Growth of ^{14}C-labelled Starch Granules in Potato Tubers as Revealed by Autoradiographs

By

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With 6 Text-figures

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Introduction

The way starch granules grow has always been a subject for much speculation. In his classical work, Arthur Meyer [10] gave strong arguments in favour of growth by apposition (centrifugal deposition of successive layers). This theory is capable of explaining many observations [1] and implies structural equality of all the layers of a granule (water content excluded) as has been demonstrated for potato and other starches in general, with the notable exception of waxy maize [2]. Growth by apposition is irreconcilable with the existence of an outer membrane with special properties [3].

In contrast, growth by intussusception (deposition of starch inside the granule) is believed to require the presence of such a membrane [14], which often in the past has furnished investigators with a convenient explanation for various observations made during the swelling of starch granules (for a recent example see [9]), although its existence cannot be demonstrated [1, 3].

Even when growth takes place by apposition, the process may involve rapid periodical crystallization of the granule from a coacervate, as suggested by MacMasters (see [2]), or this process could be a slow one, layer after layer being deposited through the same fundamental process of crystallization from a saturated solution.

It has not been possible to watch starch granules growing in living tissues with the indirect method of observation available, and it was there-

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fore necessary to find a method which would make the growth of a starch granule more directly visible. It was believed that this could be done if the substrate was labelled and growth followed by autoradiography. The technique, as developed by Pelc [12] offered great possibilities [5] and was therefore applied.

Materials and Methods

Young potato plants, grown from tubers, were put into the assimilation chamber of an apparatus, developed by one of us [6] and allowed to photosynthesise in an atmosphere containing $^{14}CO_2$, using activities in the range 75–400 μC, and total CO_2 concentrations from 0.1–1.5% (calculated for empty apparatus).

Parts of the stems were shielded from the light to prevent photosynthesis, and surface activities of these parts, and of leaves, stolons, and tubers, were measured with thin mica and window counters at various periods after the treatment. In some cases, roots and tubers were washed free of soil, in other cases plants were left undisturbed, and returned to the greenhouse, where they stayed for as long as 8 days before further investigation. As a rule plants were not de-starched before treatment. The flower pots were wrapped in plastic material, to avoid loss of $^{14}CO_2$ into the soil.

A thin slice was cut from the middle part of each tuber and autoradiographs were prepared from these slices on X-ray film. In some cases one half of the tuber was dried, and starch was isolated from the other half, in other cases the whole tuber was used for starch isolation. For some of the bigger tubers, (maximum weight 17.5 g.), specific activities of ground dry tissues and the corresponding starches were measured at infinite thickness and compared. The size of the tubers investigated varied from 4–34 mm. on the long axis. Extracts were made of some of the dried material by refluxing in 80% alcohol. After concentrating *in vacuo* the residue was taken up in a little water. Sugars present were separated by filter paper chromatography, using a pyridine-butanol-water mixture as solvent. Spots were located with α-naphthoresorcinol or benzidine as spraying reagents and also by means of autoradiography.

The stripping-film technique of Pelc [12] was applied to dried-in starch preparations on slides covered with an adhesive layer. Starch granules were studied before and after treatment with saliva at 40° C., and also after partial gelatinization with or without simultaneous action of saliva.

Results

A. General distribution of activity

Surface activity of the leaves dropped sharply during the first 10 hours, and then more gradually during the following 10 hours, tending to approach a constant residual level at about 1/3 the initial value. In the meanwhile, darkened portions of the stem increased in surface activity, so that evidently active material was transported downwards.

The most surprising result was, that even after 8 days the activity of the tubers varied over a very wide range, some being highly active, whilst others were quite inactive. There was no relation between tuber size and degree of activity. This result still remains unexplained. The starch in the mother tuber remained inactive.

Autoradiographs of slices developed after 2 days exposure time showed a weak initial equal distribution of activity throughout some tubers, with the periderm standing out clearly. In this stage (I), the specific activity of the dried tuber tissue was of the order of 1 μC/g., all of this activity being in the cytoplasm, and none in the starch granules or parenchyma cell wall material.

For other tubers of the same plant, this equally distributed activity was found to increase in intensity, until a specific activity (dried material) of the order of 10 μC/g. was reached, beyond which value starch granules also tended to become active (stage II). At still higher specific activities starch granules became highly active, a process which gradually extended throughout the tuber, starting at the end opposite the stolon (stage III). Chromatograms showed that of the sugars present, sucrose carried the bulk of activity, with very little activity in the glucose spots, and still less in the fructose spot.

B. Starch granules

1. *Untreated*

Active stolons (specific activity about 13 μC/g.) were found to carry inactive starch granules. In tubers of similar specific activity (stage II)

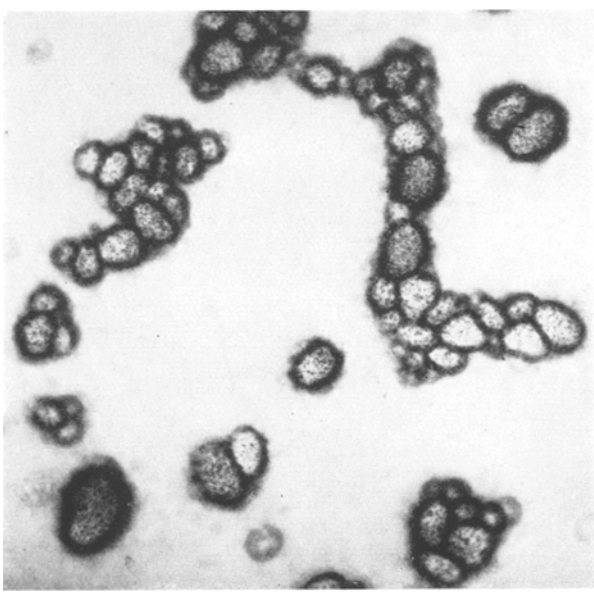

Fig. 1. Radioactive starch granules from a potato tuber. Specific activity of dried tuber tissue 60 μC/g., that of the starch 88 μC/g. 250×.

a great many starch granules were still inactive, and the remainder might show various levels of activity, from barely perceptible to more clearly visible, with sometimes a sprinkling of highly active granules.

In very active tubers, practically all granules were active, but they again showed various intensities (Fig. 1). There was no relation between degree of activity and size of the starch granule.

Individual granules showed highest activity at the distal end (Figs. 2 and 4). If the activity had been equally distributed throughout a granule,

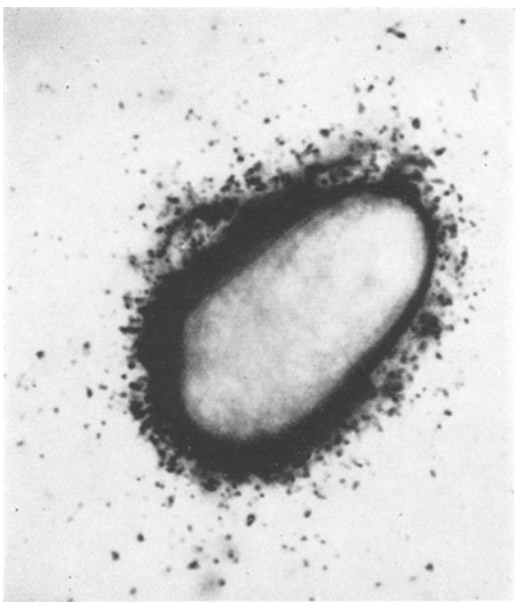

Fig. 2.

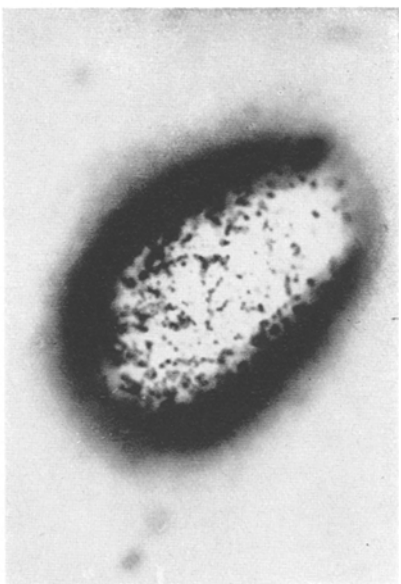

Fig. 3.

Fig. 2. Labelled starch granule showing greater activity at distal end, focussed on margin. 1250×.

Fig. 3. The same starch granule as in Fig. 2, but focussed on upper surface. 1250×.

one would expect a greater concentration of silver grains above the centre of the granule. In reality, the concentration is greater along the margin (Fig. 3). The combination of these facts provides a strong argument for growth by apposition, when we realise that layers are broader at the distal end and more crowded and thinner at the proximal end. A. Meyer has already attributed this to the unequal distribution of amyloplast material.

In a number of cases some starch granules were found to be much more active than was expected from the study of granules from tubers of similar specific activity. Their activity often had a very irregular distribution, e. g., in the shape of cross bands (centre of Fig. 4). It is possible that these strong localised activities were due to residues of amyloplasts adhering to the starch granules. Detection of activity in granules

was much more difficult in tissue smears or in paraffin sections, the active cytoplasmic material masking the granules effectively. It was possible to

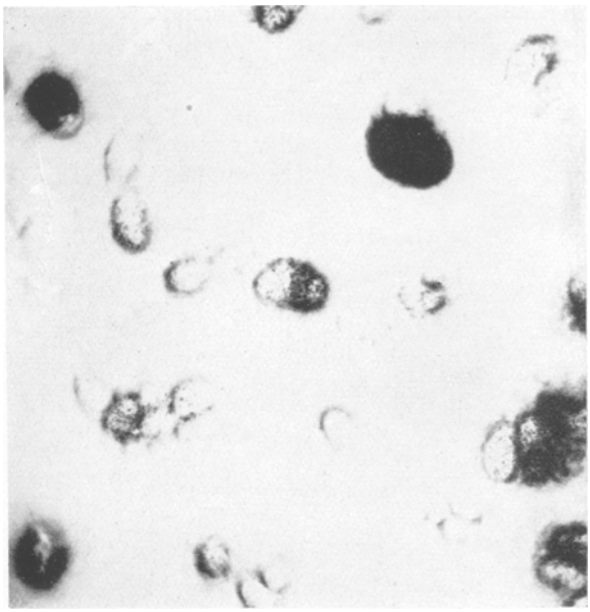

Fig. 4. Possible amyloplast residues of high activity attached to some granules. Other granules show the ordinary activity at their distal end. 250×.

"clean" the granules by treating the smears with a solution of trypsin at 40° C., but the protein, which precipitated after the unavoidable drying process, could not be washed out.

2. *Swollen and saliva-treated granules*

For the following experiments the same starch sample (see Fig. 1) was used throughout. The distal end of the swollen sac-shaped granule (heated in water) showed a markedly greater activity as compared to the proximal end, until the sac became too much extended. As a consequence, it was often possible to indicate the site of the distal end even in gelatinised starch granules. During swelling, much material (mainly the linear fraction) goes into solution, and therefore these preparations showed a high background.

When starch granules were subjected to the action of saliva at 40°, corrosion took place as described earlier [1, 3]. The corroded residues gradually became inactive, the process starting at the proximal end (Fig. 3). Agglomerates of active material were often found near the inactive residues. When starch granules are heated in water at not too high a temperature, they may become partly gelatinised, the proximal end swelling first. When the same experiment is done in saliva, those swollen proximal ends will be degraded rapidly, leaving an unswollen residue with

the inner mass exposed. Wherever such residues were found, the inner mass showed no activity (Fig. 5). As was to be expected, the dissolved material gave a high background in such preparations, which is also shown in a potograph of the same granules, focussed at a higher level (Fig. 6). This comparison is necessary as the film bulges over the relatively big objects.

Discussion

All observations point to the conclusion that the growth of all starch granules, small and large, in potato tubers takes place by apposition. The newly formed layer is thickest at the distal end and there we find the

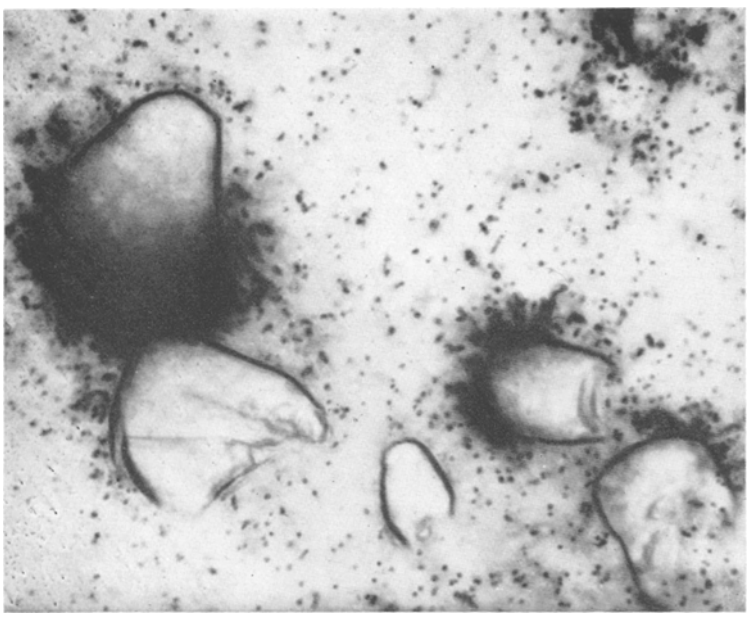

Fig. 5. Starch granules of potato after labelling and treatment with saliva near gelatinization temperature. Top left: activity disappears first at the proximal end; active material of the distal end partially dispersed. To the right a granule showing the inactive inner substance. Below: two residues which have become inactive. Focussed on margin. 1250 ×.

greatest activity. The inner mass of the granule, whenever exposed, shows no activity. When the outer layers are removed by applying amylase action, the thinnest parts at the proximal end lose their activity first, and at last all activity disappears.

This result clearly is in contradiction with the existence, sometimes assumed, of an outer membrane with special properties.

That some tubers have not become active after 8 days is a mystery that can only be solved by further studies of carbohydrate transport into the tubers under different conditions. In the meanwhile, the varying activity

displayed by tubers harvested from one plant, made it possible to follow different stages of carbohydrate influx and starch deposition simultaneously. From their comparison we can reconstruct the following picture.

Sucrose travels down the stem and enters the tuber, spreading evenly throughout the parenchyma. Very likely amyloplasts, which contain the necessary enzyme systems, convert sucrose, by a process as yet unknown, to a starch-like substance, and this starts to crystallise out when a certain concentration has been reached. The fact that starch granules only very

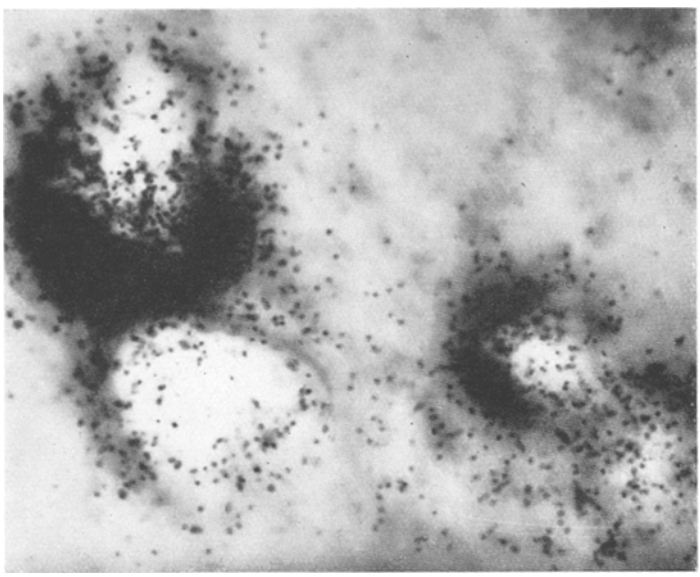

Fig. 6. The same granules of Fig. 5 focussed on the upper surface. 1250 ×.

gradually become active, points to the possibility that starch is deposited in very thin lamellae, which gradually build up a layer. This process starts at the far end of the tuber, as in radishes [11], and proceeds more rapidly along the peripheral parts of the potato than in the central part, in the direction of the stolon. There is a possible relation to the discovery that phosphorylase is most active in the tips and buds of the tubers [8].

The indications are that starch deposition is a process which is mainly dependent upon the amount of sugar available, and that the latter bears no relation to the day-night alternation. As there does not appear to be a fluctuation in total phosphorylase activity during 24-hour periods [4], factors controlling substrate transport and concentration have to be studied. In our experiments it is difficult to relate these factors to an inner rhythm of the plant, as proposed by Hess [7], because all tubers behaved in a different way.

It is interesting to note that sometimes no starch deposition was found in the first swellings of the stolons, although according to Plaisted [13] these small tubers should have been in a period of rapid starch accumu-

lation. In contrast, large tubers of the same plant might show intensive starch deposition, although here the process should be slower and more constant than in the tiny ones.

Evidently the influx of sugar may stop for a long time, and as a result the deposition of a layer can be a very slow process; in other cases it might proceed more rapidly.

Summary

Experiments have been presented which indicate that the layers of potato starch granules are built up by a gradual process of apposition. This process is dependent upon the supply of carbohydrates to the amyloplasts. The stripping film technique has made it possible for the first time to make starch granule growth more directly visible.

Acknowledgments

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