CXXXVI. STUDIES IN PHOTOSYNTHESIS.
II. THE FIRST SUGAR OF CARBON ASSIMILATION
AND THE NATURE OF THE CARBOHYDRATES
IN THE NARCISSUS LEAF.

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INTRODUCTION.

IN spite of the large number of investigations that have been made from time to time on the nature of the carbohydrates in the green leaf and the sequence of events that leads to their synthesis, there is at the present time no unanimity of opinion with regard to the nature of the first sugar formed in photosynthesis. Such an investigation is beset with a number of difficulties. The chemistry of the mesophyll cells is complex, for within them protein synthesis and respiration are taking place at the same time and the matter is still further complicated by the translocation of these substances to the storage organs.

The products of photosynthesis, either direct or indirect, are hexoses, disaccharides and polysaccharides. It would be expected on purely chemical grounds that the simplest members of this series, the hexoses, would be the first sugars formed in the assimilating organs of the green plant and that these in turn would suffer condensation to disaccharides. Nevertheless, the majority of workers on this problem of plant physiology have reported in favour of the hypothesis of sucrose being the first sugar of photosynthesis. Sucrose is a product peculiar to the plant world and has a wide distribution in every group of green plants.

It is not proposed here to give a detailed review of previous work on this subject, a large amount of which is of little value for a variety of reasons. The first adequate investigations on the quantitative relationships of the sugars of the leaf were carried out by Brown and Morris [1893] on *Tropaeolum majus*. In such work as this, it is very necessary that the leaf material should be killed instantaneously so that enzymic changes are prevented. Brown and Morris killed their material by drying in a steam oven between 75° and 80°. The carbohydrate values were calculated as percentages of the dry weight and the carbohydrates found were starch, maltose, sucrose, glucose and fructose.

From the variations in the amount of sucrose in leaves and the small

amounts of glucose and fructose found under different conditions Brown and Morris claimed that sucrose and not hexose was the first sugar of photosynthesis. It was considered that when the concentration of sucrose reached a certain value it was converted into starch, while for purposes of translocation it was first inverted to glucose and fructose and removed as such to the roots.

Stiles and Jørgensen [1916] have pointed out in a critical discussion of this work that the values recorded by Brown and Morris for the glucose and fructose fractions of their preparations are extremely doubtful. Davis, Daish and Sawyer [1916] were quite unable to find maltose in their preparations from *Tropaeolum* leaves and concluded that the method of killing by heating in a steam oven had resulted in a certain amount of degradation of the starch by diastatic enzymes.

Parkin [1911], from his investigations of the monocotyledonous plant, *Galanthus nivalis* (snowdrop), also supported the views of Brown and Morris that sucrose and not hexose was the first sugar of photosynthesis. In monocotyledonous leaves, the complications introduced by the presence of starch are eliminated, for, as a general rule, the leaves of this group of plants do not form starch and only a quite negligible amount is to be found in the guard-cells of the stomata.

Parkin found that as a general rule the percentage of hexoses remained fairly constant in amount during any single day in the spring, whereas the sucrose exhibited marked fluctuations, increasing during the day and diminishing during the night. With advance in the season the proportion of hexose to sucrose was found to increase. Parkin concluded that his values justified the suggestion of Brown and Morris that sucrose was the first sugar of carbon assimilation. Although this view is possibly justified from the experimental figures, the intervals of time that were allowed to elapse before each observation was taken were far too long to allow of deductions of this nature being made from them.

A highly elaborate attempt to settle this problem was carried out at the Rothamsted Experimental Station by Davis, Daish and Sawyer [1916] on the leaf of the mangold. These workers estimated sucrose, maltose, glucose, fructose, starch, pentoses and pentosans. The leaf material, after the midribs had been removed, was killed by throwing it into boiling 95 % alcohol containing a little ammonia.

Collections of the leaves were made at 2-hourly intervals over 24-hour periods. Three such determinations were made, in August, September and October respectively. The mangold leaf was found to contain starch only in the early stages of growth. In each of these experimental runs the sugars of the leaf lamina and petiole were treated separately and the results were expressed as a percentage of the dry weight.

In general terms it was found that in the early summer the soluble sugars (hexoses and sucrose) increased in the light to a maximum about noon and then fell off fairly regularly till the following dawn, and the concentration of sucrose

was always in excess of the hexose values and the percentage of pentosans remained fairly constant. No maltose was found to be present.

In the second experimental run (September) the hexoses were found to be in excess of sucrose and both the curves showed maxima at 2 p.m., 6 p.m. and 2 a.m. The results obtained in October were much the same as those obtained in September. Like Brown and Morris and Parkin, Davis, Daish and Sawyer were of the opinion that sucrose was the first formed sugar and that inversion of the sucrose to glucose and fructose took place prior to translocation, since hexoses were found to be in excess of sucrose in the petiole.

The problem of the first sugar has been investigated from a different aspect by Weevers [1924] who employed plants with variegated leaves. In all, some twelve different species were investigated. In every case, save two (*Aesculus Hippocastanum* and *Cornus sanguinea*), both hexoses and sucrose were found in the green parts and only sucrose in the variegated portions. In *A. Hippocastanum* and *C. sanguinea* the variegated parts also contained a small amount of hexose. Since the variegated portions of these plants contain no chlorophyll and photosynthesis is impossible in these regions, it would seem a significant fact that no reducing sugars were found in these parts. The evidence from this fact alone is not conclusive, but in a second experiment, Weevers found that the leaves of the variegated form of *Pelargonium zonale* when placed in the dark for 48 hours were completely deprived of all sugars. At the end of this period the plants were allowed to assimilate, and it was discovered that the first sugar produced was a hexose and not sucrose.

A re-investigation of the sugars in the mangold has been carried out in America by Tottingham and his co-workers [1926]. The sucrose content of the leaves was found to vary very irregularly and over a considerable portion of the experimental period closely followed the course of the reducing sugars. Occasionally, however, it varied inversely as the hexose values. In view of these results, they considered that hexose and not sucrose was the first sugar of carbon assimilation.

At the conclusion of our own work an important paper on this subject appeared in America by Clements [1930]. Like ourselves (see below) he measured the fluctuations in the sugar fractions at hourly intervals in the leaves and petioles of *Helianthus annuus*, *Soja max* and the potato over 24-hour periods, and considered from the fact that the sucrose maxima were only reached after a long period of assimilation that simple sugars (pentoses and hexoses) and not sucrose are the first-formed carbohydrates in the leaf.

From the highly conflicting views that have been expressed in the literature on the nature of the first sugar of carbon assimilation, it was thought interesting to re-investigate the question and the results are recorded below.

EXPERIMENTAL.

The plant employed throughout this investigation was the daffodil, *Narcissus Pseudo-Narcissus* L. (vars. Emperor and Sir Watkin). It was decided to use

a monocotyledonous rather than a dicotyledonous plant to obviate the complications introduced by the presence of starch. The *Narcissus* leaf, like the greater number of monocotyledonous leaves, only forms hexoses and sucrose and contains but a negligible amount of starch in the guard-cells of the stomata. Further points in favour of using a monocotyledonous plant, such as *Narcissus*, are firstly, the high percentage of sugars in the leaf, and secondly, the plants are available for use in the early part of the year.

The experimental work was carried out at the Chelsea Physic Garden and the Royal Botanic Gardens, Regent's Park. The bulbs were specially selected and planted out in rows 18 in. apart and the plants were placed at a distance of 12 in. from each other.

Preparation of leaf material.

One of the main problems confronting us was to devise a satisfactory method of killing the leaves. Brown and Morris killed their material by placing it in an oven at 80°. But even with the leaves of *Tropaeolum*, which possess very thin laminae, maltose was found to be present. This maltose was subsequently shown by Davis, Daish and Sawyer to be due to diastatic activity at the high temperature employed and was not normally present in leaves. Parkin, on the other hand, found that little inversion occurred in the leaves of *Galanthus nivalis* in similar circumstances. This method of killing, however, is clearly inadmissible in the case of *Narcissus* leaves. The leaves are very thick and sappy and it is on this account unlikely that placing them in a temperature of 80° or 100° will bring about instantaneous death without inversion of sucrose. We therefore had to resort at first to the method used by Davis, Daish and Sawyer with certain slight modifications.

The leaves were first frozen at - 16° in a mixture of salt and ice for 6 hours. They were then ground with sand and the temperature was never allowed to rise to more than - 3°. The pulverised mass was thrown into boiling 95 % alcohol containing a little ammonia to neutralise organic acids and to stop enzymic activity. After boiling for half an hour, the leaves were extracted in a Soxhlet apparatus for 4-6 hours with the same alcohol used for killing. It was found that the preliminary grinding with sand rendered unnecessary the long extraction period (12-18 hours) used by Davis, Daish and Sawyer. The sugars were completely removed from the leaves in 4-6 hours. This method very unfortunately suffers from the defect that appreciable amounts of formaldehyde and acetaldehyde are produced. At the end of the extraction period, the solution showed a marked acid reaction, and, when the alcohol had been distilled off under reduced pressure and the residue treated with lead acetate to remove colouring matter and protein, it was found that both distillate and sugar solution contained aldehydes.

In the preliminary work of this investigation, commercial 95 % alcohol was used for killing the leaf material. After a time it was found that the alcohol residues obtained from the distillation of the leaf extracts possessed a powerful

smell of formaldehyde. The presence of formaldehyde was confirmed with Schiff's test and dimedon. To test further whether *Narcissus* leaves were peculiar in this respect of producing aldehydes other leaves were used, *e.g.* *Hedera helix*, *Abutilon* and *Pelargonium zonale*. Positive reactions were obtained in all cases and the reaction was particularly marked with *Hedera helix*. It is possible that the chlorophyll of the leaves oxidises the alcohol partly to aldehyde. In a second series of experiments absolute alcohol was diluted to 95 % and the same procedure as above carried out. In this case no formaldehyde was formed, but a considerable amount of acetaldehyde was obtained. The presence of either aldehyde will vitiate the hexose values obtained by copper reduction methods. To test this point we took samples of leaves and treated them with the alcohol method and compared the figures with those obtained by the method which we ultimately used in this investigation (see below). The figures given are representative of a large number of analyses which we carried out.

100 g. of leaves taken, chopped fine and mixed. 50 g. samples used for each test. Results expressed as a percentage of "residual dry weight."

Sample A. (Killed in alcohol.)

Hexose 18.3 %.	Sucrose 4.4 %.
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Sample B. (Killed in boiling water.)

Hexose 15.5 %.	Sucrose 4.7 %.
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Difference Hexose 2.8 %.	Sucrose 0.3 %.
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It will be seen that, although the sucrose value was almost unaffected, the hexose differed by 2.8 % in amount. This large difference in the values given for the hexose sugars was found in every case.

To discover whether the presence of aldehyde affected the optical rotation of sugar solutions, a 500 cc. sample of sucrose, fructose, and glucose was made up which gave a rotation of $\alpha_D = +26'$. The solution was now evaporated under reduced pressure at 50° to 200 cc. and then made up to 500 cc. once more. The rotation was again $\alpha_D = +26'$. A similar test was carried out after the addition of a small amount of acetaldehyde. Before evaporation the rotation was $\alpha_D = +29', 3''$. After evaporation under reduced pressure and making the volume up to 500 cc., rotation $\alpha_D = +36', 0''$. The presence of the aldehyde therefore also affects the polarimetric readings. Thus the figures obtained by Davis, Daish and Sawyer for total hexoses and also for glucose and fructose based on a copper reduction method and optical rotation must be looked upon with suspicion.

The following method was found to overcome this difficulty. The leaves were first frozen in the manner described above, ground up with sand and thrown in small quantities at a time into boiling water containing ammonia. They were allowed to boil gently for 10 minutes and then filtered through muslin and carefully squeezed. The squeezed mass was allowed to remain in cold water for 2 hours (cf. Parkin) and the process of filtering and squeezing was repeated. Four such treatments were found to remove completely all the sugars of the

leaf. The filtrates were mixed and distilled under reduced pressure to about 200 cc. bulk. 5 cc. of lead acetate solution were now added to clear the solution. An excess of lead acetate is to be avoided as it tends to destroy fructose. With this amount of lead acetate only a minimum quantity of lead remained in the solution and this was removed with sodium phosphate. The liquid was filtered off and washed free from the precipitate and made up to 500 cc.

Analysis of sugars.

Total hexose. For the analysis of total hexose and sucrose, Benedict's solution was employed. The method is susceptible of great accuracy and with the modifications we were able to introduce is very rapid, so that a number of determinations can be carried out in a short time. Although the white precipitate of copper thiocyanate gives a clearer end-point than Fehling's solution, it was still found difficult to see when the last trace of blue had disappeared from the Benedict's mixture. It was found, however, that if 20 cc. of a saturated solution of potassium thiocyanate containing 20 % of sodium carbonate were added to the Benedict's solution, this precipitate was removed and the end-point became very sharp. The addition of the excess of thiocyanate in no way interferes with the accuracy of the method as the following figures obtained with known sugar solutions show:

Glucose, 0.1 % solution.	Found 0.1 %.
„ 3.0 „	„ 2.99 %.
Sucrose, 0.1 „	„ 0.099 %.
„ 2.0 „	„ 1.996 %.

By carrying out the following procedure the analysis was greatly expedited and as many as thirty or forty estimations could be effected in the course of a day. 25 cc. of Benedict's solution were pipetted into a conical flask and 20 cc. of the potassium thiocyanate solution added. The mixture was raised to the boiling-point and 10 cc. of the sugar solution were run in. When the whole was gently boiling, it was back-titrated with a 0.5 % solution of glucose (25 cc. of Benedict = 10 cc. of 0.5 % glucose sol.).

Sucrose. The sucrose was inverted in a separate portion with 10 % citric acid and the process described above repeated. Invertase was first employed for the inversion, but the citric acid and invertase values agreed so well that the citric acid was always employed and had the added advantage of rapidity.

Glucose and fructose. Attempts were made to determine the amounts of glucose and fructose in the leaf preparations. The iodimetric method of MacLeod and Robison [1929] was first utilised. Although the method yielded admirable results in our hands with mixtures of pure glucose and fructose in distilled water, it gave very discordant results with preparations from leaf material. The presence of the slightest trace of lead at once vitiated the method and even after all the lead had been removed with hydrogen sulphide and the solution subsequently aerated for 60 hours to remove all trace of the gas, discordant results were still obtained owing to the presence of traces of hydrogen

sulphide. The removal of the lead with sodium carbonate or oxalate is inadmissible with this method. Polarimetric methods were also abandoned as discordant results were again obtained. It was therefore decided to estimate only total hexose and the values recorded for reducing sugars include both glucose and fructose. No pentoses were found in our preparations.

Expression of results.

Previous workers have expressed their results as a percentage of the total dry weight of the leaf material (*i.e.* material dried to constant weight at 100°). The dry weight of a leaf, however, depends on two separate factors: the material already present and the sugars formed in assimilation. Dry weight does not therefore give a constant basis for the calculation of results, as the sugar values will vary with the rates of assimilation, respiration and translocation. As Mason and Maskell [1928] have pointed out in their work on translocation in the cotton plant, "residual dry weight," *i.e.* dry weight less total sugars, gives a better basis for the estimation of sugar percentages, since presumably residual dry weight will be a constant value over a short period of time. Chibnall [1922] has suggested that wet weight would present a more constant basis for the calculation of fluctuations in leaf products, since the variation in the water content of the leaf, relative to the total water present, is very small indeed. Further, the leaf tends to lose water and gain carbohydrates by day and to gain water and lose carbohydrates by night, on this account the total variation in water content will be, in general, less than that of dry weight. Nevertheless, there is little to show what this variation really is. Throughout this work, except where otherwise stated, we have calculated our sugar values as a percentage of the residual dry weight.

Method of experiment.

Errors due to sampling were small (1–2 %). In the field the leaves were chosen at random, one leaf from each plant, chopped fine and mixed. Samples were withdrawn for dry weight estimations and 50 g. samples were taken and accurately weighed for determinations of sugars. Continuous records were made of temperature and humidity, and stomatal aperture was measured by a Knight's porometer.

Previous workers have always determined their sugar values at comparatively long intervals of time, but it was considered advisable to estimate sugars at hourly intervals.

Before an experimental run, the plants were approximately graded as to height and then artificially darkened for periods that varied between 72 and 24 hours. All determinations were submitted to statistical treatment by the methods given by Fisher [1925].

The experimental work to be discussed falls under two heads.

- (1) Sugar fluctuations before the flower buds had opened.
- (2) Sugar fluctuations after the flower buds had opened.

Exp. 1. (Chelsea Physic Garden.) March 31, 1930.

The flower buds and stalks were removed, the plants were graded as to height and then darkened (March 28) at 10 a.m. The experimental run was begun at 10 a.m., March 31. The day was sunny at the beginning, but later clouded over and there were several heavy showers of rain. No porometer readings were taken on this occasion. Determinations of sugars were made at hourly intervals.

Table I.

	Time	Hexose %	Sucrose %	Total sugars %
Friday, Mar. 28, 1930	10 a.m.	2.65	7.80	10.45
Monday, Mar. 31, 1930	10 a.m.	6.24	4.1	10.31
	11 a.m.	5.30	5.8	11.10
	12 noon	5.40	5.2	10.60
	2 p.m.	6.80	8.5	15.30
	3 p.m.	3.90	6.6	10.50
	4 p.m.	4.80	11.7	16.50
	5 p.m.	3.60	7.7	11.30

Fig. 1.

The results are graphically shown in Fig. 1. At the commencement of the run, the hexose was in excess of sucrose and at the end of the first hour, the former had fallen, while the latter showed a rise in concentration and the values of hexose and sucrose were approximately the same at the end of the second hour. At 2 p.m. both hexose and sucrose showed a sharp rise and thereafter the sucrose was always in excess of the hexose. It is a significant fact that the sucrose values only gradually reach a maximum, and the highest peak is finally attained after 6 hours of assimilation (4 p.m.). Between 12 noon and

1 p.m. there was a heavy shower of rain. Presumably during this period assimilation would be at a minimum (see below) and yet the sucrose values showed a continuous rise. Two maxima are shown on the curves: the first at 2 p.m. when the hexose value was 6.8 % and the sucrose 8.5 %, and the second at 4 p.m. when the hexose was 4.8 % and the sucrose 11.7 %. After each of these maximum points had been attained there was a fall in the concentration of both hexose and sucrose.

This experimental run, while giving no definite evidence for hexose as the first sugar of the leaf, nevertheless confirms the observations of Parkin that sucrose is in excess of hexose before the flowering period. It also shows that it is improbable that sucrose is the first sugar of photosynthesis, as the maximum sucrose values are only reached after a number of hours of assimilation and lastly, the rise in sucrose concentration is unaffected by rain.

Exp. 2. (Royal Botanic Gardens, Regent's Park.) May 8, 1930.

In Exp. 1 the sugar fluctuations were followed from a period of bright sunshine to late evening after the plants had been in darkness for 72 hours. In this second run, the plants were first allowed to flower and then flowers and flower stalks were removed before the determinations were made on the leaves. Analysis of the flower stalks showed that sucrose was in excess of hexose, whereas in the corollas, which contain no chlorophyll, only a trace of reducing sugars was found.

It was decided to darken the plants for 24 hours instead of for a longer time. The plants were again approximately graded as to height and then darkened. Continuous records of temperature and stomatal aperture were made. The experimental run began at 10 a.m. and was discontinued at 2 p.m. All samples were taken at hourly intervals.

Table II.

Thursday, May 8, 1930

Time*	Hexose %	Sucrose %	Total sugars %
10 a.m.	16.40	6.20	22.40
11 a.m.	17.50	5.30	22.80
Noon	11.80	7.50	19.30
1 p.m.	12.10	9.20	21.30
2 p.m.	19.80	11.70	31.50

* Summer time.

Fig. 2 shows the values for hexose, sucrose, temperature and stomatal aperture graphically expressed. The sugar percentages show that once the flowers had opened, hexose was in excess of sucrose in the leaves, a result in agreement with Parkin and also with other workers. During the first hour there was an increase in hexose and a fall in sucrose. If a hexose be the first sugar of assimilation, this result is to be expected. From 11 a.m., onwards to 2 p.m., there was a continuous rise in sucrose. The hexose, on the other hand, fluctuated markedly. At 11 a.m. there was a particularly heavy shower of rain and decrease in the light intensity, and during this time the hexose value

dropped by nearly 6 %. The sucrose, on the contrary, was not affected and rose steadily throughout this rainy period. Again, if a hexose be the first sugar of assimilation this result is to be expected. A curious point was noticed here, that the stomata of *Narcissus* are relatively insensitive to shock for they

Fig. 2.

only slightly closed during the rain, which beat down with considerable violence.

Although this experiment yields very definite evidence that a hexose is the first sugar of assimilation, it was considered that a run extending from early dawn to late evening would yield results of still greater significance.

Neither Exp. 1 nor Exp. 2 was extended over a sufficiently long period of hours to allow of any correlation coefficients being calculated that would be statistically significant. It was also thought possible that in a run extending over a large number of hours, it might be possible to see whether there were any lag periods between hexose increase and sucrose increase and *vice versa*.

Exp. 3. (Royal Botanic Gardens, Regent's Park.) May 20, 1930.

All plants were graded as to height on the day before the experimental run. The flowers and flower stalks were then removed and the plants were artificially darkened at 10 a.m. The coverings were removed at 10 p.m. on the same evening and the determinations were commenced at 3.45 a.m. on the following morning before daylight. Continuous records were made of humidity, temperature and stomatal aperture.

Table III.

Tuesday, May 20, 1930

Time*	Hexose %	Sucrose %	Total sugars %
3.45 a.m.	18.20	5.30	23.50
4.45 a.m.	16.70	6.50	23.20
5.45 a.m.	17.00	7.02	24.02
6.45 a.m.	18.70	7.06	25.76
7.45 a.m.	17.50	5.50	23.00
8.50 a.m.	18.10	7.10	25.20
9.50 a.m.	16.60	6.90	23.50
10.45 a.m.	19.10	6.10	25.20
Noon	18.90	7.80	26.70
12.55 p.m.	15.30	9.20	24.50
1.50 p.m.	17.80	12.40	30.20
2.45 p.m.	19.50	11.40	30.90
3.30 p.m.	18.00	10.00	28.00
4.30 p.m.	14.10	11.70	25.80
5.45 p.m.	13.70	10.80	23.50
6.45 p.m.	13.30	12.20	25.50
7.45 p.m.	12.90	13.40	26.30
8.45 p.m.	14.10	13.40	27.50
9.40 p.m.	11.80	10.40	22.20

(Results calculated as percentage of residual dry weight.)

Table IV.

Tuesday, May 20, 1930

Time*	Hexose %	Sucrose %	Total sugars %
3.45 a.m.	1.700	0.522	2.222
4.45 a.m.	1.600	0.664	2.264
5.45 a.m.	1.550	0.640	2.190
6.45 a.m.	1.700	0.640	2.340
7.45 a.m.	1.800	0.576	2.376
8.50 a.m.	1.700	0.712	2.412
9.50 a.m.	1.700	0.712	2.412
10.45 a.m.	1.800	0.570	2.370
Noon	1.750	0.712	2.462
12.55 p.m.	1.500	0.900	2.400
1.50 p.m.	1.500	1.150	2.650
2.45 p.m.	1.700	0.998	2.698
3.30 p.m.	1.800	0.998	2.798
4.30 p.m.	1.550	1.282	2.832
5.45 p.m.	1.550	1.234	2.784
6.45 p.m.	1.400	1.282	2.682
7.45 p.m.	1.350	1.378	2.728
8.45 p.m.	1.350	1.282	2.632
9.40 p.m.	1.300	1.140	2.440

(Results calculated as a percentage of wet weight.)

* Summer time.

Figs. 3 and 4 show these results graphically expressed for the values calculated on residual dry weight and wet weight respectively together with the curve for stomatal aperture.

The correlation coefficient (r) for hexose and sucrose was $r = 0.71 \pm 0.12$. This value is statistically significant and shows that there is a direct correlation between these two classes of carbohydrates. The correlation between hexose and temperature was $r = 0.47 \pm 0.18$, whereas between sucrose and temperature the value was $r = 0.3 \pm 0.02$. The latter value is not statistically significant, the former barely so. Nevertheless, there is a closer correlation between hexose

and temperature than between sucrose and temperature. There was no correlation between humidity and dry weight over the total experimental time (19 hours), $r = 0.35 \pm 0.206$, nor between 3.45 a.m. and 2.45 p.m.,

$$r = 0.331 \pm 0.26,$$

but there was a significant correlation between these values for the period between 2.45 p.m., and 9.40 p.m., $r = 0.8839 \pm 0.089$ (see Fig. 7).

Fig. 3.

Fig. 4.

An examination of Fig. 4 shows a marked fluctuation in the hexose values, whereas those of sucrose only begin to show a continuous rise after 10.45 a.m. There is a sharp rise in the hexose value at 9.50 a.m. which is followed by a fall in the sucrose concentration and there is a lag period of 1 hour before the sucrose begins to mount once more. We have already observed that there is a correlation between the hexose and sucrose values ($r = 0.71 \pm 0.12$) and

that there is a lag period of 1 hour between increase in hexose and increase in sucrose (9.45 a.m. and 10.45 a.m.). We should therefore obtain a higher correlation between hexose and sucrose if the value for sucrose be shifted forward a period (4 hours). The result of making this shift was to increase r from 0.71 ± 0.12 to 0.77 ± 0.106 . Variations in hexose are therefore reflected in sucrose and the lag period is significant. Rain at 10.45 a.m. led to a fall in the hexose, but the sucrose still showed a rise. Between 1.50 p.m. and 3.30 p.m. there was an increase in hexose, whereas after a lag period of 1 hour the sucrose fell. The maximum hexose percentage was obtained at 2.45 p.m., and thereafter there was a continuous fall. The sucrose, on the other hand, was still increasing in amount till the maximum percentage was reached at 8.45 p.m. when it crossed the hexose curve, after which it fell away. During the early hours of the morning, 4.45 a.m. to 6.45 a.m., when the stomata were actively opening, the hexose was increasing in amount and the sucrose decreasing.

Consideration of Fig. 4 based on wet weight follows in general outline the results obtained with residual dry weight, although the fluctuations are not so marked for either hexose or sucrose. Nevertheless, an examination of the hexose curve and stomatal aperture shows that when the stomata were actively opening there was increase in the hexose, while the sucrose remained relatively constant. Again, the rise in sucrose between 7.45 a.m. to 8.45 a.m. shows a marked lag behind the increase in hexose. As in Fig. 3, there is a continuous increase in sucrose after 10.45 a.m. and a corresponding fall in hexose, and the continuous fall in hexose after 3.30 p.m. is accompanied by a continuous increase in sucrose to a maximum at 7.45 p.m. The stomata started to close at 5.45 p.m. and the hexose values had begun to fall an hour before this period, whereas the sucrose still continued to rise.

Examination of the hexose and sucrose curves, based on either residual dry weight or wet weight shows that there is a continuous negative drift with time in the hexose values ($r = -0.84$) through the day and a continuous positive drift in the sucrose concentrations ($r = +0.875$). Parkin in one of his experiments found the same result, but interpreted it as showing that sucrose was the first sugar of assimilation. On the assumption that hexose and not sucrose is the first sugar and that an equilibrium condition exists between them this result is to be expected.

The hexose values, based on residual dry weight show three maxima, the first at 6.45 a.m. (18.7 %), the second at 10.45 a.m. (19.1 %) and the third at 2.45 p.m. (19.5 %). After each of these maxima has been attained, the sucrose values show a continuous rise for periods varying between 4 and 5 hours. It would therefore appear that the hexose reaches a certain definite concentration and then suffers condensation to sucrose.

The curves for total sugars, based on residual dry weight and wet weight respectively, show a very considerable shift in the maximum position. In the case of the residual dry weight curve (Fig. 5) the maximum total sugars are found at 2.45 p.m., whereas with wet weight (Fig. 6) the maximum is not

attained till 4.30 p.m. This result is no doubt due to the marked correlation between dry weight and humidity that was found to exist after 2.45 p.m.

Fig. 5.

Fig. 6.

DISCUSSION.

The utilisation of a leaf such as that of *Narcissus*, in an investigation of this nature, reduces the problem of the first sugar of assimilation to its simplest proportions and the complications introduced by the presence of starch are eliminated. Both Brown and Morris, who worked on a dicotyledonous leaf, and Parkin, who used a monocotyledon, claimed that sucrose was the first sugar and that it was inverted to glucose and fructose for translocation to the storage organs. The value of this work, apart from errors of technique in the case of Brown and Morris's estimations, is largely diminished by the fact that the leaf

sugars were only determined at long intervals of time. Again, the figures obtained by Davis, Daish and Sawyer are vitiated by faulty technique as far as the reducing sugars are concerned. But even if this be allowed for, an examination of their curves gives no conclusive evidence for the production of sucrose as the first sugar of photosynthesis. In fact the curves obtained for the October experiment show very marked lags between the hexose and sucrose, and the latter lags behind the former.

Fig. 7.

The claim that carbohydrates are translocated to the storage organs as glucose and fructose has not been substantiated by the recent work of Mason and Maskell on carbohydrate transport in the cotton plant. Here the sugars leave the leaf as sucrose. It is possible so far as the values recorded by Davis, Daish and Sawyer are concerned that inversion of sucrose had taken place in the petioles due to the method of killing and that it was on this account that the hexose values exceeded the sucrose percentages. The petioles of the mangold are, relatively to the leaf lamina, comparatively massive organs and it is probable that they were not instantaneously killed and that for a short time, owing to high temperature, invertase activity was high.

Clements [1930], who worked with three different species and, like ourselves, estimated sugar variations hourly, considered that his data supported the view that simple sugars (pentoses and hexoses) precede the formation of sucrose in the leaf and that the latter subserves the function of a temporary storage product and is also the mobile form of sugar in the plant. We are in agreement with the general interpretations of this worker. It is unfortunate, however, that he should have employed the alcohol method of killing the leaves. The error introduced by the presence of aldehyde is very material. Moreover, the question whether pentoses be a direct product of photosynthesis is doubtful; we were quite unable to find any pentoses in our preparations. He also puts forward the claim that hemicelluloses show fluctuating values in the

leaf and form an important storage product. The method of estimating hemicellulose was to hydrolyse the leaf residue with 2.5 % sulphuric acid. This method is not advisable, since not only will hemicelluloses suffer hydrolysis, but so will pentosans and possibly the cellulose of the leaf cells. Clements does not define the term "hemicellulose" and it is impossible to say what value he attaches to it, and certainly his analytical procedure allows of little weight being attached to his figures for these bodies. It is also unfortunate that he should have calculated his results on a wet weight basis and not as a percentage of the residual dry weight, and lastly no attempt was made to submit the data to statistical treatment.

The evidence presented in the present investigation can leave little doubt that a hexose precedes the formation of sucrose in the green leaf. There is in the first place a direct correlation between these two classes of sugar. There is also a greater correlation between temperature and hexose than between temperature and sucrose. It is true that the former correlation is barely statistically significant, but on the other hand, plants such as *Narcissus* and other bulbous monocotyledons are in active growth in the cold seasons of the year and it is therefore possible that temperature does not play as great a part in their metabolic activities as in summer vegetation. There is a significant lag between increase in hexose and increase in sucrose, and even in the case in which sucrose was in excess of hexose in the leaf, *i.e.* before flowering, the sucrose values only slowly drifted towards a maximum value.

The investigations of F. F. Blackman and Matthaei [1905] on the assimilation rate of *Helianthus annuus* under natural conditions showed that there was a marked fall in the assimilation rate during rain. This condition is typified as far as our hexose curves are concerned. In every case at the onset of rain the hexose fell and the sucrose was unaffected and showed a continuous rise. Lastly, there is a continuous negative drift of hexose from the beginning of photosynthesis to darkness, and a positive drift of sucrose during the same time—a result to be expected if the hexose be continuously suffering condensation to sucrose.

If the synthesis of sugars in the leaf be looked upon as an equilibrium reaction between hexose and sucrose:

and if it be further supposed that when the hexose reaches a certain concentration there is condensation to sucrose, or if it fall below a certain value the sucrose is inverted to hexose, then this result is also paralleled in our curves. In Exp. 3, three maxima were found for the hexose values and after each there was a continuous rise in the concentration of sucrose. Priestley [1924] has taken much the same view for the synthesis of sugars in the leaf. He considered that since sugars are formed as intermediate steps in the formation of more complex anhydrides, such as starch, it is necessary that these reactions should take place smoothly and rapidly.

With regard to the function of sucrose in the plant, Priestley has put forward an unusual suggestion and one for which we can find no experimental support, namely, that sucrose is a by-product of ageing protoplasm in meristematic cells and that as these cells become vacuolated sucrose is released. It would thus appear from this view that sucrose at the growing meristems of the plant arises as a catabolic product. As Clements has pointed out, this view at once eliminates the places of storage, as, for example, in the sugar cane and sugar beet, and also eliminates the leaves, for there is a considerable amount of sucrose present in these parts. It may well be asked how the sucrose is formed in these regions.

The argument advanced by Davis, Daish and Sawyer for sucrose as the first sugar, based on the fact of the well-known enol conversion of glucose to fructose and mannose and the absence of mannose from leaves, does not apply here. The enolic form of glucose can only exist in alkaline solution, whereas the reactions in the leaf are taking place in acid media.

If a hexose be the first formed product of photosynthesis, a further difficulty has to be contended with, for not one, but two hexoses, glucose and fructose, must be formed to give cane sugar by condensation. In this connection, Nef [1913] showed that aqueous solutions of *d*-glucose, *d*-fructose and *d*-mannose, in the presence of a 0.05 equivalent of calcium hydroxide at the ordinary temperature, gave mixtures composed of *d*-mannose, *d*-glucose, *d*-fructose, *d*-pseudofructose, as well as α -, β - and *d*-glucose. Similar results have been obtained by Wilbur and Spoehr, who have been able to bring about interconversion of sugars in the presence of solutions of disodium hydrogen phosphate and in neutral mixtures of this salt and sodium dihydrogen phosphate. Again, in the animal economy, fructose is converted into glucose. On these grounds, if glucose be first produced in the leaf, the mesophyll must be possessed of a special and at present unknown mechanism to convert part of this glucose to fructose and other hexoses. Nevertheless, mannose as such has never up to the present been isolated from the leaf. Its recognition should be simple as it is the only known hexose that forms a sparingly soluble hydrazone.

The analytical data presented in this paper show that a hexose is the sugar first formed in the leaf in photosynthesis. We would therefore suggest that glucose is the first sugar formed and that part of this is converted into fructose. Condensation now takes place to sucrose and it is as sucrose that translocation takes place to the bulbs. During the early growth period of the plant, up to the formation and opening of the flower, an excess of sucrose is present in the leaves and masks the hexose, possibly owing to the high needs for sucrose in flower formation. Sugar is translocated to the flower as sucrose. It will be remembered that we always found sucrose to be in excess of hexose in the flower stalk and only a trace of hexose was present in the corolla, a region in which no photosynthesis can take place. At the termination of the reproductive phase, large amounts of carbohydrate will be needed for the dormant period of the plant and growth in the subsequent season. At this stage hexose

exceeds sucrose in the leaves, perhaps owing to the greater amount of assimilation at this time.

SUMMARY.

An investigation has been made of the carbohydrates in the leaf of the daffodil, *Narcissus Pseudo-Narcissus* L. Only hexoses and sucrose were found to be present. Evidence is presented to show that the first sugar of photosynthesis is a hexose and not sucrose.

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