THE INFLUENCE OF ENVIRONMENT ON THE SHELL STRUCTURE OF STARCH GRANULES

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ABSTRACT

It is well known from light microscope studies of potato starch that the granules formed in a constant environment (of light and temperature) have a ring formation indistinguishable from that of granules formed under field conditions. Electron microscope studies have confirmed that normal potato starch granules have a fine shell structure not usually resolved by the light microscope, and also that shells do not develop in barley granules grown in a constant environment. The paper presented here reports a further study of the dependence of shell formation on environment. Potatoes were grown in a constant environment and starch granules from the newly formed tubers were examined in the light microscope, and in the electron microscope after corrosion by acid. No difference between these granules and normal granules was observed; both wide (light microscope) rings and fine lamellae developed in both granules. Parallel studies were made on wheat starch granules. In this case, shells were not differentiated in granules that developed in a constant environment, but they could be produced at will by imposing a dark period. Thus, shell formation in potato granules must be controlled by an endogenous rhythm, whereas in wheat granules it must be controlled by external environment.

INTRODUCTION

Towards the end of the last century Meyer (16) suggested that the ring structure of starch granules resulted from a day-night alternation in the supply of starch precursors. If this were so, the granules would be built up of daily growth rings, or shells, when considered in three dimensions. However, evidence has accumulated over the years (see 8) that shell formation in starch granules is independent of day-night alternation, as shells are also seen in potato starch granules that have developed in a constant environment. The only conflicting observations have been those of Bakhuyzen (5), who saw no rings in wheat starch granules that developed in constant light and temperature. The subject has recently received further consideration

since it has been possible to employ the high resolving power of the electron microscope in the study of the shells of starch granules. Thus it was shown (8) that no shell structure was present in starch granules of barley that had been grown in a constant environment cabinet. The thirty-five-year-old results from studies with wheat were thus confirmed. There was, however, an indication that cereal and tuber starch granules were not comparable in respect to shell formation. Frey-Wyssling and Buttrose (11) then did an electron microscope study of normal potato starch granules and found, in unswollen granules, two classes of shell as revealed by acid treatment; on the one hand there were shells that were spaced widely at

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the rate of less than one per micron, and on the other there were shells with fine lamellae (making up each shell of the first class). The widely spaced shells corresponded, in their dimensions, to the rings seen in the light microscope, which develop independently of day-night alternation. It was possible that the shells with finer lamellae corresponded to the shells of cereal granules in relation to environment. An investigation has therefore been made of the structure of starch granules from potatoes grown in a constant environment, and a parallel study has been made of wheat starch granules.

MATERIALS AND METHODS

Potato (*Solanum tuberosum*) tubers were planted in earthenware pots and placed in a glasshouse for two weeks. They were then transferred to a growth cabinet and exposed continuously to white fluorescent tube lighting at a temperature of 18° ± 1 °C. Growth of the plants was poor, with responses to constant environment similar to those recorded for tomatoes (13). The newly formed tubers were harvested on the 60th day after planting, when tubers up to 1.5 inches in diameter were obtained.

Wheat (*Triticum vulgare* var. Gabo) was grown in earthenware pots in the glasshouse until shortly before heading. The plants were then transferred to the growth cabinet, where they received until maturity the same light and temperature treatment as the potatoes.

Small cubes were cut from tubers or endosperm, air-dried, and treated with 8 per cent hydrochloric acid at 38°C for 48 hours. They were then thoroughly washed, dehydrated, and embedded in methacrylate, and sections were viewed in an electron microscope.

Gravimetric measures were made of the effect of acid corrosion on wheat starch granules. Grains were milled, blended in a water-toluene (6:1) mixture, and centrifuged, and the starch was purified by repeated washing and sedimentation and dried *in vacuo* at room temperature. Aliquots (43 mg) were treated in stoppered centrifuge tubes at 38°C with 8 per cent hydrochloric acid (3 ml) for 48 hours;

the residual starch was washed by repeated suspension and centrifugation, and weighed.

RESULTS

The appearance of potato starch granules developed in a constant environment, as seen in the light microscope, is shown in Fig. 1. As found by previous workers, the rings in such granules, when seen in optical section, are indistinguishable from those seen in granules from outdoor-grown potatoes. The three large granules grouped at the centre of the figure are about 20 μ long, and appear to have from 4 to 6 clear rings spaced 3 to 4 μ apart. When viewed in the electron microscope, all such starch granules are structureless, without any indication of shell formation. Their appearance following acid treatment is shown in Fig. 2. In all essential respects, this appearance cannot be distinguished from that of normal potato granules after acid corrosion (see 11). The granule shown is in the earlier stages of corrosion, and the fine lamellae are only starting to become visible towards the periphery. The granule section in Fig. 2 has a length of 13 μ, and can thus be compared with the smaller granule at the bottom (centre) of Fig. 1. In the light microscope, one can see possibly three distinct rings in a 13 μ granule, and in Fig. 2 are found three obvious shells with a width of from 2 to 3 μ. The observation of immediate interest, however, is that the fine lamellae making up each large shell have developed even in a constant environment.

Fig. 3 shows a typical section of a wheat starch granule, formed under normal outdoor conditions, which has been treated with acid. The shells, seen in section as rings, become evident early during treatment as the acid-susceptible portions of each are attacked. The three peripheral dark bands are especially clearly defined and, unlike other bands towards the centre, have a remarkably uniform width of 800 to 1000 A. These are macromolecular dimensions, and it could be suggested

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FIGURE 1

Starch granules from a potato tuber developed in constant environment, as seen in the light microscope. × 1400.

FIGURE 2

Potato starch granule identical in origin with those in Fig. 1, but viewed in the electron microscope after acid corrosion. Boundaries of shells, corresponding to those seen in the light microscope, indicated by arrows. × 12,000.
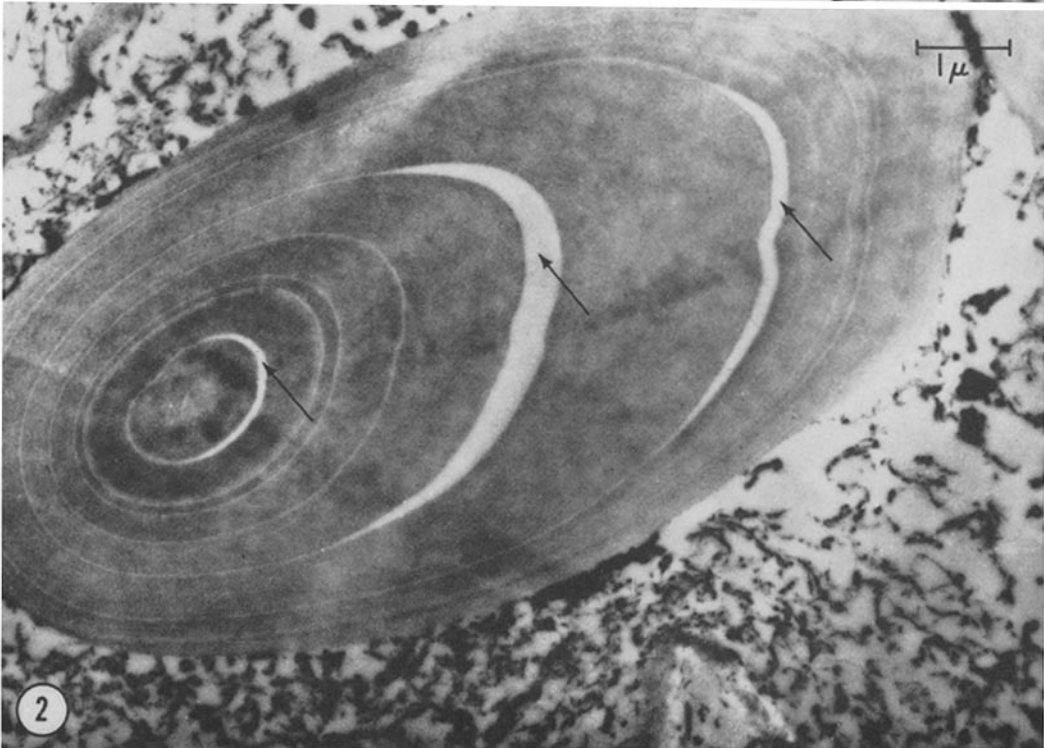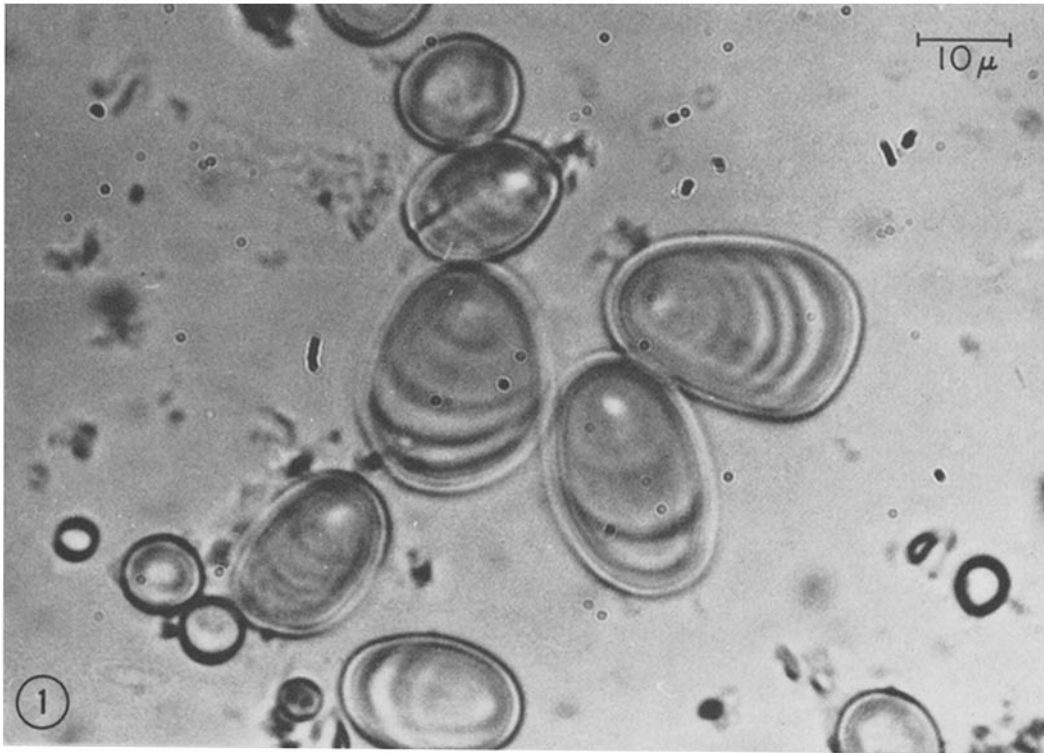
that molecules are more uniformly organized in the peripheral layers than towards the centre.

The appearance of "constant environment" wheat starch granules is seen in Fig. 4. There is no indication of a shell structure, either in the large granule or in the neighbouring small ones. This confirms earlier results obtained with barley (8). In passing, it is interesting to note what appears to be a fissure running in from the pointed edge of the large granule (see also Fig. 5). This fissure must correspond to the median plane of weakness developed during growth of the granule (8). Such a fissure is typical of this material and must represent a differential action of acid in the median plane. The starch residue in Fig. 4 has a fine granular appearance: the particles seen most clearly towards the periphery have a diameter of 250 to 300 A and are separated by somewhat smaller distances.

As it could be argued that the failure to observe shells in constant environment wheat granules was due to the failure of acid to attack the granules, the loss in dry weight of these granules during acid corrosion was compared with that of normal granules, and the results are presented in Table I. A check showed that the number and sizes of the granules had not decreased, so that the measured loss in weight must represent material leached out of the granule structure. Assuming that the granules shown in Figs. 3 and 4 are representative of their classes, it could be said that about three-quarters of the former had been solubilized and about one-half of the latter. In the case of the normal granule this is immediately apparent, but in the case of the constant environment granule there is no visual evidence. In the latter there is typically no variation in electron opacity from the periphery to the centre, nor any indication of acid attack on the outer surface. It must be assumed that the acid attack takes place uniformly throughout the granule, but at a slower rate than in the normal granule.

The foregoing results showed that day-night alternation was associated with the formation of

shells, and constant environment with their absence, in wheat starch granules. The final proof lay in producing shells to order. Wheat plants were held in the growth cabinet with constant light and temperature until 14 days after anthesis, and then they were treated as follows:

| Day 14 to day 15 | 24 hours darkness |
" 15 " " 17	48 " light
" 17 " " 18	24 " darkness
" 18 " " 20	48 " light
" 20	Ears removed and allowed to dry in darkness

The temperature during the dark periods was constant and the same as that during light exposure. Starch from the dried grains was treated with acid in the usual way. A portion of a granule is shown in Fig. 5. Changes in electron opacity can best be followed at the left-hand edge. The periphery is relatively dark, followed in turn by a light, a dark, a light, and a dark band, the granule then appearing homogeneous to the centre. This sequence can then be considered in reverse order in conjunction with the light treatment imposed. The granule would not have been initiated earlier than 6 days after anthesis (see 15), and possibly several days later than that. A possible interpretation is that from initiation to the 14th day a shell-less granule was built up, and that on withdrawal from light the inner dark band was laid down, as starch precursor supply diminished. Further, it is possible that, with resumption of photosynthesis on the 15th day and continuing to the second dark period, the next paler band was deposited, followed in turn by the darker band associated with the second withdrawal from light, then a pale band of fresh deposition, and then the peripheral dark band associated with declining deposition following harvest of the whole ear. This interpretation is supported by the observation (see especially the right-hand edge of the section) that the sharpest change in contrast is from dark to pale going outwards, and it would seem most

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FIGURE 3

Portion of a normal outdoor-grown wheat starch granule following acid corrosion. Three peripheral bands indicated by arrows. × 7000.

FIGURE 4

Portion of a large wheat starch granule and neighbouring small granules developed in constant environment. No rings visible. × 12,000.
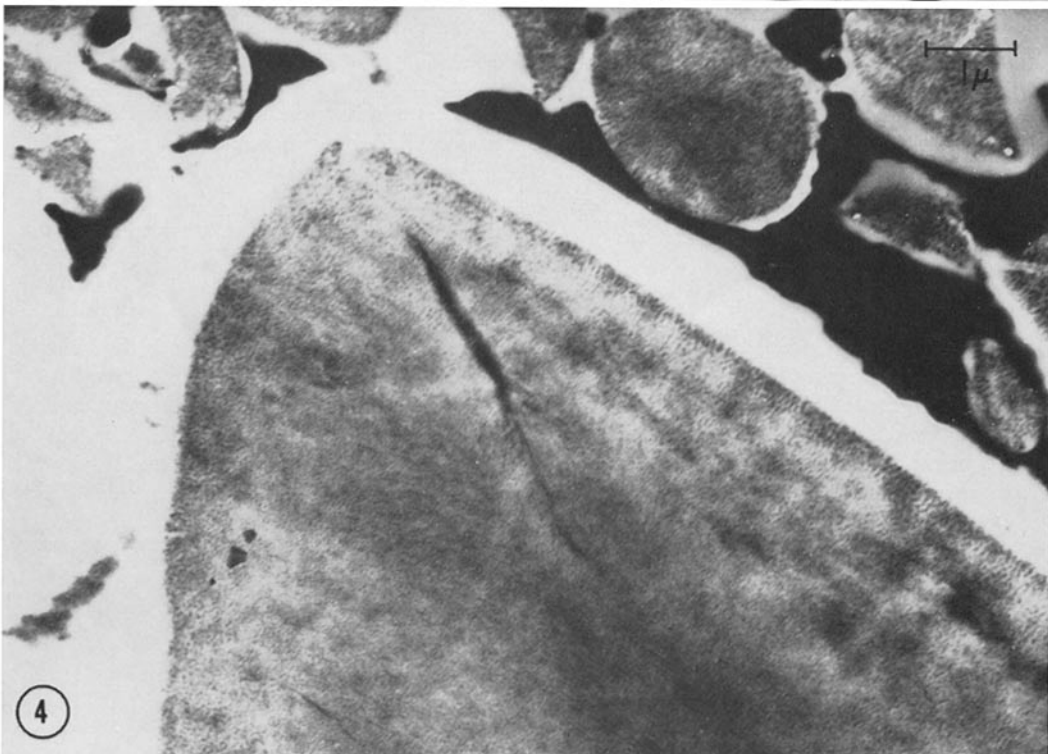
likely that this would correspond to the dark-to-light environmental change. The central portion deposited before day 14 appears relatively dark in the section shown in Fig. 5, but in the average section it approached the paler bands in electron-scattering power, and the three dark bands stood out in contrast. The shells of wheat granules must then be daily growth shells, and comparable with daily growth rings of cotton fibers (1).

The granule shown in Fig. 5 is typical of this material, but forms such as that seen in Fig. 6 are also observed. In this case the dark bands are much narrower. The difference might be due to the sectioning angle of the oblate spheroid granule.

TABLE I

Loss in Dry Weight of Wheat Starch Granules (43 mg Initial Weight) Treated with 8 Per Cent HCl for 48 Hours at 38°C

	Dry weight loss	
	mg	%
Normal granules	31.7	74.0
Constant environment granules	22.2	51.6

or to the age of the granule and its growth activity. A complete shell (light plus dark band) in Fig. 5 has a width of about 0.35 μ, while in Fig. 6 the width is about 0.5 μ. These figures are similar to those from an earlier work (15) where the calculated diameter increase of barley starch granules from the 15th to the 17th day was 0.6 μ and 0.4 μ for granules initiated at about 7 days and 10 days, respectively, after anthesis.

DISCUSSION

The main result of this work is the finding that whereas the alternation of day and night has no influence on the production of shells in potato starch granules, it is responsible for their production in wheat granules. Conclusions that shell formation is related to an endogenous rhythm

(7, 12) cannot therefore be applied to starch granules in general. In the case of granules from the potato stem (12), it has been calculated that 1.3 shells (as seen in the light microscope) are deposited per 24 hour period. This result can only apply to the wide (1 to 2 μ) shells, their deposition being regulated therefore by a rhythm of an 18.5 hour cycle. If these measurements are reasonably accurate, it can now be seen that to account for the finer lamellae there must be a short term endogenous rhythm, with a cycle of only 2 hours or so, imposed on the longer rhythm.

In discussing the difference between the shells of potato and wheat granules, the simpler case of wheat may be considered first. The classical idea that daylight photosynthesis provides an abundant supply of starch precursor, resulting in dense packing of starch molecules, followed by a fall-off in supply during darkness, with a consequent looser, more hydrated molecular packing, is consistent with the present findings with wheat granules. Thinking along these lines with regard to potato starch granules, one must assume that it is not a block in carbohydrate precursor manufacture that is responsible for shell differentiation, but rather a rhythmic block in its translocation, or in its conversion to starch. There is evidence of spasmodic translocation of sugars, and starch deposition, in the potato tuber itself (4). In this connection it should be noted that the tuber granule is relatively remote from photosynthetic organs supplying it, whereas the cereal granule is relatively close (10). However, as shelled starch granules can form not only in tubers but also in stems of potatoes growing in a constant environment (12), it may well be that a block to deposition occurs within the leucoplast.

If, in fact, shells in potato starch granules result from a rhythmic regulation of starch molecule production, the packing density of starch molecules across the shell could vary. Thus structurally there may be no basic difference in the shells of wheat and potato starch granules, but only in the mechanism regulating their structure. There

FIGURE 5

Portion of a wheat starch granule grown in constant environment but given two dark periods before harvest. Suggested relationship of rings to environment indicated: L, light; D, darkness. × 25,000.

FIGURE 6

Another wheat starch granule of the same origin as that in Fig. 5. × 28,000.
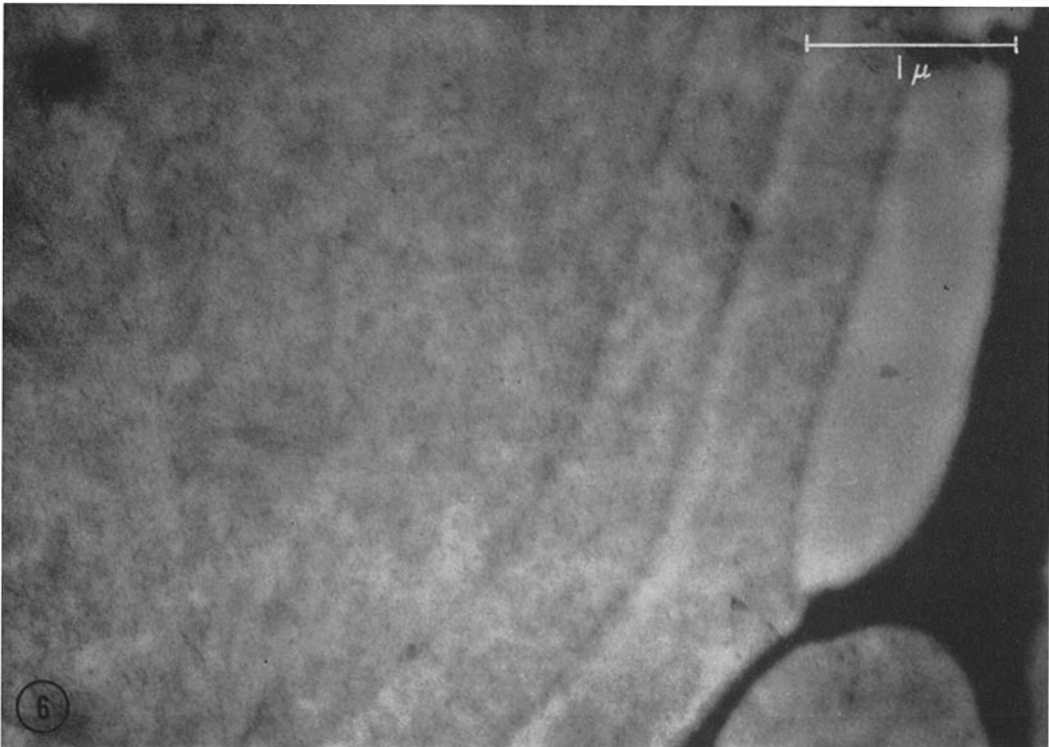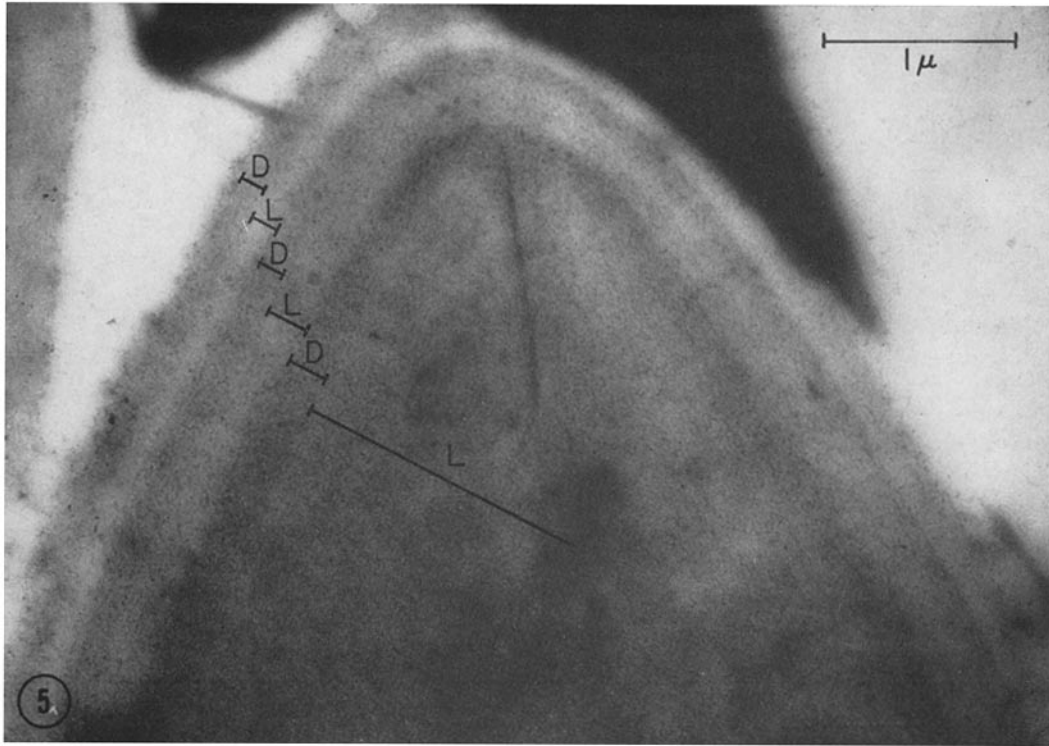
remains the fact that two different kinds of shell appear in the potato starch granule. After acid treatment the finer lamellae appear similar to those seen in shells of wheat granules, while the wider structures are anomalous. There is another notable difference between the two starch types, in that cereal starches as a class give an A x-ray pattern and tuber starches a B pattern. X-ray diffraction studies of acid-corroded granules might indicate whether there is any relationship between shell type and diffraction pattern. It is significant that x-ray patterns of bean starch can change with change in the temperature during plant growth (14), so that there are now at least two cases in which environment influences starch granule structure.

In considering relative susceptibility to acid attack across a granule shell, there is a tendency, when viewing the electron micrographs, to associate residual starch with high electron-scattering power, and degraded areas with low electron-scattering power. In discussing these micrographs, it was suggested above that electron-opaque portions corresponded to the outside of shells, and electron-transparent portions to the inside. There is thus a tendency to assume that acid preferentially attacks starch deposited at the inside of a shell. However, enzyme corrosion patterns (8) show that the inside of a shell is most resistant to amylase attack, becoming steadily more susceptible radially, with a sudden discontinuous rise in resistance at the next outer shell. Conclusions as to susceptibility to acid corrosion must therefore be cautious. On the one hand, it is possible that acid can hydrolyze where amylase cannot, and vice versa. On the other hand, it should be noted that untreated starch normally appears without contrast in the electron microscope, possibly owing to vaporization, whereas, as is seen in the micrographs, acid-corroded starch has a reasonable contrast. Residues of acid corrosion appear therefore to be more stable than native starch when exposed to an electron beam. Thus, pale bands in Figs. 2 and 3, for instance, could possibly represent unaffected starch, and the darker bands the more corroded portions.

In this discussion it has been assumed that the radial gradient demonstrated in shells of starch granules by enzyme and acid corrosion reflects a molecular packing-density gradient and not a molecular type gradient. This assumption that a molecular type gradient does not exist is based on the generally accepted view (2) that the two molecular types, amylose and amylopectin, are intimately mixed, that their relative proportions in wheat starch remain unaffected by changing day length (17), that the gradient is most strongly developed in waxy maize starch lacking amylose (8), and that their concurrent deposition in the granule is crystallographically possible (9). However, in view of a recent suggestion (3) that there is an amylose-to-amylopectin gradient from the inside to the outside of a shell, the assumption was reconsidered. According to this suggestion, it could be that amylose is laid down only at the beginning of a starch deposition phase and is followed by amylopectin. Where, as in the present work, a continuous starch deposition is induced, an abnormal amylose-amylopectin ratio should result. To determine whether this is so, the light transmission curve of iodine-stained (6) constant environment wheat starch was compared with that of control wheat starch. The curves were indistinguishable, indicating a normal ratio of the two molecular types when no shell structure exists.

The conclusion is drawn that shells in wheat starch granules are due to a gradient in packing density of the mixed amylose and amylopectin molecules, the packing density being related to the photosynthetically controlled supply of starch precursor. A further conclusion is made that shells in potato starch granules are controlled by an endogenous rhythm, unrelated to photosynthesis. It is suggested, however, that this rhythm also controls the supply of starch precursor, with a resulting variation in packing density of starch molecules.

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