CCXXX. SYNTHESIS OF SUCROSE IN PLANT TISSUE.

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THE transformations which different sugars undergo in plant tissue, independently of the photosynthetic process, are as yet imperfectly understood. There is every reason to assume that all the individual carbohydrates in plant material are capable of conversion into each other, though the experimental evidence at hand is insufficient to warrant definite conclusions in individual cases. This is also true of the most important hexose sugars, glucose and fructose. It is known that starch is readily formed from fructose when leaves that have been previously deprived of starch are kept in fructose solution in the dark. On the other hand, in wilting leaves starch is converted into sucrose. Similarly in the artichoke tuber, inulin—a condensation product of fructose—is in part converted into glucose [Colin, 1919]. It is also known that fructose is converted into glucose in the mammalian body, but evidence is still wanting to show whether a similar transformation takes place also in plants.

Although the formation of starch in leaves which are floated on solutions of various carbon compounds in the dark has been extensively studied by numerous workers, very little attention has been paid to other synthetic processes which possibly occur parallel with the synthesis of starch. In view of the very frequent occurrence of sucrose in various plant juices it was therefore of interest to study whether measurable amounts of sucrose were formed in leaves which were previously deprived of starch and then artificially fed with either glucose or fructose solutions in the dark. Such a synthesis would naturally involve a preliminary conversion of each of these hexose sugars into the other.

In fact, our experiments showed that sucrose is readily formed both from glucose and from fructose, the rate of synthesis being approximately equal in both cases.

In this connection it is interesting to note that Burkard and Neuberg [1934] have recently found in sugar-beet leaves phosphoric esters of both glucose and fructose.

EXPERIMENTAL.

Red clover and wheat were used as test plants as they produced a perfectly homogeneous material for analysis. Wheat proved to be the better of the two, owing to its more rapid growth and the fact that it did not contain appreciable amounts of reducing sugars. The plants were grown under greenhouse conditions in quartz sand in earthenware jars. Each jar contained about twenty wheat plants or four red clover plants. The experimental procedure was briefly as follows.

Red clover. An equal number of leaves was picked from each jar and the petioles were cut off. The leaves were rapidly chaffed, weighed and placed in a vacuum-drying apparatus which was previously heated to 100°. After 3 hours' drying *in vacuo* at 100° the material was weighed and ground to a powder, the dry matter content of which was determined from a small sample. After the leaves were picked, the jars were immediately placed in the dark for 24 hours in order to deprive the leaves of starch, whereupon an equal number of leaves was again picked from each jar and dried in the above manner. The remaining leaves were then cut off with their petioles and placed in a 10 % sugar solution for 24 hours in the dark. Care was taken that only the petioles came into contact with the sugar solution. For analysis, the petioles were cut off and the leaves were weighed, dried and ground to powder.

Wheat. The procedure followed was similar to that described above, except that three plants were taken from each jar, the leaves being cut for analysis. The sugar content of the leaves was determined again after 24 hours in the dark, whereupon the remaining plants were cut and placed in 10 % sugar solution so that the ends of the stalks reached some 5 cm. below the surface of the solution while the leaves did not come into direct contact with the liquid. After a further 24 hours in the dark the leaves were cut off from the stalks and analysed.

Estimation of sugars.

Total sugars. About 0.5 g. of the dry leaf-powder was weighed into a 150 ml. Erlenmeyer flask and hydrolysed on the water-bath with 30 ml. of 3 % HCl for 3 hours. The mixture was then filtered through asbestos, and the residue was washed repeatedly with small amounts of hot water, the volume of the liquid being made up to 100 ml. In this solution the total sugars were determined by Bertrand's method and calculated as glucose.

Soluble sugars. About 3 g. of the leaf-powder were weighed into a 150 ml. Erlenmeyer flask. 50 ml. of $M/15$ KH_2PO_4 solution were added, and the mixture was heated to boiling. After cooling, 0.5 ml. of toluene was added, and the flask was kept in an ice-chest overnight. The mixture was then filtered through asbestos, and the solution was made up to 100 ml. by repeated washing with hot water. The extract so obtained contained small amounts of protein which were removed with kaolin according to Michaelis and Rona [1908], and the liquid was centrifuged. Equal amounts of the protein-free extract were then taken for the estimation of (a) reducing sugars, (b) total soluble sugars, (c) sucrose. Reducing sugars were determined directly in aliquots of the solution by Bertrand's method and the total soluble sugars after hydrolysis by HCl on the water-bath for 15 minutes. For the estimation of sucrose an aliquot was treated with 0.5 ml. of toluene and 0.5 ml. of a concentrated invertase preparation¹. The solution was kept at 32° for 1.5 hours, after which it was analysed for sugar according to Bertrand. The sucrose content of the solution was calculated as invert sugar from the differences in the amounts of Cu reduced by a given amount of the extract before and after the hydrolysis by the enzyme preparation. All values are given in percentages of dry matter.

RESULTS.

Table I shows the results from an experiment in which 9 jars of red clover were used (May 7th, 1934).

¹ Invertinpräparat "Merck," No. 9098, supplied by E. Merck, Darmstadt. This preparation effects a complete inversion of sucrose in a few minutes, while maltose is not attacked.

Table I.

	Normal leaves	After 24 hours in the dark	After further 24 hours in 10 % glucose solution
Dry matter of the leaves, %	16.1	15.0	20.5
Reducing sugar	1.78	1.12	9.15
Sucrose	2.21	0.62	6.0

Other sugars were not determined. The results show that there is a striking accumulation of material in the leaves kept in glucose solution. The major part of the material is in the form of reducing sugar (probably glucose). There is, however, also a distinct formation of soluble, non-reducing sugar which is hydrolysed by invertase. 10.5 g. of dry leaf-powder were obtained from the leaves fed with glucose solution. Hence the amount of sucrose synthesised was 630 mg.

The following experiment was made with 240 wheat plants, cut about 2 weeks before blooming (May 23rd, 1934). The last portion of the leaves was divided into two lots, of which one was placed in 10 % glucose solution, the other in 10 % fructose solution.

Table II.

	Normal leaves	After 24 hours in the dark	After 24 hours in 10 % glucose solution	After 24 hours in 10 % fructose solution
Dry matter of the leaves, %	12.5	11.2	15.6	15.7
Total sugars	15.8	13.6	27.2	25.3
Total soluble sugars	3.65	Traces	15.7	15.0
Reducing sugar	Traces	0	9.1	8.25
Sucrose	3.52	0	6.35	6.0

It is particularly interesting to note that the whole amount of the synthesised, non-reducing sugar is hydrolysable by invertase, as shown by the fact that practically identical values were obtained when the extract of the powder was hydrolysed by HCl or by the invertase preparation. Thus, for instance, the following values were obtained for the leaf-powder in the experiment on fructose.

2.5 g. of the dry powder (dry matter 95.8 %) were extracted with KH_2PO_4 solution, and the volume of the extract was made up to 100 ml. as described above. 10 ml. of the protein-free extract, after hydrolysis by HCl, reduced 69.9 mg. Cu, corresponding to 35.9 mg. of glucose or 15.0 % of the dry matter. After hydrolysis by the invertase preparation, 10 ml. of the same extract reduced 68.9 mg. Cu, corresponding to 14.75 % of glucose in the dry matter. In the experiment with glucose the corresponding values were: 15.7 % after hydrolysis by HCl and 16.05 % after hydrolysis by invertase. The total amounts of sucrose synthesised in the above experiments were 775 mg. (from glucose) and 707 mg. (from fructose).

The results given in Table II also show that the synthesised sucrose actually originates from the simple hexoses (glucose and fructose, respectively) as the leaves contained only traces of soluble sugar when kept in the dark for 24 hours.

The fact that identical results were obtained on hydrolysis by HCl and by invertase also shows that the synthesised sucrose actually is present as such and not as a constituent of higher polysaccharides (raffinose, gentianose, etc.).

Other soluble disaccharides or polysaccharides were not formed. The above findings are best explained by assuming that a mutual conversion of glucose into fructose and *vice versa* takes place in plant tissue.

Research on the subject is being continued.

SUMMARY.

Sucrose is synthesised both from glucose and from fructose when leaves of red clover and wheat plants, previously kept in the dark for 24 hours, are placed in 10 % solutions of these hexoses for further 24 hours in the dark. In both cases the amount of sucrose synthesised was about 6 % of the dry matter of the leaves.

REFERENCES.

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