CCXLIV. STUDIES OF THE PHYSIOLOGICAL IMPORTANCE OF THE MINERAL ELEMENTS IN PLANTS¹.

VI. THE INFLUENCE OF POTASSIUM CHLORIDE ON THE RATE OF DIASTATIC HYDROLYSIS OF STARCH.

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THERE appears to be good evidence for the activation of animal diastases by a variety of salts, chlorides being the most effective [Haldane, 1930]. The results so far recorded for plant diastases are not so unanimous. Thus Sherman and Thomas [1915] reported an increased rate both of starch disappearance and of sugar production when numerous salts were added to malt diastase, but Sherman and Tanberg [1916] found no acceleration of sugar formation by the diastase of *Aspergillus Oryzae*, though Kellerman [1903] had already reported such an effect. These scanty and contradictory results suffer further from the fact that the acidity of the digests with which they were obtained was neither controlled nor observed, so that complete reinvestigation of the problem with all experimental precautions seemed the only means of reaching a satisfactory solution. This was undertaken as a contribution towards the study of the physiological importance of those elements which are derived by plants from the soil, and which remain in large part as simple salts within plant cells.

EXPERIMENTAL.

All the material used in this investigation was derived from a 1932 crop of potatoes var. King Edward. The potatoes were stored in a dark cellar and, for a short time before use, in the laboratory. All the experiments to be described were carried out during the months May–July 1933. At this late stage the tubers had produced small sprouts and were perceptibly softening due to loss of water. Large shoots 9–12 inches long with small leaves were obtained from some of the tubers by planting them in shallow boxes of sandy soil and keeping them moist. Three sorts of tissue were thus available and were used for the experiments, *viz.* tubers, small sprouts, and fairly large shoots.

Enzyme extracts were obtained from the tubers by peeling, mincing and finally pressing out the juice. During these operations the material was kept flooded with toluene to prevent bacterial contamination; this also had the further result of excluding air and so preventing blackening of the extract by oxidase actions which were otherwise very strong. The extract was allowed to

¹ Previous papers of this series have appeared in the *Ann. Bot.* 1930 *et seq.*, and the *New Phytologist* (in the press).

stand for half an hour, and the liquid could then be decanted free from suspended starch. A slightly different method was necessary with both the large and small shoots, which were ground vigorously with water (15 g. fresh weight with 25 cc. toluene and water) and the extract then filtered through muslin. Subsequent dilutions of the extract were made varying with the particular experiment to be performed.

Diastatic activity was examined in three different ways:

- (1) by the rate of disappearance of starch;
- (2) by the rate of formation of sugars from starch;
- (3) by the rate of formation of sugars from dextrin.

The rate of disappearance of starch was measured by Wohlgemuth's method carried out in narrow glass tubes enabling small quantities and relatively thin layers of solution to be used. This was necessary owing to the opacity of some of the enzyme solutions. The digests used contained 0.2 cc. of 0.5 % solution of Lintner's soluble starch + 0.05–1.00 cc. enzyme extract + 0.05 cc. toluene with water to make up to 1.25 cc. The tubes were incubated at 35° for a suitable time as determined by preliminary experiments; iodine was then added to each tube. The tube containing the weakest enzyme solution causing complete disappearance of the starch was then noted and the strength of the original enzyme extract calculated.

The rate of formation of sugars from starch was measured by the Hagedorn-Jensen method as modified by Hanes [1929]. Incubation was carried out again at 35°, the time period required usually being 24–48 hours. Preliminary experiments showed that these intervals lay on the linear portion of the hydrolysis progress curve. Lintner's soluble starch (supplied by B.D.H.) was again used as substrate. Three digests were used for every estimation.

Commercial preparations of dextrin could not be used for the third set of experiments as they were found to contain a strong inhibitor of the enzyme. No linear phase existed in the progress curves of their hydrolysis, which was very incomplete. The inhibitor could not be removed by electrodialysis or by controlling the p_H . Dextrin solutions were, therefore, prepared in the laboratory from Lintner's starch by the following method. A thick paste was prepared by dissolving 12.5 g. of starch in 250 cc. hot water, boiling for 10 minutes and cooling to 35–40°. The paste was strained through muslin while still warm, and 0.25 g. of an active takadiastase preparation (presented by Parke, Davies and Co.) added in 25 cc. of water. The progress of hydrolysis was followed by spot tests with iodine outside the solution. When the iodine colour had entirely disappeared, but the red dextrin colour was still present, the enzyme was destroyed by boiling for 2 minutes. The dextrin solution thus prepared contains a large amount of reducing sugars which carries the determination off the scale of the Hagedorn-Jensen method. This was removed by fermentation with an equal volume of 15 % yeast suspension for 3 hours at 35°. A stream of air was bubbled through the flask throughout the 3 hours. The yeast could not be removed by the usual method of flocculating with alumina cream since after such treatment the dextrin preparation inhibited the hydrolysing enzyme. Separation was effected by 2 minutes' boiling followed by centrifuging at about 3000 r.p.m. A clear solution of the dextrin with only slight reducing powers and no enzyme inhibitor could then be decanted. Using this solution as substrate the rate of sugar formation by the potato extract was determined as with the starch solutions except that the incubation time required was only 1 or 2 hours.

The action of potassium chloride on the enzyme activity was first examined by adding 5 cc. of 0–20 % potassium chloride to 5 cc. of enzyme extract and after a thorough mixing adding 2 cc. of this mixture to 50 cc. of substrate solution. In all the experiments recorded here the original concentration of potassium chloride used was 5 % since this amount was found to be optimum. The final concentration of potassium chloride was 0.19 % in the digests where sugar formation was examined. In the Wohlgemuth experiments in which the concentration of enzyme was made to vary the amount of potassium chloride was kept constant relative to the amount of enzyme. Control experiments were performed by adding 5 cc. of distilled water to the enzyme extract.

All the extracts were strongly buffered by the natural buffer systems at p_H 6, and the frequent colorimetric determinations carried out showed that this did not vary with the addition of

potassium chloride or any of the other experimental manipulations. The optimum p_H of the potato diastase was found to lie between 6 and 6.5. It was therefore unnecessary to add artificial buffers which inevitably complicate the system when salt effects are under investigation. Every precaution was taken by the use of toluene and by boiling the solution whenever possible to avoid bacterial infection.

In the following paragraphs the results are expressed in terms of the ratio:

$$\frac{\text{rate in presence of potassium chloride}}{\text{rate in absence of potassium chloride}}$$

This method is adopted as it conveys all the essential facts and avoids difficulties that would otherwise arise in expressing absolute rates in units applicable to all the experiments.

Tubers.

The rate of starch disappearance in the presence and absence of 5 % potassium chloride as measured by the Wohlgemuth method in the form already described showed an increase due to the salt. The ratio of the two rates of breakdown was about 1.8.

The corresponding rates of sugar formation, determined with the same enzyme extract, also showed an increase in the presence of the chloride. The ratios of the two rates were 1.97 (24 hours' digestion) and 1.85 (48 hours' digestion).

The Wohlgemuth results can only be regarded as very approximate but in view of later evidence the similarity of the ratios acquires a certain significance. Numerous repetitions of the sugar determination with further tuber extracts gave similar results.

The rate of sugar formation from dextrin was also examined and the results were in sharp contrast with the above. Two separate experiments each containing three digests gave the following results:

	Exp. 1	Exp. 2
$\frac{\text{Rate with KCl}}{\text{Rate without KCl}}$	1.03	0.98

There is therefore no activation of the enzyme causing the formation of sugars from dextrans.

Small sprouts.

Three experiments on the rates of starch breakdown by extracts of these shoots gave the following ratios:

	Exp. 1	Exp. 2	Exp. 3
$\frac{\text{Rate with KCl}}{\text{Rate without KCl}}$	1.5	1.5	1.7

Considerable activation took place, therefore, though probably not so much as in the tuber extracts.

The formation of sugars from starch on the other hand showed no acceleration due to potassium chloride. Experiments were performed with extracts from green shoots produced in the light and from aetiolated shoots formed on tubers kept continuously in the dark. The result was the same in both.

	Enzyme extract from green shoots	Enzyme extract from aetiolated shoots
$\frac{\text{Rate with KCl}}{\text{Rate without KCl}}$	1.00	1.02

This it will be noticed is in sharp contrast with the corresponding result for tuber extracts where 80–100 % activation took place.

The production of sugars from dextrin was not activated:

	Exp. 1	Exp. 2
$\frac{\text{Rate with KCl}}{\text{Rate without KCl}}$	0.95	1.00

Large shoots.

The values obtained for the ratio of the rates with and without potassium chloride were:

	Starch disappearance	Formation of sugar from starch	Formation of sugar from dextrins
	1.3	—	—
	1.1	1.3*	1.00
	1.5	—	0.85
	1.2	—	—
Mean	1.3	1.3	0.93

* Average of four titrations (two digests).

Activation is evident in the rate of starch disappearance and in the rate of formation of sugar from starch, but not in the formation of sugar from dextrin.

The amount of activation is less than in tubers or in younger sprouts, but it is noteworthy that, as with tuber extracts, the amount of activation of sugar formation is equal to the amount of activation of starch breakdown.

DISCUSSION.

It is clear from the above results that the diastatic hydrolysis of starch must be considered in at least two stages, each stage involving its own enzyme. One enzyme responsible for the immediate breakdown of starch molecules to form "dextrins" is capable of activation by potassium chloride (and perhaps other salts) while another enzyme concerned with the production of sugar from the dextrin formed by the first enzyme is not capable of such activation. This is true of all the material we have examined as may at once be seen from the foregoing section. The rate of formation of sugars from a starch substrate expresses the summation of these two processes and the results obtained here are at first sight less harmonious. Extracts from tubers and the larger shoots showed an activation but extracts from small shoots showed none. A simple explanation is provided, however, if we suppose that the tubers and large shoots contain relatively little of the starch-destroying enzyme and relatively much of the sugar-forming enzyme, and that the contrary relationship holds in the young sprouts. Then in the young sprouts where the starch-destroying enzyme is supposed to be comparatively plentiful an activation of this first enzyme will have no effect on the rate of sugar formation because it will still be restricted by the less active and inactivable second enzyme.

To clinch this argument it is necessary to show that the actual quantities of enzyme as shown by their activity in the presence of excess substrate have the appropriate relative values. To do this directly it would be necessary to convert the amounts of starch destroyed into their hexose equivalents, using some arbitrary factor for conversion, and to rely on the values supplied by the Wohlgemuth method. A more reliable comparison, having regard to the experimental data available, can be made by comparing the two methods of sugar production. When the first enzyme is more abundant and the "dextrinase" enzyme is controlling the rate of reaction the ratio

$$\frac{\text{Rate of sugar production from starch}}{\text{Rate of sugar production from dextrin}}$$

must be 1; conversely when the first enzyme is the less plentiful the ratio will fall below unity. The values calculated from the experimental results were:

Tubers	...	...	...	0.050
Small sprouts	...	...	...	1.149
Large shoots	...	...	...	0.118

and therefore provide striking verification for the theory.

All our results are thus explained by the fact that the starch-dissolving enzyme of diastase can be activated by potassium chloride but that the "dextrinase" component cannot. It is interesting to note that the apparently discordant results of earlier workers can all be explained in the same way. All the experiments performed with a method measuring the rate of starch disappearance indicated an activation by the salts employed [malt diastase, Sherman and Thomas, 1915; takadiastase, Sherman and Tanberg, 1916; potato diastase Haehn and Schweigart, 1923]. Apparently contradictory results were obtained, however, when the rate of sugar formation was taken as a criterion, and apart from possible deficiencies of technique, such as lack of control of acidity, these differences may well have been due to different proportions of starch-dissolving and sugar-forming enzymes in the preparations used by the different workers.

SUMMARY.

1. The effect of 5 % potassium chloride on the activity of potato diastase was examined in extracts of tubers, young sprouts and older shoots.

2. It was found that the rate of breakdown of starch was always accelerated, but that the formation of sugars from dextrin obtained from the starch was never accelerated.

3. In the presence of an excess of the substrate, the rate of production of sugars from starch was accelerated only if the rate of starch breakdown was slow relatively to the rate of formation of sugars from dextrin. This is to be expected from the previous results assuming that the dextrin is an intermediate product in the hydrolysis of starch to sugars. It furthermore affords a simple explanation of the apparent discrepancies between the results of previous workers.

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