CLXVI. PHOSPHATASE DISTRIBUTION IN SOME HIGHER PLANTS.

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THE important metabolic role of phosphorus is fairly well understood in the animal kingdom and in some of the fungi but is still relatively obscure in the higher plants.

Among the methods which might be used to obtain more information as to the significance of phosphorus for plant life is that of following the provision of mechanisms for its utilization by different parts of the plant at different stages of development. An attempt is made to adopt this method in the work reported. The most important mechanism for the utilization of phosphorus by plants and animals is believed to be the synthesis and hydrolysis of phosphoric esters by the group of enzymes termed phosphatases.

If it be granted that the availability of phosphorus for the plant does indeed depend on this mechanism then, since it is a general observation that the appearance of the necessary enzyme in biological situations is coincident as to time and place with the occurrence of its substrate and reaction products, a study of the presence and concentration of phosphatase in the different regions of the plant at successive stages of its life history would not only elucidate the role of phosphatase in the higher plants but might also provide data for an attack on the general problem of their phosphorus metabolism.

Unfortunately as yet we can seldom isolate enzymes in a pure form, nor can we determine them by purely chemical methods. The only practical way to study their presence quantitatively is to determine their activity, i.e. the rate at which they decompose suitable substrates, and this method has been used in this investigation.

Method.

Various extracting liquids were tested but in no case did any solvent remove the enzyme completely from the plant tissue. For this reason powdered tissue, in place of the usual extract, was incubated with the substrate. To obtain the powdered tissue fresh plant material was ground to a paste which was spread out in a thin layer on a clock glass and dried in a rapid current of air at room temperature. The dried material was then ground lightly to a fine powder. This procedure was found to be satisfactory and did not entail any serious loss of enzyme activity. The true dry-matter content of this powdered tissue was determined by heating aliquots in an oven at 56° to constant weight.

As substrate for the determination of the enzyme activity 5% sodium β -glycerophosphate was chosen for several reasons: (1) it is a naturally occurring compound, (2) it is stable in acid solution, (3) it is readily obtainable, (4) other investigators working with plant phosphatases have used it successfully.

The substrate solution was adjusted to pH 5.8 because, as seen from the figures in Table I, the optimum pH for the activity of bean phosphatase on sodium β -glycerophosphate lies between pH 5.5 and 5.9. Whilst the optimum pH

for the activities of the phosphatases of the other plants used in this investigation was not determined, the substrate was always adjusted to pH 5.8. This procedure will, however, not affect the validity of the conclusions because no attempt is made to compare phosphatase activities as between different plants.

Table I. *The effect of pH on the activity of plant phosphatase obtained by a chloroform-water extract of the "Canada Wonder" bean.*

Average pH	mg. inorganic phosphorus released. Time of hydrolysis	
	$\frac{1}{2}$ hour	2 hours*
4.2	0.12	0.33
4.6	0.20	0.63
5.0	0.29	0.84
5.5	0.32	0.96
5.9	0.33	0.94
6.2	0.30	0.87
7.2	0.10	0.26

* Average of two experiments.

For the quantitative estimation of phosphatase activity duplicate portions of 0.5 g. of the powdered tissue in 15 ml. of distilled water were brought to 37.5° in a water-bath. The substrate, 10 ml. of 5% β -glycerophosphate solution at pH 5.8, previously warmed to the same temperature, was then added. Three 3-ml. samples of each were taken at intervals of 5 min. and delivered instantly into 10 ml. of 7% trichloroacetic acid. The inorganic phosphate released by the action of tissue phosphatase was estimated by the Briggs [1924] colorimetric method in each duplicate. Control experiments were always conducted to determine the inorganic phosphate of the plant tissue itself. The amount of enzyme present is expressed as the number of mg. of inorganic phosphorus released in 1 min. by the action of the phosphatase present in 1 g. of dry plant tissue on 2% sodium β -glycerophosphate. This figure was obtained from the progress curve by interpolation.

Experiments were performed which show that the inorganic phosphorus released under these conditions, making due allowance for experimental error, is proportional to enzyme concentration, Table II.

Table II. *Relation of the plant phosphatase activity to its concentration.*

Amounts of powdered plant tissue used (g.)	Phosphorus released							
	Zero time	5 min.		10 min.		15 min.		
		mg.		mg.		mg.		
		x/c		x/c		x/c		
0.07	0.00	0.87	12.4	1.50	21.5	2.19	31.2	
0.06	0.00	0.74	12.3	1.37	22.8	2.00	33.3	
0.05	0.00	0.67	13.5	1.22	24.4	1.75	35.0	
0.04	0.00	0.46	11.6	0.95	23.7	1.42	35.5	
0.03	0.00	0.38	12.8	0.75	24.9	1.08	36.0	
x = Phosphorus released.				c = Weights of tissue.				

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EXPERIMENTAL PROCEDURE AND RESULTS.

Bean, potato, radish and wheat plants were used in these studies. Three different populations of beans were grown, two of the variety Canada Wonder and one of the Pencil Pod type. One population of Canada Wonder bean and the Pencil Pod beans were grown in soil. The second population of Canada

Wonder beans was grown in a culture solution. For the study of the initial stages of growth, however, the beans were germinated and grown in moist saw-dust or cotton-wool.

The culture solution consisted of:

Calcium nitrate	...	...	4.0 g. anhydrous salt
Potassium nitrate	...	...	1.0 g. " "
Potassium dihydrogen phosphate	...	...	1.0 g. " "
Potassium chloride	...	...	0.5 g. " "
Magnesium sulphate	...	...	1.0 g. " "
Ferric chloride	...	...	0.0336 g. " "
Sodium citrate	...	...	0.648 g. crystalline salt
Distilled water	...	...	6 litres

Sodium citrate was used to form a soluble complex salt with iron and so to prevent the formation of the insoluble iron phosphate.

Canada Wonder beans grown in soil.

The results for the experiments on the Canada Wonder beans grown in cotton-wool and soil are shown in Table III.

Table III. *Phosphatase content of the different parts of Canada Wonder beans grown in cotton-wool.*

Days between planting and sampling	Part of plant	Phosphatase mg. P released by phosphatase in 1 g. of dry matter per min.
Ungerminated	Testa	—
	Cotyledon	Trace
	Germ	0.91
7	Testa	—
	Cotyledon	Trace
	Leaves	3.19
	Roots	4.89
12	Cotyledon	1.28
	Leaves	2.78
	Hypocotyl	5.05
	Roots	4.20
18	Cotyledon	2.36
	Leaves	4.65
	Stem	4.70
	Root	4.11
Canada Wonder beans grown in soil.		
21	Cotyledon	3.19
	Leaves	6.62
	Stem	4.51
	Root	2.84
28	Primary leaves	5.65
	Young leaves	4.65
	Stem I	3.45
	Stem II	3.13
	Roots	2.73

It will be noticed that no phosphatase was found in the testae. In the cotyledons little enzyme was detected during the initial stages of germination, but later an appreciable quantity of phosphatase appeared. As soon as the young plant had developed sufficiently to make separation into leaves and roots

possible, the root tissue was found to possess a greater phosphatase activity than the leaves. As the leaves developed, the phosphatase activity increased, whilst in the roots it declined. In the hypocotyl and the stem, as in the case of the roots, the concentration of enzyme was relatively high during the earlier stages of development of the plant and fell off rapidly as the plant matured. An experiment dealing with ungerminated seed was also carried out in the following way. The seed was soaked for 8 hours to soften the testae which were then removed. The embryo was dissected out from the cotyledons and the phosphatase activities of the testae, the cotyledons and the embryos were separately determined. It was found that the embryo alone showed any appreciable phosphatase activity, and only traces were found in the cotyledons. To judge the enzyme concentration in any particular tissue according to the phosphatase activity shown by that tissue may be open to the criticism that the possible presence of inhibitors is not taken into account. The following experiment was conducted to detect the presence of inhibitors in the cotyledons. Digest flasks were made up with both powdered cotyledon tissue and young bean plant tissue, and the phosphatase activity of the combined enzyme preparation was compared with the phosphatase activities of the cotyledons and the plant separately. The results are shown in Table IV.

Table IV. *The effect of cotyledon tissue on the activity of phosphatase present in other parts of the bean plant.*

Sources of phosphatase	mg. of phosphorus released			
	Zero time	5 min.	10 min.	15 min.
0.05 g. of powdered tissue of the young bean plant	0.00	0.86	1.47	2.17
0.05 g. of powdered cotyledons	0.00	0.26	0.53	0.79
0.05 g. of powdered tissue of the young bean plant, and 0.05 g. of powdered cotyledons	0.00	1.01	1.95	2.76

The figures show that the sum of the amounts of inorganic phosphorus released by the actions of the cotyledon and the plant separately was equal to the amount of phosphorus released by the combined actions of the cotyledon and plant. If inhibitory substances were present in the cotyledons, they would presumably have affected the activity of phosphatase derived from the young plant. As no such inactivation was demonstrated, it may be concluded that no inhibitors were present and that the apparent low activity of the enzyme in the cotyledons does indeed indicate that only small quantities of phosphatase are elaborated during the early stages of germination.

The possible effect of variation in the magnesium content in different parts of the plants on the enzyme activity must be considered. Munemura [1933] has shown that plant phosphatase, like animal phosphatase, is activated by magnesium. He showed that at the optimum *pH* the maximum increase in activity obtained is only of the order of 33 % above the dialysed control with 0.002 *M* MgCl_2 . Concentrations of MgCl_2 from 0.02 *M* to 0.00002 *M* in the digest did not vary the activity of the enzyme more than 25 %. As the variations in the phosphatase content found in the different parts of the plant were of the order of 100 %, the differences demonstrated could not be due to variation in magnesium content of the plant tissues. Latshaw & Miller [1924] and Fonder [1929] showed that variations in concentration of magnesium in different parts of the plant are comparatively small. In experiments preliminary to the work

here reported 0.002 *M* MgCl_2 in the digest of undialysed extract of ungerminated beans increased the activity of the enzyme only 10%. The concentration of magnesium in digests may obviously vary greatly with no significant change in the activity of the phosphatase.

Canada Wonder beans grown in culture solution.

Phosphatase determinations on the population of Canada Wonder beans grown in culture solution were made at irregular intervals until the plants had completed their full growth and withered. The results of these determinations on the different parts of the plants are presented in Fig. 1.

Fig. 1. Phosphatase activity of the bean plant grown in culture solution. Ordinate: mg. inorganic P released from sodium β -glycerophosphate solution by phosphatase in 1 g. of dry matter per minute. Abscissa: days after planting.

Except during the early period of growth there was little phosphatase activity in the roots. It amounted to only about 20% of that found in the leaves. The phosphatase concentration in the stem was low except in the early stages of growth, yet it was higher than in the root. The phosphatase activity in the stem, after falling to a low level at the end of the third week, remained at that level with slight variations throughout the growing period. The leaves appear to be the chief centres of phosphatase activity and, except for the first few days after germination, they have been shown to possess at least twice as much phosphatase activity as the stem tissue. The leaves were subdivided into two classes. The first class consisted of the primary simple leaves. In the second class were placed the compound leaves. At the first sampling, 9 days after the beans were planted, the phosphatase activity in the leaves was comparatively low, only two-thirds of that present in the stem. It must be borne in mind that at this early stage, the leaves are only partially developed and are still between the cotyledons. The true leaf function is not fully developed. 5 days later, when the primary leaves were well grown, the phosphatase activity had doubled, but as the plants became older it showed a gradual decrease.

Pencil Pod beans grown in soil.

Only a few pods were obtained from the beans grown in culture solution because flowers do not give rise to fruit very readily under these conditions. For this reason a population of beans was grown in soil during its entire life

history. The variety "Pencil Pod" was used in this set of experiments because this variety grows more vigorously and more evenly than the Canada Wonder beans under greenhouse conditions. The results of the determinations made on Pencil Pod beans are given in Table V.

Table V. *Phosphatase content of the different parts of Pencil Pod beans grown in soil.*

Days between planting and sampling	Part of plant	Phosphatase mg. P released by phosphatase in 1 g. of dry matter per min.
13	Leaves	5.18
	Stem	1.93
	Root	1.29
20	Primary leaves	2.48
	Compound leaves	2.72
	Stem	0.67
	Root	0.74
40	Flowers	1.02
	Primary leaves	2.85
	Compound leaves	2.61
	Stem	0.63
52	Flowers	1.76
	Younger pods	1.51
	More mature pods	1.47
	Leaves	1.66
	Stem	0.68
59	Beans	0.47
	Pods	1.08
	Leaves	1.54
	Stem	0.66
72	Cotyledons without plumules	0.32
	Cotyledons with plumules	0.33
	Leaves	1.08
	Stem	0.87
	Root	0.52

The phosphatase distribution was found to be very similar to that found in the population grown in the solution. In these experiments it was possible to make two determinations on flowers and pods. The results show that phosphatase is present in these parts of the plant. It is present, in fact, in quantities which are much greater than those found in the stem or root during the later stages of its life history, although only one-fourth to one-fifth of the maximum amounts found in the leaf. The developing bean itself contains very small quantities of the enzyme. One experiment was conducted in which the newly forming embryo was removed from between the newly forming cotyledons, and the concentrations of phosphatase in the separated cotyledons were compared with the phosphatase content of the intact bean. No significant differences were obtained in the phosphatase activities of the two preparations.

The potato tuber.

In the bean experiments the greatest phosphatase concentration was found in the leaves. This fact suggested that the enzyme might be connected with the carbohydrate metabolism of the plant. It was of importance to determine whether the concentration of phosphatase varies with the change of reserve carbohydrate, such as starch, to a more soluble and utilizable form; especially since phosphorus is always associated with this polysaccharide. Phosphatase

activity in potato tubers was therefore determined. Analyses were made twice, 36 and 44 days after planting when shoots were well developed. The tubers were planted in sawdust instead of soil to facilitate the removal of the entire plant at sampling, and were kept in the greenhouse. In determining the phosphatase activity of the tuber the outside corky tissue was removed because it would have been erroneous to consider this inert material as part of the metabolically active tissue. The results of the experiments are reported in Table VI.

Table VI. *The phosphatase contents of the different parts of potato grown in sawdust.*

Days between planting and sampling	Part of plant	Phosphatase mg. P released by phosphatase in 1 g. of dry matter per min.
36	Tuber	Trace
	Leaves	1.45
	Stem	0.81
	Root	Trace
44	Tuber	0.28
	Leaves	1.23
	Stem	0.46
	Root	0.17

The distribution of phosphatase was found to be similar to that found in the bean. The phosphatase concentration is greatest in the leaves, the activity of the enzyme in the stem is weaker, whereas in the tuber and the roots the activity of phosphatase falls to a very low level. It appears therefore that phosphatase is not concerned with the process of transformation of starch into a less complex carbohydrate since the enzyme was present in very small concentrations in the tubers where this process takes place.

The radish.

It would have been of interest to study the phosphatase content of the potato tuber during the reverse process, that is during the period of deposition of starch, but practical difficulties precluded the performance of this experiment. Instead, experiments were conducted with radishes to determine whether, during the deposition of carbohydrate food reserve in the swollen part of the hypocotyl, the phosphatase increases in that region. A variety of radish was selected that would produce a "bulb" under greenhouse conditions. The seed was planted in light soil in wooden boxes, and after 10 days the small plants were planted out to allow unrestricted development. Samples were taken at this stage and the results are given in Table VII.

The leaves had the highest concentration of phosphatase. The petioles had half that concentration, and in the root it was about 40 % lower than in the petioles. 2 weeks later another sample was taken. The plant was subdivided into the leaf laminae, the petioles, the swelling hypocotyl and the roots. The leaf again was shown to have the highest phosphatase activity, whereas in the petioles and the swelling hypocotyl it was much weaker. The enzyme activity in the root was weaker than in the petiole. When the "bulbs" were well developed 2 weeks later another sample was taken. Allowing for experimental error, no change in the concentration of the enzyme in the different parts was noticed. If the phosphatase concentration in any organ is taken as a criterion of its importance for the metabolic process of that organ, the results of the experiments on radishes indicate that phosphatase plays no important part in the deposition

Table VII. *The phosphatase content of the different parts of radishes grown in soil.*

Days between planting and sampling	Part of plant	Phosphatase mg. P released by phosphatase in 1 g. of dry matter per min.
11	Leaves	3.84
	Petioles	1.68
	Root	1.15
25	Leaves	2.20
	Petioles	1.37
	Swollen hypocotyl and root	1.03
	Root	0.94
38	Leaves	2.13
	Petioles and stem	1.73
	Swollen hypocotyl and root	0.99
	Root	1.09

of sugar in the swollen hypocotyl, for the concentration of the enzyme in this part of the plant was never higher than in the petiole which is morphologically similar to it.

Wheat.

The plants so far studied, bean, radish and potato, belong to the dicotyledons. It is of interest to learn whether the distribution of phosphatase in the monocotyledons is similar.

Wheat of the variety Marquis was selected as representative of the monocotyledons. It was impossible to continue the experiments with wheat to the stage of maturation because the entire wheat population was attacked by loose smut soon after "heading". Three plants were grown in each pot. 2 weeks after seeding, samples were taken and phosphatase activity was determined on three portions of the plant: (1) the leaf laminae, (2) the leaf sheath and all the tissues enveloped by the leaf sheath, (3) the root. In the root it was found to be very low (Table VIII).

Table VIII. *The phosphatase content of the different parts of Marquis wheat grown in soil.*

Days between planting and sampling	Part of plant	Phosphatase mg. P released by phosphatase in 1 g. of dry matter per min.
14	Laminae of leaves	0.79
	Sheath	0.98
	Root	0.56
45	Laminae of leaves	0.76
	Sheath	0.82
	Root	0.24
61	Head	0.89
	Leaves	0.46
	Sheath	0.47
	Stem	0.50

The concentration of phosphatase in the leaf sheath was higher and equal to that in the laminae. 31 days later the same subdivision of the plant was made. The results did not differ greatly from the previous sampling.

16 days later, when the wheat was headed, another sample was taken. The concentrations of phosphatase in the head, the leaf laminae, the leaf sheath and the stem were determined. The leaf sheaths, the leaf laminae and the stem all

gave approximately equivalent results; the enzyme concentration in the head was however considerably greater than in the other parts. In sampling, the whole head was ground up, as it was impossible to obtain enough material to subdivide the head into its component parts. The relatively high concentration of phosphatase in the head is of interest, but unfortunately only one determination was possible owing to the destruction of the crop.

Wheat was planted in sawdust to study the early stages of development. The relative distribution of phosphatase in the different parts of the plant was similar to that found in the wheat grown in the soil, although the amounts were all larger, Table IX, because the samplings were performed during the earlier

Table IX. *The phosphatase content of the different parts of Marquis wheat grown in sawdust.*

Days between planting and sampling	Part of plant	Phosphatase mg. P released by phosphatase in 1 g. of dry matter per min.
4	Grain	0.52
	Aerial portion	1.40
	Root	0.99
6	Grain	1.00
	Aerial portion	1.79
	Root	1.11

stages of development. Enzyme concentration was determined on the different parts of the sprouted grain 4 days after planting. At this stage the roots were well developed and the first leaf was just protruding beyond the leaf sheath. The figures show that the sheath with the developing leaf had the highest phosphatase concentration. The enzyme was less concentrated in the roots, and the grain had about half the amount of phosphatase found in the root. 2 days later another sample was taken. Increases in enzyme concentration in the plumule and the remains of the grain were noticed. The wheat plant as a whole appeared to have a relatively high concentration of phosphatase during the first few days of germination, the amount of enzyme then decreasing; the decrease being first noticed in the roots. These observations are in accordance with results published by Hirsch [1931] and by Ay [1930].

DISCUSSION.

The methods used in obtaining the results reported were always similar and therefore the concentration of phosphatase which was found in any part of a plant can be compared with those found at different stages of the life history of the same or other parts of the plant. Although plant tissues have highly generalized functions and the metabolic processes are common to most cells, yet the intensity of these processes varies in the different parts of the plant during the various stages of its life history. Furthermore photosynthesis takes place only in the green portions of the plant.

Several workers have reported the importance of phosphates in plant carbohydrate metabolism. Lyon [1924] and Chatterjee [1933] have found that the rate of respiration in plants is increased by phosphate. Cockfair [1931] and Barrenscheen & Pany [1930] have shown the presence of hexosephosphates in assimilating plants. Cockfair showed in the tomato plant that, during assimilation in the light, hexosephosphates increased in concentration and during darkness they decreased.

It would thus appear that phosphates play an important role in both the catabolism and anabolism of the carbohydrates in the higher plants. The photosynthesis or the anabolic process of carbohydrate metabolism in the plants takes place only in the green portions of the plant and chiefly in the leaves. The leaves, especially during the earlier part of their development, are the parts of the plant which have the greatest rate of respiration, and it was in the leaves of the beans, the potato and the radish that the phosphatase activity was found to be greatest.

As the highest phosphatase concentration in the plant is found in the leaves, the site of vigorous carbohydrate metabolism, it is reasonable to assume that phosphatase as well as phosphates may play a role in the carbohydrate metabolism of the higher plants.

In the wheat plant it was found that the phosphatase concentrations in the leaf laminae, leaf sheaths and stem are approximately equal to one another. Morphologically and physiologically the leaf sheath and the leaf are very similar to each other. The stem, on the other hand, does not play an important role in photosynthesis, but in the case of wheat it contains a considerable amount of meristematic, quickly growing and rapidly respiring tissue at the nodes. Actually the phosphatase concentration of the stem was equal to that of the leaf sheaths and leaf laminae but, since the experiments with the wheat population were not conducted to the stage of maturation of the crop, it is possible that the phosphatase concentration in the stem would have dropped appreciably below that of the leaves after the stem had completed its growth.

The phosphatase concentrations in the stem and petioles of the bean, potato and radish were lower than that in the leaves. It was found that, in the case of the bean plant which was studied more thoroughly than the other plants, the phosphatase concentration of the hypocotyl was comparatively very high. As the stem matured, however, the phosphatase concentration decreased greatly although it was always higher than the concentration of the enzyme found in the roots. The high concentration of phosphatase in the hypocotyl may probably be explained by the fact that at that stage of development this portion of the plant is growing very rapidly and consequently respiring very actively.

These results conform generally with the hypothesis that phosphatase may be concerned with carbohydrate metabolism. The concentration of phosphatase in the roots of the bean, potato, radish and wheat is lower than that found in the leaves and stem. The actual presence of phosphatase in the roots can be explained if one accepts its role in respiration, but the enzyme may have a further function. Heck & Whiting [1927] and Weissflog & Mengdehl [1933] have shown that organic phosphorus compounds are readily available to plants, and it is possible that the root phosphatase may be concerned in the process of absorption by the plant of these organic compounds from the external medium.

Although the experiments reported in this paper were concerned with the distribution of phosphatase in the plants, the mere study of relative concentrations of the enzyme in the various parts of the plant at different stages of the life history was not the objective. As has been already pointed out in the introduction, the work was undertaken to throw more light on the role of phosphatase and consequently on the phosphorus metabolism of the plant. On the basis of the experimental results it seems possible that phosphatase plays a part in the carbohydrate metabolism of the higher plants, but further work is necessary to confirm the validity of the suggestion. The significance of the work reported lies in the fact that it is possible to show that phosphatase is active in the plant throughout its life history. Although the presence of phosphatase in the leaves had been previously known, most of the work which could throw some

light on the physiological role of the enzyme was carried out on ripening and germinating seeds. From the work of other investigators an impression had been created that phosphatase is concerned with the synthesis of organic phosphorus compounds as a store of that element in the ripe seeds, and that during germination the enzyme hydrolyses these organic compounds to release the phosphate radical for the use of the developing embryo. Phosphatase is, however, present in all parts of the plant and the fully developed leaves have the greatest concentrations. It must therefore play an important part in the metabolism of the plant throughout the whole of its life cycle.

SUMMARY.

Analyses of beans, potatoes, radishes and wheat for phosphatase content were made and the results are reported.

The bean. 1. Phosphatase activity in the dormant bean seed is low. The embryo contains a much higher concentration of the enzyme than the cotyledons.

2. In the germinated seed the enzyme concentration is much greater than in the dormant seed. The hypocotyl and the stem have the greatest concentration of enzyme, but the concentration falls off rapidly after a short period of growth. The concentration in the roots is not quite so high as in the stem. In the leaves the phosphatase concentration is low, but it is higher than that found in the cotyledons. The very low concentration of phosphatase in the cotyledons during the early stages of germination is not due to the presence of inactivators.

3. In the leaves, after they have emerged from between the cotyledons, the phosphatase activity gradually rises and reaches a maximum after a few days. The concentration of enzyme in the leaves at its optimum is higher than that of any other part of the plant throughout its entire life history.

4. As the plant matures the phosphatase concentration in the leaves decreases gradually, but in the stem and root it falls very rapidly, although it is always higher in the former than in the latter.

5. In the flowers the enzyme concentration is low and is comparable with that found in the leaves of a fully matured plant. The phosphatase content of the pods is equal to that of the flowers. The newly forming beans have a very low enzyme concentration.

The potato. In the potato plant which is sprouting from the tuber the greatest enzyme concentration is found in the leaves, and much smaller concentrations are found in the stem, whereas in the tuber and roots only slight traces can be detected by the experimental methods employed.

The radish. In the radish phosphatase is present in all parts of the plant, the greatest concentration being found in the leaves. In the petioles there is also a considerable amount of the enzyme. The root and the swollen hypocotyl have much smaller concentrations of phosphatase than the petioles. The radishes were grown for one month, during which time three samplings were made. The results did not vary throughout this period.

Wheat. 1. In the wheat grain the enzyme increases in concentration during germination. The greatest phosphatase content, however, is found in the plumules. The concentration of the enzyme in the rootlets was intermediate between that of the grain and the plumule.

2. When the aerial portion could be subdivided into two parts, the laminae and the leaf sheath with the tissues they enclosed were examined. No significant differences were noticed in the enzyme concentrations of those two parts of the plant.

3. At heading the phosphatase concentrations in laminae, leaf sheath and stem are equal and are lower than that found in the head.

4. The trend of phosphatase concentration in the life history of the wheat plant is similar to the trend in the bean plant, for the concentration of the enzyme in the young plants is high and decreases to a low level during maturation.

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