the smaller side has already undergone this change.
however, do not swell in anhydrous liquids such as pure glycerol; though in reagents like potassium hydroxide swelling begins at the injured spot (11). In water, swelling does not extend into the interior of the granule and then gradually throughout the whole of the granule as one would expect if a limiting membrane were the only mechanism that prevented the swelling of uninjured granules. Nor does the swelling spread circumferentially to involve the material between the laminae that have been fractured, as one would expect if the laminae were separated by membranes. These phenomena are particularly striking in granules broken in two by a fracture passing through the hilum. The two halves swell and disperse only over the surface of the fracture and for a short distance beneath. The halves do not swell en masse. They retain a normal appearance and their optical properties do not change except at the swollen fractured-surface region (31). HUSS (41), moreover, has demonstrated that triturated potato starch suspended in water occupies a greater volume than an equal weight of uninjured starch because a part of it is swollen. Indeed, the degree of swelling depends upon the extent of the injury, for the swollen portions do not wholly disperse. An extensively injured granule may swell to very nearly the size it would have reached if boiled in the uninjured state, as was established by measurement with a micrometer eyepiece (31). This is apparently because the natural granule contains α-amyllose (amylopectin), which swells in cold water but does not disperse.

4 There is much confusion in the nomenclature. It seems generally agreed that all starches contain a substance easily dispersed in water. For it, MEYER (64) introduced the term, β-amyllose, which is still very generally used, though sometimes this substance is simply termed amylose.

Many starches also contain insoluble material, which MEYER termed α-amyllose, but later MAQUENNE termed it amylopectin. At that time, it was not yet known that some starches contain combined fatty acid of high molecular weight, while others contain none but usually instead phosphoric acid. The presence of phosphoric acid was discovered first, and it was believed that its presence was characteristic of α-amyllose or amylopectin, so-called. SCHOCH (85, 105), however, has shown that in potato starch there is no insoluble fraction. According to this view, amylopectin is not a chemical individual, but merely β-amyllose esterified to different degrees with phosphoric acid, or β-amyllose in different degrees of association esterified with phosphoric acid. KADEN (44), working under the direction of C. J. LINTNER, has questioned its very existence. Moreover, some native starches apparently contain no insoluble material at all, but consist wholly of β-amyllose (38).

The fatty-acid compound occurs preformed in cereal starches; it is insoluble. It is the only insoluble substance whose existence preformed in native starches has not been questioned. It follows that it is the only substance generally admitted to occur in native granules which conforms to the specifications as regards insolubility laid down for amylopectin by MAQUENNE, who introduced this term.

It seems, therefore, inadvisable to continue to employ the term amylopectin, for not all starches contain preformed insoluble material, though they may contain combined phosphoric acid. Further, amylopectin, so-called, has none of the chemical characteristics
The swelling of injured granules seems to be quite analogous in character to that of granules gelatinized by heat. The two sorts of swollen granules behave similarly with respect to staining and they exhibit similar optical properties. Tannin acts only upon that part of the granule which is injured in the same manner in which it acts upon boiled granules (14). Intact granules do not stain with such dyes as Congo red (54), which are among the colloidal dyes, though granules are stained by other dyes. FISCHER (27) found that, in granules impregnated with these dyes, picric acid precipitates the dyes in crystalline needles arranged radially. This may be taken as evidence of radial structure of the granule.

Injured granules, however, stain easily with the dyes, like Congo red, that do not stain intact granules, the staining being restricted to the injured part (27, 31, 41, 112). Heat-gelatinized granules stain like the swollen, injured portions of unheated granules, but the staining tinges the whole of the granule (30). HUSS (41) has, indeed, proposed the use of this phenomenon as an indicator to fix gelatinization temperatures. Congo red stains only the swollen parts of granules; allowing the granule to remain in the dye solution for 48 hours does not result in the penetration of the dye much, if at all, beyond the swollen area. The dyed material appears granular, taking the dye unevenly, whereas granules gelatinized by heating stain uniformly and appear more homogeneous.

Furthermore, Congo red dissolved in anhydrous glycerol does not stain injured granules, nor does it stain whole granules. After standing some time, however, the glycerol takes up water from the atmosphere and the injured granules begin to swell.5

The swollen portion of an injured granule loses its birefringence exactly like a granule gelatinized by heat. Here it should be pointed out that KATZ and HANSON (50) believe that wet grinding as employed by ZWICKER and by them acts in a way different from dry grinding as employed by SPONSLER (95) and by ALSBERG and PERRY (7). KATZ and HANSON found that wet grinding does not destroy birefringence (black cross with nicols crossed) or the x-ray diagram. As we shall see, dry grinding does not destroy birefringence either, except in the injured portion. In starch ground wet, change of pectin, nor does it serve similar functions. It has seemed best, therefore, to continue the practice of the writer and his co-workers in following TAYLOR and his co-workers (102–106) and SHERMAN and his co-workers by employing the term α-amylose for the insoluble fraction, and the term β-amylose for the soluble fraction. It is recognized that this practice is open to the objection that the Greek-letter prefixes have no essential connection with a stereochemical difference of the units composing the starch constituents, and that KATZ and WEIDINGER (53) have questioned the identity of TAYLOR's substances with the correspondingly named ones of MEYER.

5 According to FISCHER (27, p. 71), air-dry granules swell appreciably in a mixture of equal volumes of anhydrous glycerol and water.
in birefringence in the injured portion is not manifest because the injured portion swells and mostly disperses (cf. pp. 298–299). According to Sponsler, sufficiently severe dry grinding destroys the x-ray diagram. According to Katz and Hanson (cf. also 51), wet grinding does not. This difference is probably due not to difference in mode of action of the two methods of grinding, but to the fact that wet grinding, as employed by Katz and Hanson, did not disintegrate the granules nearly as completely as the very long dry grinding employed by Sponsler. Starch subjected to a shearing pressure (gleitendem Druck) of 20,000 atmospheres loses its x-ray diagram (65).

The behavior of the swollen, injured part of a granule is similar to that of a granule swollen in toto by heating in water. Everything indicates that the swelling of injured granules is brought about by the same mechanism as gelatinization by heat. This suggests that neither gelatinization nor the alteration of the x-ray diagram brought about by gelatinization as described by Katz and co-workers is accompanied by chemical alteration of the molecule in the strict meaning of the word.

Membranes

Many investigators (65) assume the existence of an outer, limiting membrane to explain some of the phenomena above described. In fact, there seems to be an outer, visible membrane, probably part of the protoplast which according to Hanson and Katz (34), may be more or less lost in the process of preparation. According to Zwikker (112, pp. 61, 68), the plastid is removed in the preparation of starch. It is obviously a delicate structure. Perhaps it affects some of the physical properties of starch. Thus Lachele (55) found that the granules of a commercial sample of wheat starch (Merck's), although neutral in reaction, became ruptured when swollen by boiling in water for 20 minutes, whereas a sample prepared by the method of Rask and Alsberg (75), which employs no other reagents than 1 per cent. sodium chloride solution, water, alcohol, and ether, did not. Naturally, the plasticities of the two starch preparations were different. Possibly the difference in their behavior was due to the removal in one case and the preservation in the other of a natural, outer membrane or coating. In any event, such an outer coating can be but little concerned in the phenomena under consideration. If it were responsible for them, then commercial starches which contain granules that have lost this membrane in whole or in part (cf. 34) would contain an appreciable number of granules which are easily stained and disperse in water. As a matter of fact, such starches contain few such granules, and these are always plainly injured ones.

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The membrane in gelatinization.—The view that natural granules have a strong outer membrane is based not upon seeing such a membrane with the microscope (cf. 64, p. 149) in unaltered granules, but upon observations on gelatinized granules. These observations are of two sorts: the appearance of gelatinized starch, and the distribution of phosphorus chemically determined.

Under the microscope, gelatinized granules appear to be sacs with elastic walls enclosing sol or very dilute gel.\(^7\) The existence of a membrane could be demonstrated with a dissecting microscope, the microneedles of which had been replaced by fine glass hooks. These hooks were made to puncture a sac at opposite poles. By gradually screwing the hooks apart, the sacs of potato starch could be stretched to about twice their length. If stretching is continued much beyond this point, the sac ruptures suddenly as if made of over-stretched elastic. If the stretched sac is not ruptured and the strain is then relieved, it resumes its original shape. By means of a resistance wire, the paste was heated on the stage of the microscope and the manipulations repeated. It seemed a little easier to deform the warm granules, but the difference was not great. It was not possible to decide whether cold, gelatinized granules contain gel and hot ones sol (32).\(^8\) There can be no doubt, then, that gelatinized granules are surrounded by an elastic, limiting membrane. It is this fact, principally, that has led to the inference, erroneous as we shall see, that this membrane preexists in natural granules.

Phosphoric-acid content of membrane.—The membrane of gelatinized granules has been reported to contain phosphoric acid and has been assumed to consist of amyllopectin which many investigators identify with the insoluble material of the granule. However, some starches consist wholly of \(\beta\)-amylose (cf. 38).\(^9\) The belief that amyllopectin is the insoluble material of starch and

\(^7\) That this is the structure of gelatinized starch is also shown by the fact that the consistency of starch paste depends upon the maintenance of this structure (cf. 3, 34). Paste loses plasticity if the swollen granules are ruptured. Grinding in a pebble mill, for example, converts paste into a more or less viscous liquid, because the sacs collapse, thus no longer occupying nearly all the space of the system (7).

\(^8\) Analogous observations were recorded by HESS and RABINOWITSCH (39), who examined granules that had first been gelatinized in water and then put into dilute alcohol. This may account for some of the minor differences between their observations and those of Griffith.

\(^9\) In this connection, it is worthy of note that in the only starch (rice) in which the ash of the glutinous and non-glutinous varieties was compared, the non-glutinous variety had, on the average, more phosphoric acid and silicate than the glutinous variety. But some samples of the latter contained more of both than some samples of the former (101). The glutinous variety moreover, had the greater swelling power and yielded the more viscous pastes in spite of its generally lesser phosphoric acid content, a fact which does not support the view held by Samec and others that viscosity depends principally upon the phosphoric acid-containing fraction.
that it forms the membrane of gelatinized granules has led many investigators\textsuperscript{10} to assume that the peripheral zone of ungelatinized granules also consists principally of \(\alpha\)-amylose (amylopectin). A difference in the phosphoric acid content of large and small granules of the same species has been advanced as evidence for this view. The reasoning is as follows: If \(\alpha\)-amylose is localized principally at the surface of granules, small granules of the same sample should contain a larger percentage of phosphoric acid than large ones, assuming the thickness of the membrane to be about the same in all granules, because the ratio of surface to mass is greater in small than in large granules. Small ones have a larger surface, relatively, and therefore a larger percentage of their mass should be in the membrane, if this is not thinner than that of large granules. Schoen (86), however, would attribute any greater phosphoric acid content of small grains to sorption on their greater surface. Hines made an investigation under the writer's direction upon cassava starch, separated into fractions of different granule size by air flotation in an air classifier, and failed to find any correlation between granule size and phosphoric acid content (1). It is therefore clear that there are starches for which no conclusion regarding the distribution of amylopectin can be drawn from their phosphoric acid content.

Furthermore, evidence controverting the view that small granules contain more phosphorus than large ones is to be found in the behavior of granules when boiled in water. If \(\alpha\)-amylose (amylopectin) were localized at the periphery, one would expect those natural granules to have the thickest and strongest membranes that have the greatest phosphoric-acid content. One would expect such granules when boiled to hold together better than granules with a lesser phosphoric-acid content. As a matter of fact, there is no such relationship between phosphoric-acid content and resistance to boiling. Potato starch with a fairly high phosphoric-acid content disintegrates rapidly, whereas tapioca, also a root starch but with a much lesser phosphoric-acid content (106), does not (31).

Moreover, Maquenne and Roux (60), who first estimated the amylopectin content of granules, believed that it is intimately mixed with the other constituents. Schoch (85), in his study of the distribution of phosphoric acid, reached the conclusion that phosphoric acid is quite irregularly distributed through the natural granule.

\textsuperscript{10} For example, (66, p. 216). Ling and Nanji (57), however, hold a different view. They suggest "... that 'amylose' as it exists in starch granules is present in more than one physical state and possibly in different degrees of hydration ... about 25% of the 'amylose' exists as a crystalloidal phase in the form of spherites constituting a core round the hilum of the granule. This portion is readily extracted by water or by dilute alkali. The remainder, which presents a colloidal phase, is dispersed uniformly in the amylopectin layers. It appears to exist either as a solid solution or to be so strongly adsorbed on the amylopectin as to resist extraction."

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THE MEMBRANE AN ARTIFACT.—In summary, it may be said that there is no doubt that gelatinized granules are surrounded by a fairly strong membrane. The evidence that in ungelatinized granules amylopectin is concentrated in a peripheral membrane is doubtful. This, together with the absence from natural granules of a visible membrane of the thickness and toughness of the membrane of gelatinized granules, leaves the question of the existence of such a membrane open.

But the facts regarding swelling, shrinking, and staining, above arrayed, cannot be reconciled with the assumption that natural granules are surrounded by a membrane which is impermeable to many dyes, and to the starch substance itself. If the resistance of intact granules to staining and to more than moderate swelling and their insolubility in cold water were due merely to the existence of such a simple membrane, then in an injured granule with a locally ruptured membrane staining should be general throughout the granule and swelling in cold water should be general also. As we have seen, none of these phenomena occur. They are strictly localized to the site of the injury. As we shall see later, the birefringence of granules, as well as their solubility, is affected in an analogous way.

It is conceivable that the granule is surrounded by a membrane so rigid that it prevents mechanical disorganization except at the site of the injury where the membrane is broken. This is, however, improbable, because if there were a rigid membrane permeable to water this should either prevent shrinking or else one should be able to observe a separation of the shrunken granule substance away from the rigid membrane. It is improbable that a rigid membrane and the granule substance both shrink to exactly the same degree. Certainly in a dried granule no separation between membrane and granule substance is to be seen. If there is a limiting membrane which is elastic, like the membrane surrounding a gelatinized granule, it could hardly account for the strict localization of the swelling to the site of injury.

Some investigators have assumed the existence of a simple system of membranes represented perhaps by the laminae or rings. If there is such a system, the laminae are hardly a part of it, for unlaminated starch swells like ordinary starch (12). Moreover, a granule broken in two by a fracture passing through the hilum has all of its rings opened up. Any simple membrane system that might be present would be opened up widely so as to permit extensive, if not complete, swelling and staining. As we have seen, these phenomena are strictly limited to the immediate region of the fracture. Certainly, a series of concentric membrane rings could not explain the facts. Nor could a series of radial membranes. Nothing short of a system consisting of concentric, hollow spheres, divided by many radially arranged transverse planes could explain the behavior of fractured granules.
Katz and Rientsma (52) have, indeed, suggested this very possibility. Katz (46) subsequently elaborated this idea as follows: "There might be a binding substance in a swelling substasce which binds packages of micelle together to larger, probably, but still submicroscopical units. In grown products, tissues, fibers, starch granules, this binding substance might take the form of membranes lying between the micelle or between small bundles of micelle."

Subsequently, Hanson and Katz (34) obtained support for this view by the examination of Lintner starch,11 which easily fragments into concentric rings composed of separate rectangular blocks regularly arranged radially. These blocks they believe to be composed of bundles of parallel crystals, for they are normally birefringent. They believe further that the blocks are held together in natural starch by a cementing substance, possibly amylpectin (α-amylose), which is dissolved out by the acid in the process of lintnerization. Lintner starch does not swell in cold water, but disperses to a clear solution in warm water. It does not form paste because the cementing substance which holds the granule together and swells in the process of gelatinization has been removed. It is the cementing substance, they suggest, that forms the membrane system of the natural granule.

It is altogether likely that the view that there is intermicellar substance is correct,12 but it may be doubted that it serves as a membrane system, for its presence does not seem capable of explaining the swelling of injured natural granules. Why should the cement substance swell so little in the cold in an intact granule and so greatly in an injured one? Obviously, some additional factor is involved.

On the other hand, if the natural starch granule swells greatly in water after it has been broken up by mechanical means, why does it not swell when it is broken up into blocks by Lintner's treatment? It must be either that the prolonged acid treatment so greatly modifies the granule's substance that its swelling power and solubility are radically altered, or else that, of the substances of which the granule is composed, only one, the cementing substance, swells and disperses cold. The Lintner process may remove this cementing substance.13 In that event, the other material, composing the blocks of Lintner starch, neither swells nor disperses in water, except when warmed. Thus we might explain the properties of Lintner starch; but we would not explain why natural intact granules which contain the swelling substance do not swell greatly unless warmed, whereas the same granules, still containing the swelling substance, swell greatly at once when injured.

11 Starch subjected for a long time to 7.5 per cent. hydrochloric acid.
12 Loskitt (58, p. 154) concluded from the optical properties of starch that it must be a mixture of colloidal and crystalline particles.
13 Commercial soluble starch and the artificial starch granules of Maquenne and Roux do not yield amylpectin (33).
We are thus forced to the conclusion that such membrane as natural granules may possess is not identical with the limiting membrane of heat-gelatinized granules. How is this to be explained? Obviously, by the assumption that the membrane surrounding heat-gelatinized granules is an artifact. Zwikker (112) has demonstrated that this is true. He has correctly described the origin of the membrane during gelatinization thus: "The outer parts of the grain become distended; they detach themselves at the same time from the central mass or remain adherent to it. In one case as in the other, a cavity is formed in the interior of the grain. The warm water or any other liquid that may have been used to make the starch swell is thus aspirated so to say into the interior. In passing through this wall it [the liquid] removes from it [the wall] and carries along with it [the liquid] the dispersible substances; in consequence, when the gelatinization has become complete, the membrane is only composed of the elements that are soluble with difficulty derived from the different parts of the grain and especially from the most resistant layers; all these elements are found agglutinated into a homogeneous mass."

That granules in process of being gelatinized by heat do show a central cavity which gradually enlarges, has also been observed by the writer and by many others (64). Very commonly, this cavity is at first stellate, due to the radial structure of the granule. The observations of Bakhuyzen (12) upon wheat starch devoid of laminae support the view of Zwikker. There is, therefore, no necessity to assume the pre-existence in natural starch of a rigid, limiting, outer membrane. The gelatinized granule's membrane is clearly an artifact.

On the assumption that natural granules possess no limiting membrane, the behavior of compound granules, when gelatinized, becomes understandable. After they have fallen apart into their component granules each of the latter forms its own limiting membrane when gelatinized, exactly like a simple granule. And this occurs even when no membrane separating the component granules is visible in the original unbroken compound granule.

Furthermore, occasionally two granules that lie close to one another in the same protoplast become enveloped by a capsule of starch substance which shuts them off completely from their surroundings. Meyer terms them complex granules. When they are gelatinized by heating in water the whole complex is connected into a sphere with only one outer limiting membrane (70, pp. 210, 224). If the two granules had each its own outer membrane, one would expect the complex granule to be changed into a large sac surrounding two smaller sacs—one for each of the component granules. Their

14 The writer's translation from the French original.
15 A drawing of a granule in this state is given by Hanson and Katz (34).
behavior when gelatinized is easy to explain on Zwicker's hypothesis but difficult on any other.

Unlaminated starch

The facts presented seem to warrant the conclusion that it is not a membrane system that keeps the natural granule from swelling beyond definite limits. If this is so, what is the mechanism that is responsible for this phenomenon? The hypothesis advanced in this paper presents such a mechanism: it is the radial arrangement of the crystallites and starch chains. To make clear how this simple physical arrangement explains the facts, it is best first to consider granules without laminations or rings. This is justifiable because the occurrence of laminations is accidental, rather than essential, otherwise they would never be lacking in any sort of starch. But they are occasionally missing from many sorts of starch and their absence is the rule in the root starch of Zingiber officinale (70, p. 17), in the starch of the yellow turnip, and the seed of Ruellia pavale (109). Meyer (64) attributed their formation to the alternation of day and night; moreover, Bakhuyzen (12) failed to find them in seed starch from wheat grown in an environment from which this sort of periodicity had been eliminated—that is, an environment nearly constant with respect to illumination, temperature, and humidity.

Bakhuyzen's unlaminated wheat starch has very definite radial structure. In it, the crystallites may be seen to reach from the periphery to the hilum. When these granules were carefully heated in water so that they swelled slowly, he found that they could be seen to be composed of very refractive radial needles attached to the refractive border of the granule. These needles are tapered toward the center, resembling pyramids with a base 2 to 3 microns in diameter and a length of 17 to 20 microns, which is about 45 per cent. of the total diameter of the granule. In such unheated, unlaminated starch, probably all of the needles (crystallites) stretch from the border to the hilum.

The starch elements

X-ray analysis has definitely established that the starch granule has crystalline structure. Most probably the ultimate starch elements are long units (45, 98, p. 75). It is not yet possible to say just what is their form, but it is probably not a straight chain, for since the elements of the chain are apparently held together by α-glucosidic linkages, a straight-chain structure is not possible (37). Perhaps starch chains are spirally wound (65), like the thread on the end of a bolt that receives the nut, or have a zigzag (65) shape.

See, however, (19).

It is interesting to note that spirally twisted granules have been reported by Nödeli (70, p. 31).
like the bellows of an accordion, or a meander (99) form, being fluted more or less like a sheet of corrugated roofing iron. In any event, the long chains seem to be folded or crumpled in some manner as yet unknown, whereby the molecule is shortened—compressed as it were—in the direction of its long axis, for a starch molecule consists of 8 to 10 times as many glucose residues as a cellulose molecule of the same length (99, p. 828). Perhaps the chains are crumpled and the long axis of the molecule shortened because certain of the side groups of the macromolecule attract each other strongly. This would tend to bend the long axis and shorten the molecule. It is possible the chain is also forked or branched (66, 99). Units of this type would not pack as closely as straighter chains, for example, those of cellulose. This may be one of the reasons why starch has a looser structure than cellulose, is more easily penetrated by solvents (99), and is more readily subject to mechanical influences (45, 95).

In these long units, the atoms are linked together by primary valences. It is probable that van-der-Waal or cohesive forces hold these macromolecules together in more or less parallel arrangement. Whether in this way the macromolecules are held together in separate and discrete bundles to form micelles is not clear. At present, the view seems to be gaining ground that the micelle, in general, is not always and necessarily a separate and discrete unit and that fibrous structures like cellulose need not have a completely discontinuous framework. Neale (72, 89) has suggested that in certain cases, at least, the structure is not "micellar" or discontinuous, but that, though imperfectly crystalline, it is continuous except for random breaks in the primary valence chains and that though continuous it is slightly imperfect. Taylor and Schoch (105) have suggested the presence in starch "of more or less completely esterified tri-basic phosphoric acid, either with hydroxyl groups on one chain or bridging between two chains of glucose anhydrides."

Granule structure

Everything indicates that the chains, or the bundles (micelles) into which the chains are perhaps grouped, are arranged radially. 18 Of course, the radial granule structure visible with the microscope is a coarser one than that of the macromolecules or even the micelles. Each of the visible radial needles must be regarded as a crystallite composed of many chain molecules or of many micelles of such molecules. 19

A granule thus constructed would be strongest in a radial direction. The primary valence bonds which hold the glucose residues into a chain would

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18 This is similar to the anatomical structure proposed by Meyer (64). The bundles would correspond to his trichites, but he assumed the trichites to be forked.

19 Whether the macromolecules are arranged parallel in the crystals has not yet been proved by x-ray analysis, but is probable according to Meyer, Hopff, and Mark (65).
make for greater strength lengthwise of the chain than would the secondary valences which hold the chains to one another laterally (94). In consequence, there is a tendency for granules to rift radially when subjected to mechanical strain. The force required need not be very great; as we have seen, mere thorough drying may cause radial cracks to appear. This fragility need not be taken to mean that the structure of the granule is everywhere discontinuous from micelle to micelle or that chemical cross linkages between chains are necessarily absent.

Does this suggested structure explain the shrinking and limited swelling of natural granules?

Let us first consider the shrinking of granules when dried. If the starch units are long chains, then when they swell they must increase in diameter much more crosswise than lengthwise, if indeed they lengthen longitudinally at all (96).

And conversely, they must diminish more in their transverse diameter than longitudinally when they shrink. This seems to be the usual behavior of fibers. The diameter of the wool fiber becomes about 18 per cent. greater when it is wetted, but only 1 per cent. longer (8). Therefore, it is difficult to explain the very considerable shrinkage of starch granules when they are dried (78), for the radial arrangement and the swelling and shrinking of long chains in their shorter diameter should result in swelling or shrinking in a tangential direction. As a consequence, the shrinking granule should break up into wedge-shaped pieces. This is actually the behavior of inulin spheroliths (27) and of the crystalline disks of amylodextrin (69). It is probably also a factor in the radial rifting of dried starch already noted. Since, however, the starch granule swells and shrinks in a radial as well as a tangential direction and increases or decreases its diameter, we must assume, either that the starch chains do not behave like wool fibers or else that for some reason they are prevented from so behaving.

This difficulty disappears if we assume the starch chains in the natural moist granule to be already stretched mechanically by their own swelling pressure. As we have seen, according to Staudinger and Eilers (99), the starch chain is shortened in some way, as compared with cellulose, a conclusion based in large part upon viscosity determinations of starch solutions.20 In solution, the macromolecule is under little or no mechanical strain. It is, therefore, presumably much crumpled so that its long axis is much shortened. It may, however, not be maximally contracted. There is no basis for knowing whether starch macromolecules have the same length in solution as in the natural starch granule.

20 This interpretation of the significance of viscosity measurements has, however, been questioned (18, 62).
It is obvious that at maximum contraction the region over which water or other solvent might associate itself with the macromolecule is shorter than when the macromolecule is more or less extended. In the contracted state, secondary valences are in part neutralized by the very intramolecular attraction that shortens the chain. Moisture sorbed on natural starch granules is under quite enormous pressure (77) and we are, therefore, justified in assuming that water exerts very great force in attempting to associate itself with starch. In doing so, water would endeavor to make as much room for itself along the length of the chain as possible. To this extent, the forces crumpling the chain, such, perhaps, as the intramolecular attraction of side chains for one another, would be counteracted and weakened. The chain would tend to straighten. It is, therefore, probable that starch molecules, while shorter than cellulose molecules though with the same number of glucose residues, are still, as Staudinger and Eilers believe, quite long chains when in solution. In this condition, they are neither fully contracted nor fully extended. When a starch solution is dried to a film, there may well be further contraction of the macromolecules.

If these conceptions correspond to realities, it follows that, in natural moisture-containing starch granules, the chains are extended to the degree that associated water has stretched them. But their state of extension is probably not the same as in a starch solution. Presumably, as in the case of wool, the association of water tends to thicken the diameter of the chain. We would, then, have two factors at work in opposite directions: lengthening of the chain which would tend to make it thinner, and the pressure of associated water which would tend to make it thicker.

When a cellulose or a wool fibril sorbs water, it increases greatly in its transverse axis and but slightly along its longitudinal axis. Such a fibril is free to expand in all directions except inward where it is hindered by other fibers also swelling similarly, and, therefore, increases greatly in diameter. A starch fibril is in a quite different position. Because of the radial arrangement of the granule, it is surrounded on all sides of its long axis by other fibrils also endeavoring to swell in a direction transverse to their long axes. Every macromolecule is prevented from associating itself with water to its maximum capacity because it is hampered mechanically by its position with reference to its neighboring macromolecules. That is why the intact starch granule is strictly limited in its capacity to swell despite the fact that its substance has unlimited capacity to swell and disperse. No limiting membrane is necessary to account for the phenomenon.

According to our hypothesis, the macromolecules in moist natural starch are perhaps unable to associate themselves with as much water as is possible when they are free in solution. Nevertheless they may be more extended along their long axis when in situ in the moist granule than when free in solution,
for in the granule the macromolecules squeeze one another laterally; they subject one another to strain. In consequence, they tend to push one another apart and, since this is possible only to a slight degree, they flatten and lengthen one another.

The possibilities for such lengthening are appreciable, since the chains tend, if not subjected to strain, to assume a very crumpled form. The dry granule, when moistened, tends to swell and its thickness is a function of the moisture content. Any condition such as heating that changes the moisture content, \textit{i.e.}, the association or sorption of water, changes the size of the granule; it shrinks or swells. In the natural moisture-containing granule, the chains are already stretched to some degree due to their own swelling pressure. That is why the granule shrinks when dried and swells again when moistened.

It is also the reason why the moist granule does not swell beyond a certain size. It could do so only if there were room for the macromolecules (or micelles) to thicken more; but there is no room because they are surrounded on all sides, except at the peripheral end, by other macromolecules or micelles which are also trying to thicken. Swelling, therefore, stops when equilibrium is reached between the force making for thickening and the resistance to thickening.

This equilibrium may be disturbed by increasing the kinetic energy of water as by heat. Molecular agitation which is associated directly with the coefficient of thermal expansion would be less lengthwise than crosswise of the starch chains (\textit{cf.} 97) and therefore granules tend to swell more in warm than in cold water. Greater swelling in warm water can take place only if the diameter of the granule enlarges. Since the macromolecules (or micelles) swell mostly laterally, this can take place only in the following ways: (1) The macromolecules straighten their long axes by water sorption and pressure against one another. (2) The macromolecules push one another apart, though still cohering. In consequence, the diameter of the granule increases greatly so that spherical granules are converted from solid spheres into hollow globes. (3) The granule is disorganized and more or less dispersed. (4) Or, there is a combination of these phenomena.

The first phenomenon, the straightening of the macromolecules accompanied by water sorption, probably occurs in the early stages of gelatinization and may perhaps account for changes in the x-ray diagram.

The second phenomenon, the conversion of a solid spherical granule into a hollow sphere, may actually be observed when the granule is slowly heated in water. It swells and, as we have seen, a cavity develops at the hilum, because the degree to which the chains may lengthen must be strictly limited. The granule cannot swell greatly and still retain its solid form. Spherical granules become hollow spheres. Other granules swell in a way determined
by their form and the fact that swelling develops pressure tangentially. Thus, it is well known that eccentric granules (Solanum, Phayus, cassava, et al.) swell relatively more in breadth than in length.

Katz and Hanson (50) have assumed that the swelling of solid granules to hollow spheres is a unique phenomenon, and that the only possible way to explain it is to assume some sort of growth structure in the native granule. They have not themselves determined the nature of this structure resulting from growth. Protein crystals, however, also swell with the formation of a vacuole in the interior, and may be made artificially. Schimper (83) attributed the phenomenon to the greater solubility of the material in the interior of the crystal, and this is the explanation Nögel had offered earlier for the analogous phenomenon in starch. However, the explanation offered by Zwiker and by Bakhuyzen for starch seems equally applicable to protein crystals, if one assumes that the outer layers swell sooner and tend to draw away from the center, thus creating a vacuole. It is then unnecessary to assume, as did Schimper, that these crystals consist of more than one protein, which would not be in accord with present-day knowledge. Nor is it in accord with the fact that Wyckoff and co-workers have obtained x-ray diagrams from protein crystals. Some protein crystals behave so much like starch with respect to swelling and solubility that if one has a "growth structure" then they both have. This "growth structure" is the arrangement of their molecules in the crystal. The hypothesis here presented, should it be supported by further experimental evidence, offers a simple and reasonable explanation of the phenomena.

With heating, the granule swells greatly. The second phenomenon, disintegration, has then occurred; the granule has been converted into a sac filled with dispersed starch substance; and we have the third phenomenon. The degree to which disorganization occurs is a function of the amount of water available, as has been shown by Meyer (63).

If this hypothesis should ultimately prove to be well founded, then analogies with rubber at once come to mind. The moist starch granule is elastic; it changes its shape under pressure and returns to its original form without the slightest change in its microscopic structure. Dry granules, on the contrary, are inelastic and brittle. Rubber, too, under certain conditions, loses its elasticity. Moreover, it is well known that rubber exhibits the x-ray diagram of amorphous substances unless it be stretched, in which case it exhibits a fiber x-ray diagram. When it is quite anhydrous, starch, like unstretched rubber, exhibits no x-ray diagram, or rather it exhibits the diagram of an amorphous substance (45). It acquires an x-ray diagram only if it is permitted to sorb moisture, but the diagram is not that of a fiber. It is more like that of a crystalline powder. According to our hypothesis, we may consider the macromolecule of a natural undried granule as already
partially stretched by associated water, very small quantities of which are capable of stretching the chains to give them an orderly arrangement sufficient for an x-ray diagram.

Hitherto it has not been possible to obtain a fiber x-ray diagram from starch. Neither spinning threads from starch solutions nor pressing preparations was effective (65). For natural starch granules, a fiber x-ray diagram is not to be expected. For a fiber diagram, it is necessary that the chain molecules of the material be oriented so that their long axes for the most part are parallel. In a powder consisting of starch granules, this is not the case. Nor would a single starch granule yield a fiber diagram, if it were possible to obtain an x-ray diagram with it. Although the macromolecules are probably parallel in the individual crystallites, the crystallites themselves are not parallel to one another. Their arrangement is radial. Therefore, although in the moist granule the macromolecules may be regarded as more or less stretched by their own swelling pressure, they cannot yield a fiber diagram.

Náray-Szabó (71, p. 299) and Katz and Derksen (48) have studied the loss of the x-ray diagram in quite anhydrous starch. Katz (45, pp. 3633-3635) has also described a similar behavior for gelatin and agar, and has offered the following alternative explanations: One of them is that the substance when dried changes into another modification, another phase within the meaning of the phase rule. This means that the material, e.g., agar gel, is built up of crystallites which contain water of constitution. The other alternative is that in drying no change of phase occurs (within the meaning of the phase rule) involving a discontinuous modification of individual units, but rather there is a continuous change involving a gradual loss of regularity in the lattice. Katz makes no choice between these alternatives in the case of gelatin and of agar, but in the case of starch he is inclined to believe that there may be something like true water of crystallization. Perhaps he favors this view because all starch preparations (soluble starch, amylodextrin, etc.) exhibit the same phenomenon (45). What is meant by “water of crystallization” is, of course, a matter of definition. In the case of starch, the phenomenon is probably not exactly the same as in the case of the efflorescence of an inorganic salt in which the water is held in stoichiometric proportions. Nor does the phenomenon seem to be exactly the same as that which occurs in the case of certain protein crystals which show no x-ray diagram unless great care is taken to prevent efflorescence (111). In many soluble proteins, this process does not seem to be reversible except through recrystallization; in starch it is. One thing seems certain: water is held in starch very firmly. Fischer (27) found that starch granules treated with a dilute solution of cobaltous chloride ceased to be colored rose red long before they became anhydrous. On the contrary, they turned in-
tense blue, showing that the water of partially dried starch is held so firmly it is not available to the cobaltous chloride.

It is also difficult to understand why the birefringence of the starch granules, as made manifest by the black cross, should persist in anhydrous starch, as we shall see it does, if the crystals weather. The black cross appears because the crystallites are approximately parallel, an arrangement one would hardly expect to persist after weathering. It is therefore doubtful that the behavior of starch when sharply dried is due to exactly the same phenomenon as the weathering of a crystal losing water of crystallization.

Furthermore, it is difficult to explain as a case of efflorescence (95) the loss of the x-ray diagram when natural starch is thoroughly dry-ground in a pebble mill. Our hypothesis, however, offers a basis for a common explanation for both phenomena. This hypothesis assumes that, if there are no effective contravailing forces, starch molecules tend to contract along their long axes, to crumple up. The evidence at hand and already presented does not prove this innate tendency, but it does make our assumption at least reasonable. In the natural moisture-containing granule, the macromolecules are not fully crumpled, because with the adsorbed moisture they occupy too much space for them to contract fully. They are more or less stretched. As the granule is dried, the chains become thinner, occupy less space and in consequence are able to contract further. In the process, they lose their orderly arrangement sufficiently to sacrifice their x-ray diagram. Perhaps the disorderly arrangement arises because contraction is not uniform from macromolecule to macromolecule, and therefore the diffracting groups of individual molecules change their positions with reference to one another from macromolecule to macromolecule. One need not imagine a more extensive disorganization. This idea comes to pretty much the same thing as the second of Katz's alternatives. In the moist natural granule, the macromolecules are prevented from crumpling because they can shorten only by thickening, and being associated with water, there is no space for them to shorten. If, however, the radial, parallel structure is sufficiently broken up by mechanical disintegration of the granule, there is no longer the impediment to contraction that exists in the intact granule, even in the presence of some moisture. A large number of the macromolecules are then free and the x-ray diagram is more or less lost.21

Our hypothesis is also consistent with the changes in x-ray diagram that occur in starch when it is made to swell. It is well known that stretching such fibers as wool or hair changes their x-ray pattern—presumably because

21 It would be interesting to determine whether dry grinding has an analogous effect upon the x-ray diagram of gelatin. It is possible, for Alsberg and Griffing (5) found it to have a marked effect upon solubility and gel formation. It would also be interesting to examine gelatin films made by drying soils and gels. Field (25) has shown that the former are not birefringent as are the latter.
the chain molecules of which they are composed are straightened and lengthened (9).  

Katz and co-workers (34, 45-53) have found that starch, when made to swell in water by heating, may undergo alteration of its x-ray diagram, and that in certain cases this alteration is reversible. They suggest that the change is due to the formation of different modifications of starch. The phenomenon might, however, be explained according to our hypothesis as the consequence of lengthening or shortening of the chains.

The hypothesis suggested would also explain the hysteresis which, as we have seen, hinders dried granules from returning to their original size when moistened. According to this hypothesis, the chain macromolecules shorten or crumple as the granule loses water, else the granule would not shrink. With the removal of water, the macromolecules are brought closer together than they have ever been, for it must be remembered that starch is formed in a milieu, the cytoplasm, which has a high water content. Unless starch is artificially dried, it always contains a fair amount of moisture. With the removal of moisture and the moving closer together of the contracted but thickening chain molecules, linkages through secondary valence are perhaps formed between side groups that have never before been united. As already indicated, this might also lead to distortion of the lattice and loss of the x-ray diagram. Speakman (90, 91) has assumed something of the same sort to occur in wool, heated to higher than ordinary temperature, a treatment which reduces the capacity of the fiber to sorb water hygroscopically. The black cross still remains in the anhydrous granule, for probably the contraction of the long axis of macromolecules, even though it distorts the lattice, does not greatly disarrange the parallel arrangement of the macromolecules or distort the crystallites upon which the birefringence depends.

When a granule in this state is moistened, it is necessary that these newly established linkages be broken up, if it is again to swell to its former state. Obviously, this would take time, and would occur imperfectly. Consequently, the granule would swell slowly and not fully to its original size.

We now see why injury to the granule enables it to swell at the site of the injury. The injury separates some of the macromolecules or micelles so that they are no longer surrounded on all sides by other macromolecules or micelles. They associate themselves to their maximum capacity with water. Again no limiting membrane is necessary to account for the phenomena, which are due merely to the fact that through the site of the injury the orderly packing of the chains has been disarranged. Itallie (42) has made an analogous suggestion but he also assumes the existence of a limiting membrane. It is plain why the degree of swelling in cold water depends upon the completeness of the disruption of the granules. Our hypothesis also

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22 This view was criticized by Katz (9, pp. 207-209), to whom Astbury (9, pp. 210-211) replied.
explains the observation of Katz and Hanson (50) that injured granules change the character of their x-ray diagram at a temperature 10° to 20° C. lower than that at which uninjured granules undergo this change, and also why injury wipes out to a large extent differences in this temperature which are shown by different species of starch in the uninjured state.

GROWTH

Structure of the type here suggested is consistent with what is known of the manner of growth of starch grains. If starch grains possessed an outer limiting membrane permeable only to substances of comparatively low molecular weight, it would be difficult to understand how small granules grow into large ones. There is firstly the difficulty of imagining how a limiting membrane of the type demanded by the properties of the natural granule stretches and enlarges simultaneously with the growth of the granule. Certainly, the long molecules of starch, if formed in the protoplasm outside, would find it difficult to pass through such a membrane. It would, therefore, be necessary to assume that mono- or disaccharide passes from the protoplasm across the membrane into the granule there to be united into long chains and oriented radially. This would demand that the directive forces residing in the cytoplasm act across the membrane, or else that cytoplasm itself extends through the membrane into the granule. For such assumptions, there does not as yet seem to be evidence. If, however, there is no limiting membrane of the sort under discussion, but the cytoplasm—perhaps in a condensed form—lies in contact with the growing granule, then it is simpler to picture the growth of a granule much as Sponsler (92, 93, 94) explains the formation of a cellulose fiber in the cell wall or Farr and Eckerson (22) explain the formation of the cotton fiber.

A granule might grow through lengthening of the starch chains by the condensation of a glucose or maltose residue at the peripheral end of the chain, so that the chains grow longer. Or perhaps the method of growth is more like that described for cotton fibrils by Farr and Eckerson. In that event, small starch crystallites are formed in the cytoplasm surrounding the small granule. These then arrange themselves radially around the granule and become attached to or inserted between the chains of particles already arranged radially in the growing granule.

As the granule grows, the chains must lie less closely packed at their peripheral ends. This leaves room between them for the formation of new chains by the same process by which the original chains were formed; and these new chains are also radially oriented between the outer ends of the older chains. Thus, crystallites are built up which are irregularly conical with their bases lying at the periphery of the granule. Such a form is clearly to be seen
in unlaminated starch in which the crystallites extend from the periphery to the hilum (12), a phenomenon which can be explained only on the assumption that new starch substance is added at the base of the needles.

This picture of the growth of starch granules is not the same as that suggested by Zwikker (112), but it is not inconsistent with it. Zwikker suggests that the leucoplast secretes a concentrated colloidal solution of amylose and amylopectin. Now starch lowers the surface tension of water, so that during an interruption of the activity of the leucoplast there is at first a concentration of the substance with the greatest effect on surface tension (e.g., a potassium salt of amylopectin) at the surface accompanied by its separation or precipitation. When the leucoplast again becomes active, this layer remains unaffected and the process is repeated. Zwikker, who wrote in 1921 when the x-ray analysis of organized structures had not been developed as it is today, has nothing to say regarding the formation of colloidal amylose or amylopectin. Now that we know something regarding the form of the starch unit, we can go a step farther and extend the picture to the manner of formation of the chains. It is entirely possible that surface forces play a rôle, as Zwikker suggests, in determining the parallel arrangement of the chains in building up particles of the type observed in the case of cellulose fibers by Farr and Eckerson.

If this is in fact the mechanism by which starch granules grow, there is no reason to believe that the chains or macromolecules of which the crystallites are composed are all of the same length. Indeed, the chances are greatly against it. It is probable that starch, like many other materials (cf. 98, pp. 109, 112–113; also 65), is composed of macromolecules of varying length. Neale (72) has presented evidence that this is true for the cellulose fiber. There is much evidence that this is in fact the case for starch. Thus Fouard (29; cf. 79, 81) has reported that solutions of demineralized starch can be fractionated by dialysis through collodion membranes of different permeability. The occurrence of chains of different lengths is also consistent with the views of Hanson and Katz regarding the structure of the granule. Probably the arrangement of the molecules in starch granules resulting from the manner of growth above suggested causes imperfect packing and may be a factor leading to the loose structure of granules already discussed.

Whether the elements in the unlaminated starch of Bakhuyzen extend, as they appear to do under the microscope, from the hilum to the periphery, it is not possible to decide from existing evidence. Some of the starch elements may be as long as this and consist of macromolecules which are joined together by van-der-Waal forces to form quite long fibers. This is well known to occur in the case of soaps, and has been suggested for starch (10). Moreover, it should be noted that Sponsler believes that the diffraction data obtained from x-ray analysis of cellulose may be accounted for upon the basis of chains of cellulose-unit cells of indefinite length.
THE LAMINATED STARCH GRANULE

BAKHUYZEN correlates the occurrence of the large needles in starch from wheat plants grown under constant conditions with the absence of lamination or rings. In field-grown wheat seed he finds the needles or trichites no larger than 5 microns, or about 10 per cent. of the diameter of the wheat-starch grain. Ten to 20 or more rings can be counted. Larger needles may also be observed. If such starch is carefully warmed to swelling temperature, the laminae may be observed to consist of small particles. Particles in successive rings may often be seen to be oriented along a straight radial axis, suggesting that such a string of particles was formed from a single needle which had been fragmented by heating. The parts of the needles which correspond to the non-refractive rings or laminae, however, remain.

Much evidence indicates that laminated starch is built up of bundles of parallel chains combined to form crystallites of a length about equal to the width of the laminae. If starch granules develop in the manner described for cellulose fibers by FARR and ECKERSON (22), as we have suggested, then it is quite probable that in some starches the thickness of laminae is determined by the length of the starch crystallite formed in the cytoplasm. Certainly in some, the breadth of the laminae is of the same order of magnitude (1 to 2 microns) as the longest diameter of the cellulose particle of FARR and ECKERSON (cf. also 23). In eccentric starches, the laminae at the pole nearer the hilum may be very narrow, indeed. If the growth of the starch granule takes the course suggested by FARR and ECKERSON for cellulose, then any periodicity which interrupted the process must of necessity lead to the deposition of starch substance in layers or laminae.

This view, based on the observations of BAKHUYZEN and others, is in harmony with the observations of HANSON and KATZ (34) who, as we have seen, describe the granule as consisting of short elements radially arranged and embedded in an amorphous matrix.

SOLUBILITY

It has been known since the first part of the nineteenth century that, while starch granules do not dissolve in cold water, a starch granule which has been injured by cracking, chipping, or grinding disperses more or less in water without warming. It has furthermore been shown that the degree to which the granule disperses depends upon the extent to which it has been disintegrated mechanically, for with very thorough grinding all the β-amyllose can be dispersed and extracted from corn starch by cold water (85, 102). Those who, like MATTHEWS and LOT (61), believe that only a minor part of the substance of injured granules is soluble in cold water are cer-
tainly mistaken. This solubility in cold water which results from injury to the granule is not due to alteration of the starch substance by frictional heat engendered in disrupting the granule (41, 43). Nor is it at all likely, as SAMEC (80) states, that grinding as such breaks up the starch complex into smaller aggregates, which disperse readily; the manipulation necessary to injure a granule so that some of its substance disperses is far too slight. All that is necessary is to put a little dry starch on a microscope slide, cover it with a glass cover slip, and press gently upon it for a moment, at the same time moving the cover slip a trifle sideways. Moreover, as we have seen (p. 309), merely drying sharply causes radial rifts which render some of the granule soluble.

Furthermore the colloidal solutions obtained from potato starch ground in a pebble mill do not behave as though the starch had been broken up into smaller complexes. The solutions are strongly opalescent and change rapidly. The concentrated solutions first obtained separate a solid phase until an equilibrium is apparently established such that less than 1 per cent. of starch remains in a non-opalescent or but slightly opalescent solution (24, 25). The phenomenon bears a close resemblance to that exhibited by gelatin and studied by STRAUP (100). For different starches, the equilibrium seems to be a little different with respect to the concentration of dissolved starch at the final equilibrium and to depend to some extent upon the total mass of starch in the system. Some starches require a longer time to reach equilibrium than others, and the longer the time the more difficult it is to obtain non-opalescent solutions. These, for example, are more easily obtained from maize and wheat starches than from potato starch (4, 6, 26).

Examination with the microscope shows that the dispersal of starch from an injured granule is preceded by localized swelling at the site of the injury; it does not occur anywhere else. The dispersing starch does not stream out

24 On the other hand, it must be admitted that severe dry grinding affects the properties of gels. Gelatin is but slightly soluble in cold water. If some gelatin such as is used by bacteriologists be ground dry for a long time (50 hours) in a porcelain pebble mill, it yields an opalescent solution when suspended in cold water. This solution filters with difficulty but the small quantity of filtrate is perfectly clear and not very viscous. On standing overnight, it sets to a clear gel (cf. 5). It may perhaps also prove significant that SCHOCH (85) prepared dispersions from potato starch ground for 500 hours, which did not readily retrograde, and found that 900 hours of grinding produced a certain amount of dextrinization, as evidenced by reddish iodine coloration. Moreover, TAYLOR and SALEZMANN (104) found that long grinding in the presence of moisture affected and modified the stability of starch or amylose toward alkali. Possibly such severe treatment is accompanied by a change of some of the starch from the crystalline to the vitreous state as these states are defined by BEILBY (15). This change is sometimes accompanied by changes in solubility and in heat of solution. Grinding a crystal or hammering a metal tends to cause some parts of it to flow. If a substance cannot flow by local melting, perhaps it undergoes some localized form of decomposition. Starch does not melt.
into the surrounding water as though out of a hole in a bag. Rather it behaves as though at the points of injury it were swelling by imbibition. When the imbibition has reached a certain stage, dispersion gradually occurs. The swollen portion never wholly disperses, even after days; nor does the swelling extend into the interior of the granule. The reason why cold-water solubility is related to the degree of disruption of the granules is now clear. Unless the granule has been very completely disrupted, only those portions swell that have been disintegrated; and swelling seems to be a necessary step antecedent to dispersion. It is obvious that the localized dispersion of injured granules is as difficult to explain by the assumption of a membrane or related restraining mechanism as localized swelling—and for the same reasons. It would seem that our hypothesis regarding the structure of granules, if it is satisfactory to explain the swelling phenomena, is equally satisfactory to explain why an intact granule does not disperse, but disperses at once at the site of injury when it is mechanically damaged. Injury having more or less disarranged the close-packed radial parallel chains, some of these are free to associate themselves with water along their long axes and ultimately to disperse. In this connection it may be noted that Nägeli (70, pp. 111-112) reported that saliva attacks granules tangentially, i.e., transversely to the radius of the granule, more readily than in the direction of the radius. This is what one would expect if in fact the starch chains lie radially.

**Optical properties**

Some investigators have assumed that the material in a moist natural granule, being swollen, is under a strain. To this strain, they attribute the appearance of the black cross which is so characteristic when a starch granule is viewed through the polarizing microscope with nicols crossed (20). The alleged disappearance of this cross when the granule is dried has been regarded as proof of this view. As Harrison (36) puts it: "Since the granules have a resistant outer coating, any swelling of the interior portions will produce an increase in the internal pressure. When the outer coating is broken, and this occurs when the granules are ground in a mortar, no pressure will be produced by the swelling of the interior portions, but under these conditions no cross is shown by the granules."

Harrison's observation that dry starch granules swell when moistened is undoubtedly correct, as we have seen, and so is his observation that disrupted granules may not show the black cross in polarized light. But the assumption which he and many others make that "the granules have a resistant outer coating" has been shown above to be unwarranted. His statement that the black cross becomes indistinct, if the granules are dried, is also unwarranted. Puriewitsch (74) has reported that arrowroot starch dried for 76 hours at 105° C. has exactly the same appearance in polarized light as wet
starch immersed in water for 2 days. Katz and Derksen (48, p. 109) made a similar observation.

It is probable that Harrison was deceived, because it is difficult to get a clear picture under the polarizing microscope of starch examined in air. Therefore, Griffing (31), at the writer's suggestion, heated potato starch for several days to a little over 110° C. It was then transferred as rapidly as possible, and still hot, to anhydrous glycerol or to cedar oil and examined immediately in these media; it exhibited perfectly normal birefringence.25

It has long been known that when starch is gelatinized by heating in water its birefringence is lost and the black cross disappears. Indeed, the loss of birefringence has been proposed as a criterion for fixing the gelatinization temperature (76, p. 298); but Woodruff and Webber (110) found that the temperature at which birefringence disappears varies with the amount of water available for swelling.

As already pointed out, Harrison (36) has shown that the black cross disappears when starch granules swell in the cold as the result of injury. Huss (41) made similar observations and showed that the black cross vanishes whenever the starch granule is made to swell beyond its normal size, whatever the cause. He pointed out that the disappearance of the black cross and the taking up of dyes are parallel phenomena.

Indeed, mechanical disruption greatly modifies the optical properties of granules. Harrison's observation that grinding starch in a mortar causes the black cross to disappear has already been mentioned, but the observation is much older (67). Indeed, Scheffer (82) has proposed examination in polarized light as a method of detecting injury to starch granules because such injury is difficult to recognize in ordinary light. In polarized light, injured granules appear either with but slight birefringence or else with only a part of the granule birefringent. The fact that birefringence may be lost only in part of the granule and the black cross remain in part is probably the reason why a few investigators26 have reported that injury does not obliterate the black cross. The truth seems to be that just as the degree to which a granule is soluble in cold water and swells in it depends upon the completeness of the disruption of the granule, so also does the loss of birefringence. Badly shattered granules lose their black cross and most of their birefringence, but examined with a selenite plate some birefringence may still be detected (31), and starch that has been severely ground loses its x-ray diagram (66, 95).

The localized loss of birefringence in injured granules may be demonstrated by water extraction of starch ground for many hours in a pebble mill

25 Fischer (27, p. 83), however, states that dried granules do not show lamination but appear homogeneous, and so does Någell.
26 For example, Någell (68).
until it appears to have lost the black cross. The ground starch is suspended in water and separated from the solution by centrifuging. The process is repeated till the extract is not blued by iodine. The undissolved residue, dried with alcohol and ether and examined in polarized light, appears dull white on the dark field, looking much like the ground starch before it was washed, except that there had been washed away the amorphous, granular, non-luminous material which had been adherent to the surface of the granules. When this material was mounted in cedar oil, the granules appeared dull yellow, transparent, with numerous traces of bright birefringence and distorted black-cross markings. The explanation is obvious. Grinding covered the surface of most granules with abraded starch substance, which is not birefringent or only slightly so, and therefore masked the birefringence of the material in the interior of the granules. After this had been washed away by extraction with water, the granules again appeared birefringent, although this was less pronounced and no longer typical because the granules for the most part were more or less affected by the treatment.

Apparently, anything that sufficiently breaks up the anatomical structure of the granule greatly modifies its birefringence, if it does not wholly destroy it. It is, therefore, probable that at least some of the optical properties are due to parallel arrangement of crystals or other elements in the granule. This is also the opinion of Katz (47).

We must, then, distinguish two sorts of birefringence in the starch granule—that which is due to parallel arrangement of the starch crystals, and that which is inherent in the starch substance itself and still visible with a selenite plate in badly shattered starch. The latter is relatively slight.

The black cross is due to the parallel arrangement of the starch crystals manifest through the microscope in the radial striations. It is due, therefore, to a comparatively coarse structure and disappears whenever this is disorganized. Hence, it is understandable why the black cross remains in completely anhydrous starch, which, according to Katz, no longer exhibits an x-ray diagram. In such a dry granule, the radial striation and the laminations are still visible. Conversely, when a starch granule is gelatinized without enough water to permit free swelling,27 the radial striations, the laminae, and the black cross are no longer visible but the granules still exhibit an x-ray diagram, although an altered one. It is obvious, then, that the disorganization which causes loss of the capacity to exhibit an x-ray diagram is of a finer sort than that which is sufficient to destroy the black cross.

The hypothesis herein advanced is, of course, to a considerable degree a speculation, but a speculation which seems consistent with and capable of offering a reasonable explanation of all the facts known to the writer. It

27 Katz terms this "first-degree gelatinization."
emphasizes the organization of the granule without consideration of which it seems impossible to form any sort of rational mental picture of the behavior of the starch granule. The importance of the organization of biological structure in determining behavior has been emphasized by others (40). The hypothesis offered may in the end prove to be ill-founded, but it poses many questions which can be answered by experiment and this is sufficient justification for its presentation.

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