



貯蔵器官内における澱粉の形成 (其の6) :
フォスフォリラーゼの澱粉形成との関係及び特に葉
から貯蔵器官への移動

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Starch Formation in Storage Organs (VI)

Relation of phosphorylase to starch formation, with special reference to its translocation from leaves to storage organs

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堀 七 郎 : 貯蔵器官内における澱粉の形成 (其の6)
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Introduction

Although several investigations have been made on the mechanism of tuber formation in potato plant, no full elucidation has yet been given as to the nature of this phenomenon. Recently Gregory (1956)¹ has made a noticeable study on this subject and stated that the tuber formation may be brought about by a stimulus formed or activated in the plant under some specific conditions of temperature and photoperiod. He suggested that this stimulus for tuberization may be a hormone like substance similar to that involved in the formation of flowers under the inductive photoperiod. In view of the current concept that the action upon plant of various physical agencies such as light and temperature, may be mediated by chemical substances, the view of Gregory appears highly probable. However, the chemical nature of the tuberization factor remains thus far quite obscure.

In the course of his work on the phosphorylase activity in relation to the starch formation in potato plant, the writer noticed that tubers were formed from the terminal and axillary buds of etiolated stems of the plant grown from seed in darkness and that the terminal and axillary buds were found to contain phosphorylase abundantly at the time of the tuber formation, whereas no tubers were formed from similar stems in the light, where phosphorylase was found to have disappeared from these tuber-forming tissues. These observations suggested that phosphorylase might have some relation to the tuber formation in potato plant, though this enzyme may be regarded rather as a secondary than as a primary factor of tuberization.

In the present study the distribution of phosphorylase in various tissues of potato plant was examined under a variety of conditions, whereby evidences were obtained suggesting the translocation of this enzyme through conducting tissues.

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Materials and Methods

Materials employed in this investigation were the tubers, stolons, stems, leaves, and roots of potato plant (race "Danshaku") cultivated chiefly in the field, with the exception that the excised leaves were cultured in the glass house and the etiolated stems formed from seed potatoes were grown in the laboratory.

The phosphorylase activity in the tissue was estimated either by the method previously described (Hori, 1957)² or roughly by the modified histochemical method of Yin et al. (1947)³ as follows:

Sections of starch-free tissues were incubated in a 0.5 per cent glucose-1-phosphate solution, buffered at pH 6.0 with sodium acetate. After keeping in the dark for 24 hours at room temperature (17°-20°C.), the sections were examined microscopically for the production of starch by staining with iodine. If the sections contained chlorophyll, they were decolourized with ethyl alcohol prior to the treatment with iodine. The quantity of phosphorylase within the cells was roughly estimated from the amount of starch produced from glucose-1-phosphate under comparable conditions.

Results

I. Distribution of phosphorylase in the potato plant

I) Leaf

The materials were harvested in cloudy weather because of the absence of starch in their mesophyll cells. In the leaf, phosphorylase was found abundantly in the palisade tissue, and next to this, in the spongy tissue. In the cells of these tissues, phosphorylase was found exclusively in chloroplasts, in which starch was produced from glucose-1-phosphate in so large amount that each chloroplast appeared upon treatment with iodine like a dark globule. In the lateral veins, the enzyme was found in the sieve tubes in smaller amount, but none in the vessels. Epidermal cells contained no phosphorylase except guard cells*¹ (Plate I, fig. 1).

II) Terrestrial stem

Phosphorylase was found considerably in the sieve tubes lying on both sides of the xylem throughout the stem. The products from glucose-1-phosphate in the sieve tube were almost the same in shape and iodine colour as those produced in the sieve tubes of the leaf vein, but the quantity was somewhat larger in the former than in the latter. In the starch sheath*², phosphorylase content was very small. In general the cortical and medullary cells contained it slightly, the products from glucose-1-phosphate being minute granules, stained pure blue with iodine and scattered thinly. No phosphorylase was found in the vessels and epidermal cells, except in the guard cells of the stomata. The phosphorylase content in all of these tissues was somewhat larger in the apical part of the stem than in the lower parts (plate I. fig. 6).

III) Stolon and tuber

At the tip of the stolon the enzyme was found abundantly in the sieve tubes, cortex, and medulla, but its content was low in other parts of the stolon where the occurrence was limited to the sieve tubes. The distribution of phosphorylase in the tuber as well as the method of removing starch therefrom was described previously (Hori, 1954⁴, 1956⁵).

IV) Root

The enzyme was found considerably in the phloem and cortical cells, but not in other tissues.

2. Translocation of phosphorylase in the phloem

a) Phosphorylase in the phloem

Phosphorylase was detected in all of the sieve tubes throughout the plant tissues, but its content was found to vary with the plant part as well as with the conditions under which the plant was kept.

b) Disappearance of phosphorylase from the leaf in the dark

When the plant was transplanted from the field to a pot and kept in darkness at room temperature of 17°-20°C., most of the leaves were found to turn yellow after three days. Histochemical examination of these etiolated leaves revealed that phosphorylase had almost disappeared from the mesophyll cells and lateral veins, i. e., all the chloroplasts had become empty of phosphorylase, and when treated with glucose-1-phosphate they were stained only yellowish with iodine.

c) Migration of phosphorylase in the leaf

Leaflets severed from the petiole were kept in darkness at 17°-20°C. with the petiolule immersed in tap water and the lamina exposed to air. Sections were prepared from various parts of these materials at 24 hour intervals and examined histochemically for phosphorylase until the leaflets rotted away.

Forty-eight hours after the start, it was found that the major part of phosphorylase had diffused out of the chloroplasts into the cytoplasm as evidenced by the formation from glucose-1-phosphate of minute, iodine-blue granules outside the chloroplasts and by the scantiness of such granules in the chloroplasts. Twenty-four hours thereafter, phosphorylase content in the cytoplasm was significantly diminished and the chloroplasts were entirely depleted of this enzyme, while the lateral veins became to be filled with it (Plate I, fig. 3).

After next twenty-four hours, sieve tubes of both the midrib and the petiolule became filled with phosphorylase, whereas the enzyme was entirely lost from the mesophyll cells (Plate I, fig. 5). Twenty-four hours further later, phosphorylase in the phloem was shown to be transferred to the starch sheath lying immediately outside of the phloem. The leaflets remained green during first four days, but on the fifth day, namely, one day after complete depletion of phosphorylase from mesophyll cells, they were spotted with yellow colour indicative of necrosis.

* (1) The presence of phosphorylase in sieve tubes was observed also in plants other than potato, e. g., the fruits of cucumber, maize⁶ and water melon.

* (2) The histochemical detection of phosphorylase in these starch containing cells was not always difficult, because of the distinct difference both in form and iodine colour between the products from glucose-1-phosphate and the storage starch.

These results indicate that in the excised leaflet the phosphorylase of the chloroplast was dammed up in the course of its downward translocation at the starch sheath of the midrib and petiolule due to the blockage of the passage, while in the intact plant complete disappearance of the enzyme from shoot tissues took place.

d) Change of phosphorylase content in the stem in the dark

The preceding experiment showed that the phosphorylase was dammed up at the basal tissues of the leaflet when it was severed from the stem, while it disappeared entirely from these tissues when the leaflet was attached to the stem. These observations suggest that the phosphorylase of the chloroplast might possibly be transferred from the leaf to the underground tissues passing through the stem. To test this possibility examinations were made on the change of phosphorylase activities in the stem and stolon of the intact plant which was kept in the dark.

Intact plant with several stolons was transplanted in a pot and kept in darkness at room temperature, 17°—20° C., and the stem was examined histochemically for the changes in amount of phosphorylase. Three days after the start, it was found that all the leaves turned yellow and phosphorylase was lost from their mesophyll cells with concomitant increase in phosphorylase activity in the stem as compared with the plant growing in the field. But several days later, the stem in turn became depleted of phosphorylase accompanied by some enlargement of the stolons in the soil. The tubers thus formed contained phosphorylase in nearly the same amount as the normal tuber but the starch content was considerably lower. The low starch content in the etiolated plant, in spite of the abundance of phosphorylase, is obviously due to the deficiency of the carbohydrate sources. The phosphorylase content in these tubers was examined histochemically after they had been made free of starch by keeping the plant in this manner for further seven days. That the phosphorylase content in the tuber remained unchanged under these conditions was checked separately.

e) Change of phosphorylase content in the stem of leafless plant in the light

The above experiments disclosed the formation and the downward translocation of phosphorylase from the leaf to the underground tissues passing through the stem when the plant was kept in the dark. These findings led to suppose that, if all leaves were cut off from the plant growing in the field, the stem would become free of phosphorylase which otherwise is present in an appreciable amount. To find out this possibility, experiment was carried out in the following manner:

From the fieldgrown potato plant, all of the leaves were cut off, leaving only a few unfolding ones at the stem apex. Hereafter it was necessary to repeat the removal of the leaves which were developed from the apical bud as well as grown at leafaxils.

By the histochemical examination of the sections from various parts of the stem after 10 days, it was found that phosphorylase almost disappeared from the stem except at its apical region where a small amount was still recognized. In this case, a few of underground stolons were found to have grown to tubers above 10 mm in diameter. The tubers thus grown were scanty of starch but contained nearly the same amount of phosphorylase as did the normal tuber.

f) Change of phosphorylase content in the stem cutting

The possibility of the downward translocation of phosphorylase from leaves, as indicated in the preceding experiments, was further corroborated by the following experiments.

(I) Stem cuttings 15-20 cm. long were taken from the upper part of the stems of about 50-day old field grown plants and all of the leaves were cut off from them. These cuttings were planted in tap water and kept in darkness at room temperature of 17°-20° C.

By the histochemical examination of various tissues seven days after planting, it was found that phosphorylase which was initially distributed throughout these tissues was accumulated at the lower end of the cutting, and none or very little thereof could be detected in the upper parts.

At the lower end of the stem cutting, phosphorylase was found most abundantly in the starch sheath opposite to the external phloem of the vascular bundle. From the sieve tubes of both inside and outside of the xylem, phosphorylase almost disappeared and we could recognize a transitional state, where the phosphorylase was migrating from the sieve tubes into the starch sheath (Plate II, fig. 8).

In the transverse section made from the lower end of the stem cutting and treated with glucose-1-phosphate followed by iodine, the cells of the starch sheath lying opposite to the vascular bundle appeared like a string of deep blue beads due to the presence of the products from glucose-1-phosphate densely packed within the cells. Such a string of beads did not form a complete ring but was broken at the interfascicular parts where blue starchy products were absent (Plate II, figs. 7, 9). At that time starch was entirely lost from the starch sheath.

In the longitudinal section of the same part, the starch sheath appeared as a long column filled with the similarly stained products. The length of the column measured about 1-2 cm. from the cut end.

(II) Stem cuttings with leaves, similarly taken from the plant, were planted in tap water in the same way as above and kept in darkness. In this case, the leaves turned yellow after 3 days and fell off 2 days thereafter. Upon inspection of the transverse and longitudinal sections made from the cuttings 7 days after the start of the test, it was found that the mode of phosphorylase accumulation in the starch sheath was almost the same as that in the case (I), but the amount of phosphorylase was far larger than in the case without leaves, as evidenced by the greater length of the column, 4 cm., of the deep blue stained products filled in the starch sheath. The height of this column was nearly proportional to the number of leaves on the stem cutting at the start of the test. Thus it seemed likely that the phosphorylase originated from the leaves. In the fallen leaves no phosphorylase was detected.

(III) Similar stem cuttings with leaves were planted in tap water in glass bottles which were entirely enveloped with tinfoil in order to keep away from light, thus avoiding the possible influence of light on the phosphorylase in the stem part, and kept in the light at room temperature of 18°-24° C. After 7 days some of the lower leaves turned yellow but the upper leaves remained green. By the inspection of the sections from the stems it was found that starch was abundantly formed in the cortical and medullary cells around the vascular bundles of the lower part of the stem as well as in the upper leafy parts of the stem.

These stems with leaves were made free of starch by keeping them in the dark for about 7 days, and then phosphorylase was examined. It was found that phosphorylase was stored in the starch sheath of the lower part of the stem more abundantly than in the case (II), where stem-cuttings with leaves were kept in the dark. In the stem-cutting with leaves kept in the light, the starch sheath filled with the products by phosphorylase action reached the height more than 10 cm. from the lower cut end.

All these findings seem to indicate that phosphorylase accumulates in the starch sheath of the lower part of the stem, when the stem was severed from the underground part, and that its amount was greatly affected by whether or not leaves were present on the stem.

3. Relation between the content of phosphorylase in leaves and that in storage organs

The content of phosphorylase in storage organs has been measured by Nakamura et al. (1951)⁷ with many higher plants but the measurement of its content in leaves has been limited to a comparatively small number of species.

Therefore, the author examined histochemically the phosphorylase content of the leaves of various higher plants to see if there exists any relationship between the phosphorylase content of leaves and that of storage organs. In many plants with conspicuous storage organs the histochemical examination of phosphorylase in leaves was possible, since these plants lack starch in their mesophyll cells, if harvested before daybreak. There are also many other plants whose mesophyll cells do not lose starch under the same conditions. In general they are woody plants or herbaceous ones without well developed storage organs. Although it was known that the leaves of fruit trees could be made free of starch if they were kept in the dark for a few days, these plants were not employed for the estimation of phosphorylase, because with the potato plant such a procedure has been shown to bring about the diminution of phosphorylase in leaves simultaneously with the depletion of starch.

The results are presented in Table 1. For the sake of comparison the phosphorylase content of the storage organs as determined by Nakamura et al. is shown. In some of the storage organs histochemical examination was also made by the present author and the results are given in parentheses.

Table 1. Phosphorylase content of leaves* and storage organs**.

Plants	Phosphorylase Content	
	Storage Organs** (units/ml. pressed juice)	Leaves (histochemical assay)
<i>Cucurbita moschata</i> Duch. var.	9.917 — 0.569	++++
<i>Cucumis sativus</i> L.	0.031	++
<i>Citrullus vulgaris</i> Schrad.	(slight)	+
<i>Vicia Faba</i> L.	0.62	+++
<i>Phaseolus vulgaris</i> L. var. <i>humilis</i> Alef.	2.07 — 4.20	++++
<i>Pisum sativum</i> L.	0.41	++++
<i>Vigna Catiang</i> Endl. var. <i>sinensis</i> King	4.55	++++
<i>Hordeum vulgare</i> L. var.	0.326 — 0.194	++
<i>Avena fatua</i> L.	0.140	+
<i>Panicum Crusgalli</i> L. var.	0.404	++
<i>Zea Mays</i> L.	1.40	++++

Raphanus sativus L. var.	0.17		++
Solanum tuberosum L.	3.88		+++++
Lycopersicon esculentum Mill.	none		++
Allium Cepa L.	none	(none)	-
Allium odorum L.	0.140		+
Allium fistulosum L.	0.054	(slight)	+
Beta vulgaris L. var.		(slight)	+
Dioscorea Batatas Decne.	0.08		+++++
Arctium Lappa L.	0.06	(very slight)	+
Fagopyrum esculentum Moench.	0.171		++
Daucus Carota L. var. sativa DC.	none	(slight)	++
Dahlia pinnata Cav.		(none)	+
Physalis Alkekengi L. var.	0.155		+
Lilium Maximowiczii Regel	0.684 — 0.303		+++++
Ribes grossularioides Maxim.		(none)	+
Vitis Coignetiae Pulliat.	none	(none)	+
Helianthus tuberosus L.	0.16		+++
Taraxacum platycarpum Dahlst.		(very slight)	++
Petasites japonicus Miq.		(none)	+
Wasabia Wasabi Makino	0.117		+++++
Adenophora triphylla A. DC. var.		(none)	+

* The content of phosphorylase in leaves was roughly estimated by the amount of the blue products in mesophyll cells formed upon treatment with glucose-1-phosphate followed by iodine.

Marks indicate: - none, + very slight, and the number of + relative abundance of the content.

** Data of Nakamura et al. (1951)⁷

From this table it can be seen that: (1) phosphorylase is found in the leaves of all plants tested where starch is present in some tissues or other with the only exception of onion which forms no starch at all; (2) the leaves of the plant whose storage organs are rich in phosphorylase are also generally rich in phosphorylase, while the leaves of the plant whose storage organs contain no or very small amount of phosphorylase contain only a small amount thereof. Starchy storage organs were generally rich in phosphorylase but starchless ones contained no or only negligible amount of the enzyme.

The lily is well known to form no starch in the mesophyll cells but abundantly in the guard cells, bulbs, and bulbilets. Thanks to this nature the leaves could be histochemically inspected for phosphorylase, even with the samples harvested in the daytime. It was found that the content of phosphorylase of the leaves was considerably smaller in the early morning than in the afternoon. Hence, the phosphorylase content was compared by the usual enzyme assay method between the leaves harvested before daybreak and those harvested in the afternoon with several plants including lily. The results are given in Table 2.

Table 2. Phosphorylase content of leaves harvested in the early morning (at 4 a.m., July) and that harvested in the afternoon (at 2-3 p.m.).

Plants	Content of phosphorylase in leaves (unit/ml. pressed juice)	
	harvested before daybreak	harvested in the afternoon
Solanum tuberosum L.	1.407 (100)	1.806 (128)
Vitis Coignetiae Pulliat.	0.063 (100)	0.126 (200)
Raphanus sativus L. var.	0.084 (100)	0.357 (425)
Phaseolus vulgaris L. var.	0.105 (100)	0.525 (500)
Lilium Maximowiczii Regel	0.672 (100)	1.491 (221)
Pisum sativum L. var.	0.546 (100)	1.365 (250)
Pyrus Simonii Carr.	0.420 (100)	0.546 (130)

It can be seen from this table that with every plant tested phosphorylase content of leaves is considerably lower before daybreak than in the afternoon. Further, it appears noteworthy that in the lily phosphorylase content of leaves was high despite the absence of starch in the mesophyll cells even in the daytime.

4. Influences of light and temperature on the phosphorylase activity of leaves and on the tuber formation

To examine the influences of light and temperature on the phosphorylase activity in the leaves as well as on the tuber formation, one set of potato plants was cultivated in the field and the other set in the green house where the temperature was kept by 4°–6° C. higher than that in the field throughout the day and night and the plants were exposed to natural light in the daytime and to artificial light of about 2000 Lux. of maximum intensity at night.

About 50 days after germination the leaves of each group were harvested in cloudy weather because of the absence of assimilation starch in the mesophyll cells, and examined histochemically for the phosphorylase activity.

As a result of this experiment it was found that the leaves of the field group contained abundant phosphorylase in the mesophyll cells, whereas the enzyme content was far small in the green-house group. In the latter leaves, the products formed from glucose-1-phosphate and stained blue with iodine appeared in the chloroplasts as small granules (ca. 1—2 μ in diameter), while in the former leaves chloroplasts were filled with the blue products so densely that they appeared as dark globules (plate I, figs, 1, 2).

At about 40 days after the germination numerous stolons were formed from the plants cultivated in the field, while none from those in the green house.

5. Correlation between phosphorylase and tuber formation

(I) Leafless stem cuttings about 20 cm. long, taken from the upper part of the stems of about 60 day-old plants grown in the field, were planted in the nutrient solution (60g of sucrose, 1 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.2 g of KNO_3 and 0.8 g of KH_2PO_4 in 1000 ml. of tap water), and kept in darkness at room temperature of 17°–20° C. As a control similar cuttings were planted in tap water. The nutrient solution was renewed every two days.

The stem cutting planted in the nutrient solution showed after 5 days an indication of the tuber formation from basal axillary bud. Thereafter the tuber continued to grow for about five days until it attained a size of about 9 mm. \times 12 mm. However, it did not further increase its size though the cutting was kept in the nutrient solution (Plate II, fig. 11). Histochemical examination of the stem at this stage revealed the complete disappearance of phosphorylase from all of the tissues of the stem except tuber (Plate II, fig. 10). Contrary to this, the control cuttings showed no tuber formation and phosphorylase was found to accumulate in the tissues of the basal part of the stem.

Leafy stem cuttings of similar length, taken from the plants in the same manner, were planted in tap water and kept in the dark for 5 days at the same temperature. Upon cutting off the etiolated leaves from these stems, they were then transferred to the same nutrient solution as in (I) and kept in the dark at the same temperature. These cuttings contained phosphorylase more abundantly than did the stems growing in the field. By keeping several days in the nutrient solution more than two tubers were formed on each stem cutting.

(II) Experiment was next conducted to see whether or not tubers are formed on the stem cutting even when phosphorylase is entirely absent in any tissue of the stem as starch sheath, phloem, cortex or medulla.

Leafless stem cuttings of the same length were obtained from the upper part of the stem, which had been freed from phosphorylase by repeated removal of leaves while growing in the field for more than 15 days*. They were planted in the same nutrient solution and kept in darkness at the same temperature. As a control, similar stem cuttings were planted in tap water and kept in the same way. As a result of this treatment tuber formation from these stems was abolished but an etiolated shoot was formed from the upper axillary bud and grew vigorously to 5—7 cm. long 12 days after planting, while the control cutting formed neither tuber nor shoot (Plate II, fig. 12). By the histochemical examination, the etiolated shoot thus formed was found to contain no phosphorylase in any tissue of the stem.

The results of these experiments (I and II) showed clearly that, (1) the tuber formation from axillary bud of the stem could occur only when the stem contained an appreciable amount of phosphorylase in either of the tissues such as starch sheath, phloem, cortex and medulla, and was fed sucrose in the dark; (2) the number of tubers thus formed was generally proportional to the amount of phosphorylase contained in the stem; (3) when the tuber formation occurred most of the phosphorylase present in various tissues of the stem migrated into the tuber; and (4) tubers were not formed from the stem which was free of phosphorylase.

(III) Similar experiment was performed with etiolated stem cuttings about 10 cm. long, which were taken from the dark grown plants raised from seeds. These stems contained abundant phosphorylase at the terminal and axillary buds. One set of the cutting was kept in the dark and the other was exposed to light for more than 15 days, when most of the phosphorylase disappeared from these tissues with their external appearance turning green. These two kinds of the stem cuttings, etiolated and greenish, were then planted respectively in the same nutrient solution and kept in darkness at the same temperature.

The results obtained were the same as those of the former experiment in so far as the relation between the presence of phosphorylase and the formation of tuber is concerned. Thus, three days or more after planting a small tuber was formed from every axillary bud of the etiolated stem, and a few days thereafter, also from the stem top, whereas no tuber was formed from the greenish stems which were scanty of phosphorylase.

Histochemical inspection of the etiolated stem cuttings during the course of the tuber formation showed that at first starch deposition occurred densely in the tuber-forming tissues such as the terminal and axillary buds, and thinly in the cortical and medullary cells which also contained a small amount of phosphorylase. Further, the deposition of starch in the tuber-forming tissues such as terminal and axillary buds continued to increase and resulted in the formation of the enlarged tuber.

* cf. p. 19

Discussion

The present investigation showed that in the potato plant phosphorylase was detected in all of the sieve tubes, which contain no starch at all throughout the plant. This finding does not agree with the statement of Yin et al. (1948)⁸ that phosphorylase is not detectable in that plant part which forms no starch. It seems probable that the presence of phosphorylase in the sieve tubes is not responsible for the starch formation in this tissue but that the enzyme is *en route* from leaves to the site of starch formation, since much evidences were obtained in this study that phosphorylase is transported through sieve tubes.

The results obtained in the present study indicate that phosphorylase may not be synthesized *de novo* within storage organs and that tubers are neither formed nor enlarged without phosphorylase in the stem. Accordingly all of the phosphorylases required for the starch accumulation in tuber and for its growth may be supplied to the tuber-initiating sites from other tissues.

It was observed with the plant growing in the field that before tuber formation the tissues containing phosphorylase most abundantly were the leaves, especially chloroplasts of the mesophyll cells. However, when such plant was removed from the field and placed in darkness, phosphorylase was lost from the leaf chloroplasts almost completely within a few days. Along with the depletion of phosphorylase from leaves the content of this enzyme in the stem, especially in its sieve tubes and starch sheaths, increased and many stolons containing much phosphorylase were formed. When either leaflets were severed from the petiole or the stem was cut off from the underground parts and kept in darkness respectively, the phosphorylase, initially present in the chloroplasts, was found to migrate within several days successively to the midrib, petiolule, and to lower cut end. These findings indicate not only the basipetal translocation of phosphorylase from leaves towards underground tissues but also the formation of phosphorylase in the leaves.

The possibility of phosphorylase synthesis in leaves and of its translocation into storage organs seems to be strengthened furthermore by the results: (1) in plants whose storage organs are rich in phosphorylase, their leaves contain also much phosphorylase, and even the obligate sugar leaves that do not form starch at all in their mesophyll contain significant amount of phosphorylase if the plant has some starch storing organs elsewhere; (2) there is a marked diurnal variation in the phosphorylase content of leaves, the enzyme content being considerably lower at daybreak than in the afternoon; (3) with potato stem cuttings planted in water, an approximate parallelism was found between the number of leaves on the stem and the phosphorylase content in the sieve tubes and starch sheath near the cut end where the translocated phosphorylase accumulated.

Among these results the finding is especially noteworthy that abundant phosphorylase appears in the daytime in the leaves of a lily (*Lilium Maximowiczii* Regel) whose mesophyll cell do not form starch at all. However, in the bulb of this lily a considerable amount of starch is stored. It would be difficult to explain the presence of phosphorylase in starchless leaves, unless we consider that this enzyme is synthesized in leaves, probably in chloroplasts, and translocated to the starch-forming site.

The most important part played by phosphorylase in the tuber forming process may be the conversion of sugar into starch followed by its deposition in the cells of tuber. The present experiment showed that tubers were never formed from the stem cuttings if phosphorylase was absent, despite the ample supply of sucrose to the cuttings. Similar phenomenon was observed with the potato plants cultivated in the green house. No tuber was formed from these plants whose leaves contained far less amount of phosphorylase than those of the plants cultivated in the field. Moreover, evidence was obtained that tubers were not formed without the presence of phosphorylase in tuber-forming tissue.

From the data above mentioned, it may be considered likely that the formation of starch by the action of phosphorylase may be prerequisite to the tuber formation which may be induced in the morphogenetic process by the stimulating action of so-called "tuberization factor". Accordingly, the "tuberization factor" might be in some way related to the translocation of phosphorylase from leaf to the tuber-forming tissue.

The present study disclosed that in the tuberization process the starch deposition in tuber tissue took place nearly in parallel with the accumulation of phosphorylase in the same tissue.

Accordingly, continual supply of phosphorylase to tuber from other tissues may be required for the tuber formation as well as for its growth.

Further, a reference will be made to the relation of the phosphorylase activity in the tissues to the temperature and photoperiod at which the potato plant was grown. Observations were made in this study that the content of phosphorylase in the stem, leaf, stolon and tuber was considerably reduced by the exposure of the plant either to a continuous illumination or to a relatively high temperature. As stated by Gregory (1956), his "tuberization factor" was also affected profoundly by temperature and photoperiod in a similar manner. It would appear, therefore, that the "tuberization factor" might exert its action through the mediation of phosphorylase.

All these results may lead to the conclusion that phosphorylase plays a fundamental role in the tuberization of the potato plant.

Summary

1) The behaviour of phosphorylase in the tuber forming process in potato plant was investigated.

2) In the potato plant the content of phosphorylase is the highest in the chloroplast of mesophyll cells, but a small amount of this enzyme is regularly found in nearly all of sieve tubes before the onset of tuber formation. However, when the plant is transferred to darkness phosphorylase of leaves seemed to migrate towards underground tissues of tuber formation, passing successively through the sieve tubes of leaf vein, petiole, and stem. Hence it appears possible that the leaves, especially the chloroplasts thereof may be the principal site for synthesizing phosphorylase.

3) Tubers can be formed from the terminal and axillary buds of the stem only when the stem contains phosphorylase in its tissues such as starch sheath, phloem, cortex and medulla

and fed sucrose in darkness, while no tuber is formed from buds of the phosphorylase free stem even under otherwise identical conditions.

4) From the results of this study it may be concluded that phosphorylase is important as an essential participant in the tuberization of potato plant, though this enzyme can hardly be regarded as primary factor of tuber formation.

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Explanation of Plate

Fig. 1 Transverse section of a lamina of a leaflet of potato plant cultivated in the field after treatment with glucose-1-phosphate and iodine. $\times 200$.

Fig. 2 Transverse section of a leaflet of potato plant cultivated in the green house after treatment with glucose-1-phosphate and iodine. $\times 200$.

Fig. 3 Transverse section of a lamina of a leaflet severed from the petiole and kept in the dark with its petiolule immersed in water for 2 days after treatment with glucose-1-phosphate and iodine. Arrow indicates lateral veins. $\times 50$.

Fig. 4 Transverse section of the midrib of an intact leaflet of potato plant, treated with glucose-1-phosphate and iodine. Note the mesophyll cells where the dark-stained products from glucose-1-phosphate are appearing densely. Ph, Phloem; St, Starch sheath. $\times 100$.

Fig. 5 Transverse section of the midrib of a leaflet severed from the petiole and kept in the dark with its petiolule immersed in water for 3 days, treated with glucose-1-phosphate and iodine. Arrow indicates sieve tubes. $\times 100$.

Fig. 6 Transverse section of a normal stem of potato plant cultivated in the field, treated with

glucose-1-phosphate and iodine.

St.→, Starch sheath; Ph.→, Phloem. × 50.

Fig. 7 Transverse section of the lower end of a stem cutting of potato plant, planted in water and kept in the dark for 7 days, treated with glucose-1-phosphate and iodine.

St.→, Starch sheath; Ph.→, Phloem. × 50.

Fig. 8 Transverse section of the lower end of a stem cutting of potato plant, treated with glucose-1-phosphate and iodine. Arrow indicates state of phosphorylase migrating from internal phloem to starch sheath passing through xylem. × 200.

Fig. 9 Transverse section of the lower end of a stem cutting, planted in water and kept in the dark for 7 days, treated with glucose-1-phosphate and iodine. × 15.

Fig. 10 Transverse section of the lower end of a stem cutting which was cultured in sucrose solution and formed tuber, treated with glucose-1-phosphate and iodine.

St, Starch sheath; E. Ph, External phloem; X, Xylem; I. Ph, Internal phloem. × 50.

Fig. 11 Stem cuttings from field grown potato plant fed sucrose showing tuber formation.

Fig. 12 Formation of shoot from phosphorylase free stem cuttings fed sucrose.

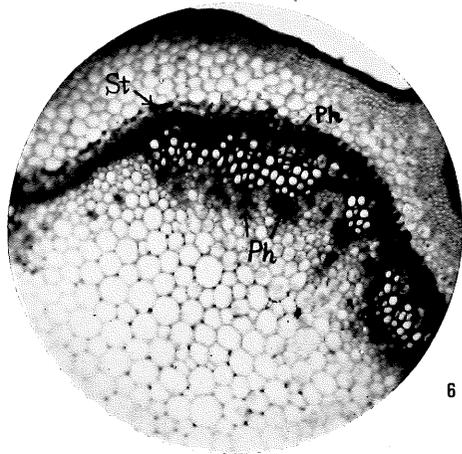
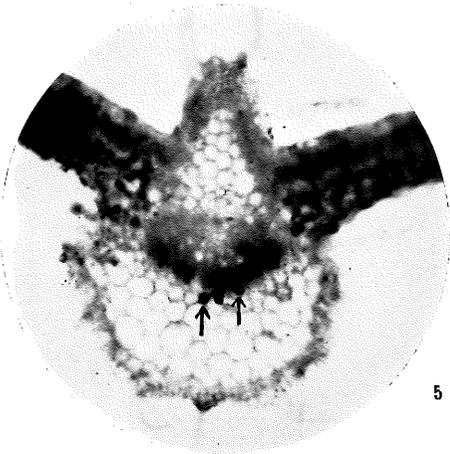
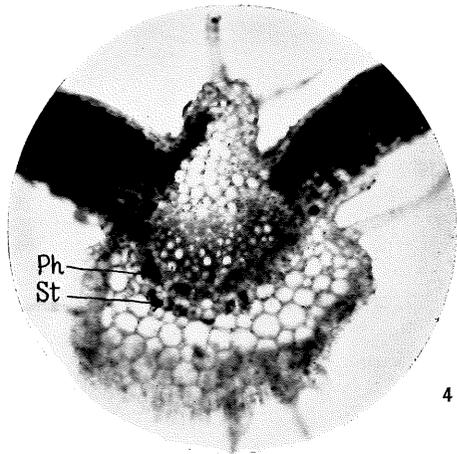
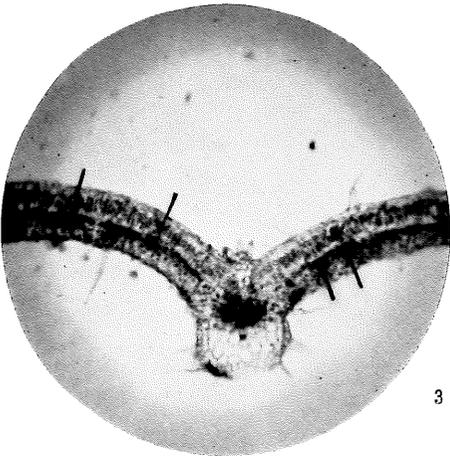
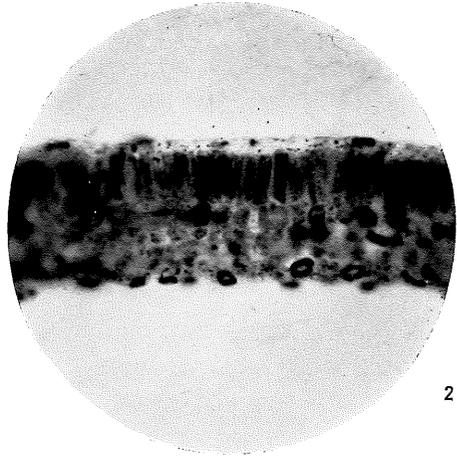
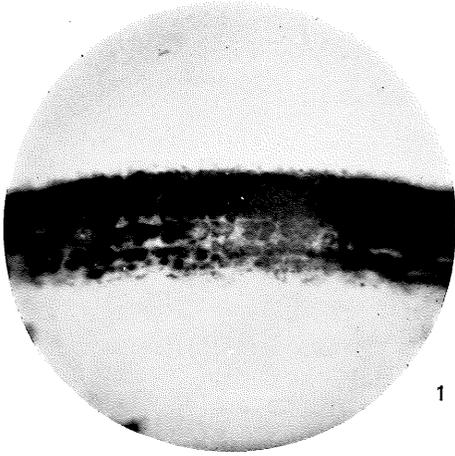


Plate Figure II

