THE CARBOHYDRATES OF THE FOLIAGE LEAF OF THE SNOWDROP (*GALANTHUS NIVALIS*, L.), AND THEIR BEARING ON THE FIRST SUGAR OF PHOTOSYNTHESIS¹

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INTRODUCTION

Since the publication of Brown and Morris's important memoir entitled 'A Contribution to the Chemistry and Physiology of Foliage Leaves',² no extensive researches by chemical methods have apparently been carried out on the carbohydrates, which arise in the green leaf as the result of photosynthesis. With the exception of the fairly recent work of Strakosch,³ which will receive attention later, little has been done either in the way of confirming or refuting the results obtained and the conclusions drawn by Brown and Morris from their study of the sugars of the foliage leaf.

The investigations about to be described were commenced with the primary purpose of testing Brown and Morris's novel view, namely, that cane sugar is the first carbohydrate to be synthesised in carbon-assimilation.

The plant used for their experiments was *Tropaeolum majus*, the common nasturtium of our gardens. It forms starch plentifully in its leaves. The carbohydrates they recognised and attempted to estimate quantitatively were five in number, viz.:—glucose (dextrose), fructose (levulose), sucrose (cane sugar), maltose, and starch. From analyses made of leaves picked at various times of the day and under diverse

1. A brief account of this work, as far as it had then progressed, was given before the Botanical Section of the British Association, Dublin Meeting, 1908. (See, for abstract, the Annual Report for that year, p. 907).

2. Brown and Morris, *Journ. Chemical Soc.*, LXIII, p. 604, 1893.

3. Strakosch, 'Sitz. K. Akad. d. Wissen., Wien,' *Math.-Nat. Cl.*, CXVI Bd., H. VI, Ab. I, p. 855, 1907.

conditions, they came to the somewhat unexpected conclusion that sucrose is the first of the five carbohydrates to appear in photosynthesis. The others they derive from it as follows:—The starch arises directly from the cane sugar. They consider that when the latter exceeds a certain concentration, the excess is condensed to starch, thus relieving the osmotic pressure of the cell-sap. The glucose and fructose come also directly from part of the sucrose through inversion. The maltose proceeds from the starch through the dissolution of the latter by the action of the leaf-diastase. Further, finding that in their analyses the fructose was invariably in greater quantity than the glucose, they put forward the view that the latter is more readily consumed in respiration.

In the discussion,¹ which followed the reading of this paper before the Chemical Society, the President of the year, Professor H. E. Armstrong, suggested in his comments the likelihood of the cane sugar arising directly from the maltose, thus opposing the view of its being the first carbohydrate of photosynthesis. Such a criticism appeared then justifiable, especially since Brown and Morris themselves had shown three years earlier² that the barley embryo is capable of changing maltose into sucrose.

From the time of Sachs several plants, chiefly Monocotyledons, have been known, which never under natural conditions form starch in their green tissue (mesophyll). The idea struck the writer that such a type of leaf would be a profitable one upon which to experiment on the lines of Brown and Morris's work. Without starch, maltose would most likely be absent from such a leaf; consequently any sucrose found to be present could not have this sugar for its origin. The analysis would also be simplified, as in all probability there would only be three instead of four sugars for estimation, viz.:—sucrose, glucose and fructose.

The Snowdrop (*Galanthus nivalis*) was chosen, partly as a pleasanter subject with which to work than the Onion (*Allium cepa*), the best known of the non-starch forming Monocotyledons, and partly because some years previously³ the writer had made an extensive microchemical study of the distribution of starch and inulin in this plant. In no case was starch ever to be recognised in the mesophyll of the leaf, nor could it be induced

1. Brown and Morris, *loc. cit.*, p. 677.

2. Brown and Morris, 'Researches on the Germination of some of the Gramineae,' *Journ. Chemical Soc.*, LVII, pp. 513-520, 1890.

3. Parkin, 'Contributions to our knowledge of the Formation, Storage and Depletion of Carbohydrates in Monocotyledons,' *Phil. Trans. Roy. Soc., London*, Ser. B., Vol. CXCI, pp. 40, 49, 53, 58, 61 and 66, 1899.

to form here by feeding with sugar solutions. My trials on this point did not meet with the success obtained apparently by Boehm,¹ who mentions the Snowdrop, among others, as capable of producing starch in its mesophyll, when the leaf is supplied with sugar solution.

Starch, however, occurs in the guard cells of the stomata, but the quantity is too small to be taken into account in chemical analysis. The bulb contains starch and inulin in abundance, but neither of these carbohydrates ever appears, in the author's experience, under natural conditions in the mesophyll of the leaf.

Thus by using a plant like the Snowdrop, the investigation of the carbohydrates of the foliage leaf becomes simplified. There are no products of starch dissolution to complicate the issue. The sugars found to be present, viz. :—sucrose, glucose and fructose, must in all probability be closely connected with the photosynthetic process. The main problem seems to centre round the origin of the cane sugar in the leaf. Does its formation precede that of the hexoses, or is the opposite the case? The results set forth in this paper favour the former idea, viz., that sucrose is the first sugar to appear in quantity in a free state in photosynthesis, and that the two hexoses, glucose and fructose, arise from it by inversion.

This research has been in progress for the last five or six years. As the leaf material can only be collected at one period of the year, chiefly in the months of March and April, the work has necessarily been somewhat protracted. The results obtained from the leaves collected one spring naturally suggest the work to be carried out when more material is at hand. This, however, cannot be obtained till the following spring, as the Snowdrop is not a plant which can be induced to produce leaves at any time of the year; and even if it could, it might not be advisable to use leaves grown under artificial conditions.

Though the author is fully aware that this investigation is by no means complete, other lines, both for purposes of verification and attack, suggesting themselves, yet the research appears to have progressed sufficiently far under present methods to warrant publication, and invite criticism. Further repetition on the same lines seems hardly necessary. Then again, opportunities for this class of practical work may cease in the near future, so that it is well to publish the results and draw the conclusions, while the experimental details are fresh in the memory.

Even though the results may be proved wrong in some respects, or

1. Boehm, ' Ueber Stärkebildung aus Zucker,' *Bot. Zeit.*, XLI, p. 49, 1883.

the interpretation put upon them unacceptable, yet I shall feel repaid if they arouse interest in this somewhat neglected branch of physiological botany.

Throughout the practical work I have had the valued and cheerful assistance of my friend Lieut.-Col. Dixon, M.A., Trinity College, Cambridge, to whom I wish to express my best thanks. As far as the experimental part of this research goes, our names might well appear jointly in the title, but he has not seen his way to consent to this, as he considers himself not competent to judge of the botanical aspect of this investigation, and thus could not hold himself responsible for the conclusions drawn or views expressed.

My thanks are also specially due to Mr. J. H. Millar, B.Sc., F.I.C., for much helpful correspondence in regard to methods for sugar estimations, particularly respecting those requiring the use of yeasts.

To Professor T. B. Wood, we are much indebted for kindly allowing us the use of the saccharimeter belonging to his department, the Cambridge School of Agriculture.

The analytical work has been carried out wholly in the Chemical Laboratory of the University of Cambridge, and we desire to express our thanks to the former and present Professors of this Department for the privilege of working there, and for the opportunity it afforded us of employing liquid air in some of our experiments.

ANALYTICAL PROCEDURE

It will be necessary to deal at considerable length with the details of the general method which has been followed in this investigation for the estimation of the sugars in the Snowdrop leaf. This branch of physiological chemistry is as yet in the tentative stage. Every step requires close study and checking, in order that some degree of accuracy may be attained. No great advance, except with respect to the introduction of discriminating yeasts, has been made since the publication of Brown and Morris's work.¹ The method they used, in the light of work since carried out on pure sugar mixtures, is open to some objections. Being aware of these sources of error we have endeavoured either to overcome them or to allow for them, hoping that this research may thereby gain in reliability, though we are quite prepared for the possibility of further sources of error being brought to light in the quantitative estimations of sugars in plant extracts.

1. *Loc. cit.*, p. 663, 1893.

Analytical work of this nature is at the best slow. Our object has been to dispense with operations which we deemed to be unnecessary refinements with respect to the degree of accuracy at present obtainable in this line of investigation. At the same time our main purpose has been to carry out the analyses of the leaf extracts as uniformly as possible, so that the relative value of the results may be as great as possible.

In a research of this kind a large number of analyses with a moderate degree of accuracy seems preferable to a few with many precautions. In the latter case, on account of the small number of experiments, the effort to eliminate small errors may allow large ones to be passed over unobserved, whereas in the former, though slight variations may continually be arising, yet, owing to the number of analyses, large errors can hardly escape detection. Our conclusions are drawn from wide differences in sugar contents. Small fluctuations do not affect the generalisations. Differences in grams of sugar, and not fractions of a gram, per 100 grams of dry leaf, form our basis for drawing conclusions.

Drying the Leaves. This research has been conducted in the main with air-dried material. The leaves were desiccated quickly at a temperature sufficiently low to prevent browning or discoloration. They were then reduced to a powder for extraction.

In the process of drying, changes may take place in the sugars present in the leaf, altering their relative proportions. The cane sugar, for example, may undergo some inversion either through enzymic action or the acidity of the cell-sap. The effect, if appreciable, will be to increase the reducing sugars at the expense of the sucrose. This possibility has been put to the test in the following way.

Experiments with Liquid Air. A batch of fresh leaves was divided into two equal portions by weight. From one part the sugars were extracted at once, and from the other after drying. In order to reduce fresh leaves to a pulp for extraction, and at the same time to prevent any enzymic action taking place, recourse was had to liquid air. The leaves were immersed in this reagent, then withdrawn, and while frozen and brittle, were quickly ground up and the whole mass at once thrown into hot water to kill the enzymes, a few drops of ammonia being added to neutralise any acidity which might have an invertive action. The sugars so extracted were in much the same proportions, as in the control extracts made from the dried leaves.

The above fears then appear somewhat groundless. The air-dried

material may be assumed to retain the sugars in somewhat the same proportions as in the fresh leaf.

Details of two such comparative analyses are given below. The leaves were picked at two different periods of the spring—for the first experiment on March 11th, and for the second on April 30th. The amounts of the sugars are calculated for 100 grams of dry leaf.

					Fresh leaf. (Ground up by means of liquid air)		Dried leaf. Control	
					Experiment I	Experiment II	Experiment I	Experiment II
Sucrose	...	...	...	...	12.84	10.46	12.74	10.42
Hexoses (reducing sugars)	...	...	...	...	5.94	12.87	5.67	12.38
Total sugar	...	...	...	...	18.78	23.33	18.41	22.8
Ratio of sucrose to hexose...	...	...	...	...	1 : 0.46	1 : 1.23	1 : 0.44	1 : 1.19

In both cases, instead of the proportion of hexose to sucrose being greater in the air-dried (control) material than in the freshly extracted leaf, it is slightly less.

The drying, then, has not resulted in any appreciable inversion of the cane sugar.

It is to be noticed, also, that the total sugar extracted from the fresh leaves in both instances is rather greater than from the dried leaves. That is to say, the extraction is most likely more complete from the fresh pulp; the relative proportions of the two classes of sugars, however, remain very nearly the same.

For each separate analysis five grams of the leaf powder were taken, corresponding to about 150 fresh Snowdrop leaves.¹ As the analyses were carried out in duplicate, double this number of leaves were required. For a comparative experiment, such, for example, as the comparison of early morning with late afternoon leaves, fully 600 were needed; and to allow for a margin of safety and the possibility of having to repeat one or more of the analyses, 1,000 leaves are not too many.

Thus a research of this kind entails the growing of a considerable area of the plants and the manipulation of a large bulk of fresh material.

For the most part the Snowdrops were grown, and the leaves collected and dried at my home near Carlisle, in Cumberland. Through the kind-

1. The number required not only varies with individual differences in the size—a minor matter—but also with the season, as the leaves continue to grow throughout the spring by basal growth. Fewer would be required, e.g., late in April than at the beginning of March to make up 5 grams of dry material.

ness of Mr. Lynch, the Curator, a plot of Snowdrops was grown in the Cambridge Botanic Gardens for my use; but the bulbs do not seem to grow so well there, so I had to rely chiefly on my Cumberland stock. Colonel Dixon also collected and dried leaves for me at St. Bees, which came in useful for preliminary and general purposes.

The Snowdrop leaf-powder is hygroscopic, like all organic air-dried material. In order to obtain absolute dryness in a hygroscopic substance, recourse must be had to desiccation in an atmosphere devoid of moisture. This research hardly warrants the employment of this refinement, but in order to have the powdered leaf samples of approximately the same degree of dryness, exactly half a gram of each sample at the time it was being used for analysis was taken and placed in a steam oven for six hours, cooled in a desiccator, and then weighed. The equivalent of the loss of weight (10-20 milligrams) suffered by the half gram is then to be deducted from the amount of air-dried leaf powder taken for extraction. Experiments on this point showed that after six hours' heating in the steam oven, most of the moisture had left the half gram of leaf powder, i.e., the loss of weight on further heating was very small.

In future work of this kind the above operation might perhaps be dispensed with, especially if the powdered leaf sample be kept for some time in a desiccator before being weighed for extraction.

Extraction. Moderately strong alcohol is commonly used for dissolving out sugars from dried and ground up plant tissues. Brown and Morris¹ in their investigation of the sugars of *Tropaeolum* leaves employed 80-85 per cent. alcohol.

The use of spirit for dissolving out the sugars necessitates the extraction of the leaf-powder previously with ether or some such solvent to remove the chlorophyll. Then further, after obtaining the alcoholic extract, the spirit has to be distilled off, in order to obtain the sugars in aqueous solution for analysis. Hence this mode of sugar extraction is somewhat tedious.

As no starch occurs in the Snowdrop leaf, the feasibility of directly dissolving out the sugars from the leaf-powders by distilled water presented itself. Both cold and hot water were tried, and the former method was finally adopted as the solvent. Thus the necessity of removing the chlorophyll was dispensed with, as this is insoluble in water, and hence the time taken over the extraction was considerably shortened.

1. Brown and Morris, p. 662, 1893.

The method of procedure was as follows :—The five grams of powdered leaf taken were well shaken and allowed to soak some time in a flask with 50 c.c. of distilled water. The extract was then filtered into a 100 c.c. flask. The residue was afterwards washed into the original vessel, well shaken and again filtered into the 100 c.c. flask. This operation was repeated twice more, but these filtrates were first concentrated down on the water bath before being added to the measured flask, otherwise the volume of the aqueous extract would have greatly exceeded 100 c.c. Thus the sample of leaf-powder taken was extracted four times with cold water, and by this means most of the sugar was obtained in solution. The aqueous extract thus procured is rich brown in colour, too deep for reading in the polarimeter; and so to remove the pigment, as well as any tannin or glucoside present, basic lead acetate solution had to be added. Five cubic centimetres of this reagent were always used. Upon filtering, a clear faintly yellow extract was obtained, containing the sugars.

The removal of the sugars by means of a water Soxhlet apparatus was tried, but abandoned in favour of cold water extraction, as less sugar was dissolved out by the former method, and some of the sucrose appeared to have undergone inversion, even though precautions were taken to neutralise any acidity. The Soxhlet extraction was continued for 8-9 hours. Below are the results of two comparative experiments on different leaf samples.

	Mode of extraction			Sucrose	Hexoses	Total sugar in 100 grams dry leaf	Ratio of sucrose to hexoses
I.	Cold water	...	...	9.29	16.35	25.64	1 : 1.8
	Water Soxhlet...	...	...	7.08	17.86	24.94	1 : 2.5
II.	Cold Water	...	...	6.17	13.72	19.89	1 : 2.2
	Water Soxhlet...	...	...	5.04	14.32	19.36	1 : 2.8

Not only has the Soxhlet water extraction been less complete than the cold water one, but also the sucrose is considerably less in amount, with a corresponding increase in the reducing sugars; thus this somewhat drastic method of extraction is to be avoided, as it conduces to sucrose inversion.

To what extent are the sugars removed by the cold water method of extraction? This point has been submitted to investigation. Though the removal of the total sugar is not quite completed by the four successive extractions, as judged by the amount of cupric reduction, yet for the purpose of this research it may be deemed near enough. The continua-

tion of the extraction beyond the fourth time would result in a little more sugar and also greater accuracy, but an inconvenient volume of liquid for concentration would be obtained, and the whole operation would be difficult to carry out in a single day.

Details of two estimations on different leaf samples are given below, showing separately in each successive extraction, after inversion, the total amount of copper reduced.

Copper in grams.				Copper in grams.			
		I	II			I	II
1st extraction	...	0.9815	0.7105	5th extraction	...	0.0333	0.0342
2nd	„	0.7035	0.5915	6th	„	0.0180	0.0112
3rd	„	0.2800	0.2425	7th	„	0.0034	0.0014
4th	„	0.1053	0.097	8th	„	0.0014	0.0007
Total for the first		—	—	Total for the second four		—	—
four extractions		2.0703	1.6415	extractions		0.0561	0.0475
		—	—			—	—

Thus the first two extractions remove two-thirds to three-quarters of the total sugar. After the fourth extraction only about one-fortieth of the sugar originally present remains in the residue, viz.: the amount corresponding to about 0.05 grams of copper, i.e., roughly speaking, 0.025 grams of hexose sugar. Hence we may consider that in the cold water method of extraction adopted, about this amount of sugar is left in the residue of the original 5 grams of leaf taken. For 100 grams this will be equivalent to 0.5 gram of sugar, and so to be nearer the absolute percentage of total sugars in 100 grams of dry leaf, about half a gram should perhaps be added to the figures given in this paper.

Does any of the cane sugar undergo inversion during the cold water extraction? As the leaf material was dried finally in a steam oven, it might be expected that the enzymes present would all be destroyed and that none of the sucrose would be inverted during extraction with cold water, provided micro-organisms were prevented from growing. This, however, we have found by experiment to be hardly the case. An extract made with cold water when compared with a control carried out with boiling water shows a slightly lower percentage of sucrose and a correspondingly higher one of hexose. The longer the cold water extraction takes, the greater the difference becomes. The following are some of the results obtained on this point:—

COLD AND HOT WATER EXTRACTIONS COMPARED

Experiment	Sucrose	Hexose	Total sugar	Sucrose difference
I. Cold	9.25	10.97	20.22	0.52
Hot	9.77	10.45		
II. Cold	15.72	9.04	24.76	0.35
Hot	15.37	9.39		

COMPARISON OF USUAL COLD WATER EXTRACTION WITH ONES LEFT LONGER

Experiment	Sucrose	Hexose	Total sugar	Sucrose difference
I. Usual	14.79	5.35	20.14	0.97
Allowed to soak extra 24 hours ...	13.82	6.32		
II. Usual	15.30	10.68	25.98	1.12
Extra 24 hours	14.18	11.80		
III. Usual	4.53	9.45	13.98	0.84
Extra 24 hours	3.69	10.29		
IV. Usual	8.43	9.53	17.96	2.72 or 0.9 per 24 hrs.
Extra 3 days	5.71	12.25		

The amount of inversion which has taken place through prolonged soaking comes out very similarly in the above four experiments, viz., about 0.9 grams for twenty-four hours.

The diminution in the sucrose and corresponding increase in the hexoses was equally observable whether the extraction was made with or without the addition of an antiseptic, it seems, therefore, due to surviving invertase in the dried leaf material. It is a recognised fact that enzymes in a dry state can resist a higher temperature than when moist.

To prevent the above error arising, the obvious course would have been the substitution of hot water extraction for cold, thus destroying any active enzyme present. There is, unfortunately, a drawback to this procedure. Once the leaf material is treated with hot water, then the decoction ceases to filter easily, and the whole extraction becomes difficult and tedious. The cold water method has, therefore, been adhered to, though aware of the error arising through the inversion of a little of the sucrose, which amounts approximately to 0.5 gram per 100 grams of dry leaf. In the leaf analysis given, the cane sugar should be, therefore, increased by about half a gram and the hexoses correspondingly diminished.

The effect of basic lead acetate. The cold water extract, though clear, is of too deep a colour for its angle of rotation to be read in the polarimeter, consequently a decolourising agent has to be employed. Both animal charcoal and basic lead acetate remove the colour. The latter has been generally used in our analyses, but experiments for comparison have also been made with the former. Basic lead acetate has the advantage over animal charcoal of removing tannin and allied matters which reduce Fehling's solution. Now basic lead acetate is a somewhat disturbing factor to introduce into a sugar mixture, but unfortunately it can hardly be dispensed with, as no adequate substitute could be found. A number of experiments have been made to test its influence on the sugar estimations, and to find out the possible errors it introduces.

As already pointed out, 5 c.c. of the standard basic lead acetate solution has, as a rule, been added to each aqueous extract made from the five grams of leaf, the whole volume being then accurately made up to 100 c.c. By a series of tests it has been found that this quantity of the reagent just suffices to remove all matter from the extract capable of being precipitated by basic lead acetate. A further addition of the lead solution causes no more precipitate to come down.

The extract is then filtered to remove the lead precipitate, and its angle of rotation at once read in the polarimeter, and its cupric reducing power estimated, without going to the trouble of removing any excess of lead that may remain in solution. The removal of the lead by sulphuretted hydrogen is a somewhat lengthy process, and the presence of this slight amount has no appreciable effect on the rotation or the cupric reduction, as the experiments described below will show.

Now it is well known that basic lead acetate affects the rotation of fructose, forming a compound with it, which has a less rotation than this sugar itself.

We have ascertained the effect on the rotation and cupric reduction of adding 5 c.c. of our standard basic lead acetate solution to one per cent. solutions of the three sugars—sucrose, glucose and fructose, respectively. The only immediate appreciable effect is a lessening of the rotation in the case of fructose and to some extent of glucose, as the following figures show:—

Without			With basic lead acetate	
	Rotation	Copper in grams	Rotation	Copper
Glucose	... + 3.10	1.828	+ 2.47	1.822
Fructose	... — 5.53	1.857	— 2.46	1.844
Sucrose	... + 3.82	—	+ 3.98	—

A slight decrease in the cupric reducing power of the 'leaded' sugar solutions (glucose and fructose) is also noticeable.

The next point to be investigated was to see if the addition of tannin to the sugar solutions and its removal by basic lead acetate altered the percentages of the carbohydrates. Tannin was added to the one per cent. solutions of the sugars just to the extent that it was all carried down in the precipitate, caused by the addition of 5 c.c. of the standard basic lead acetate solution. Any excess of lead was not removed. The rotation and cupric reducing power were found to correspond very closely, so that neither the lead precipitation nor the slight amount of lead salt left in the solution altered the percentage composition of the separate sugar solutions. Below is shown the close agreement in the figures:—

					Tannin added and then precipitated by 5 c.c. basic lead acetate	Control in distilled water
Sucrose	...	...Rotation	...	...	+ 3.76	+ 3.77
		Copper after inversion	...	...	2.011	2.011
Glucose	...	...Rotation	...	...	+ 2.74	+ 2.75
		Copper	...	...	1.742	1.757
Fructose	...	...Rotation	...	...	— 4.75	— 4.75
		Copper	...	...	1.709	1.737

A similar experiment carried out with a mixture of the three sugars showed little difference between the one containing tannin and the control. The mixture consisted *approximately* of 0.5 grams of sucrose, 0.2 glucose, and 0.25 fructose—total 0.95 grams of mixed sugars in 100 c.c. The results obtained are set forth below:—

				Tannin added and precipitated by 5 c.c. basic lead acetate	Control in distilled water
Sucrose	...	...	...	0.487	0.489
Glucose	...	...	...	0.204	0.201
Fructose	...	...	...	0.244	0.244
Total sugar				0.935	0.934

Thus the two estimations are in very close agreement. The addition of tannin and its removal from solution by the necessary amount of basic lead solution has hardly affected the composition of the sugar mixture; and further, the slight amount of lead left in the filtrate after the removal of the tannin-lead precipitate has had no influence on the estimation.

It thus appears that if the basic lead acetate solution is not added in great excess, but only in sufficient amount to precipitate tannin and allied matters, it has no appreciable effect on the sugar estimations, even

when no attempt is made to remove any lead remaining by sulphuretted hydrogen. Let us see if this is borne out by comparing a leaf extract which has been treated with sulphuretted hydrogen to remove the lead with one which has not.

	Excess of lead removed by sulphuretted hydrogen			Lead not removed
Sucrose	...	...	...	10.29
Glucose	...	...	...	3.88
Fructose	...	...	...	4.44
<hr/>				<hr/>
Total sugar from 100 grams of dry leaf	...	...	...	18.61
				<hr/>

By the extra operations required through treatment with sulphuretted hydrogen, the total sugar has undergone some reduction, but the relative proportions of the sugars remain much the same.

A number of trials have been made to see if the addition of 5 c.c. of the basic lead acetate solution per 100 c.c. of distilled water has any effect on the slight spontaneous reduction shown by Fehling's solution. By careful manipulation to make the comparisons as parallel as possible, the data show that the lead solution has a slightly lowering effect on the cupric reduction, the mean difference being 0.5 milligram. This small amount will be negligible in the sugar estimations of the leaf extracts, as much less lead will be left in the saccharine solutions.

The aqueous leaf extract reduces Fehling's reagent considerably more before treatment with basic lead acetate than after.

The following table taken from four experiments demonstrates this difference:—

AMOUNT OF COPPER REDUCED BY THE EXTRACTS MADE FROM 5 GRAMS OF FOUR DIFFERENT LEAF SAMPLES

	Before treatment with basic lead acetate			After treatment with 5 c.c. basic lead acetate	Difference
I.	...	...	1.374	1.205	0.169
II.	...	...	0.926	0.718	0.208
III.	...	...	0.820	0.656	0.164
IV.	...	...	0.734	0.509	0.225

The effect of basic lead acetate is, however, without influence on the amount of sucrose estimated by Clerget's acid method.

AMOUNT OF SUCROSE FOUND IN 5 GRAMS OF DRY LEAF

	Without lead			With lead	Difference
I.	...	...	0.629	0.628	0.001
IV.	...	...	0.576	0.569	0.007

The sucrose in the 'leaded' extracts is in both cases slightly less, due perhaps to a slight retention of sugar by the lead precipitate.

The analysis of the extract after filtering off the basic lead acetate precipitate. For the estimation of the three sugars—sucrose, glucose and fructose, in the leaf extracts, the cupric-reducing power and angle of rotation before and after inversion have provided the data, checked and supplemented by yeast methods.

Fehling's reagent has been employed throughout for the copper estimations under the uniform conditions laid down by Brown and Morris.¹ The cuprous oxide produced by the sugar was collected in a Soxhlet tube plugged with asbestos, and reduced to copper by heating in a current of hydrogen, and weighed as such. These estimations were always made in duplicate, and a maximum difference of two milligrams allowed. With extra care the duplicates can without great difficulty be brought to agree within the milligram, but for the sake of speed, as well as for the general degree of accuracy of this work, a difference of two milligrams seemed permissible.

For the calculation of the amount of the three sugars from the weight of copper reduced, the table prepared by Brown and Morris has been employed.² A different factor has thus been used for fructose than for glucose.

For the estimation of the sucrose, inversion by means of hydrochloric acid under Clerget conditions³ has been the method followed, being quicker than by using the invertase of yeast. These two ways were, however, compared, and found to agree closely.

					Clerget	Yeast
I.	Rotation...	...	...	...	2.28	2.3
	Copper ...	...	...	...	1.882	1.879
II.	Rotation...	...	...	...	2.16	2.1
III.	Rotation...	...	...	...	1.04	1.06
	Copper ...	...	...	...	1.7	1.69

Thus the two methods give practically the same data, and it may be assumed that treatment with hydrochloric acid under Clerget conditions results merely in the inversion of the cane sugar, and further, that the fructose is unaffected by the hydrolysis. Therefore the loss of rotation

1. Brown, Morris and Miller, *Journ. Chem. Soc.*, LXXI, pp. 95 and 278, 1897.

2. Brown, Morris and Miller, *ibid.*, p. 281.

3. To 50 c.c. of the sugar solution 5 c.c. of concentrated hydrochloric acid are added, and the whole heated on the water bath to 68° C. for fifteen minutes.

and the increase in cupric reduction can be considered as being wholly due to the inversion of the sucrose.

The analyses have been conducted on the assumption that the only sugars present in the leaf extracts are sucrose, glucose and fructose. The data obtained have invariably worked out correctly on this assumption, which could hardly have been the case if appreciable quantities of other sugars or soluble carbohydrates or additional substances capable of reducing Fehling's reagent or of rotating the plane of polarised light were present. But, naturally, we have not been content without additional investigations supporting this assumption.

Experiments with Yeast. A series of experiments with ordinary brewer's yeast have been carried out on the aqueous extracts of the Snowdrop leaves.

On submitting an extract, which has been precipitated with basic lead acetate and which has had the excess of lead removed, to yeast fermentation, the rotation and cupric-reducing power become negligible quantities. Hence it is to be inferred that these two properties possessed by the extract before fermentation are due to the presence of fermentable sugars only. No extraneous bodies capable of rotating the plane of polarised light or of reducing Fehling's solution to any appreciable extent appear to be present. We have, therefore, to deal solely with plastic sugars and presumably glucose, fructose and sucrose.

An estimation of the different sugars in a mixed solution can be made by taking the specific gravity and opticity before and after fermentation, coupled with inversion by means of invertase. Such yeast methods can be employed solely for sugar estimations, but the extracts require to have a total strength of sugar at least five times that possessed by the aqueous solution made from 5 grams of dry Snowdrop leaf. Hence for continuous work on these lines a much greater quantity of material would be required than was at our disposal. The yeast method, however, has been used as a check on the copper one, and has given concordant results, thus confirming the view that the three sugars—glucose, fructose and sucrose—are alone present in the extracts.

Experiment I.—

				Total percentage of sugar in 100 grams of dry leaf
By yeast and specific gravity	...	...	...	21.52
By copper method	...	...	...	21.71

Experiment II.—

					Yeast method decolourised by animal charcoal	Copper method
Sucrose	...	...	...	...	11.4	12.2
Glucose	...	...	...	...	3.33	3.33
Fructose	...	...	...	...	4.67	4.0
Total sugar in 100 grams dry leaf	...				19.4	19.53

Results obtained after decoloration with animal charcoal. As already mentioned the aqueous extract of Snowdrop leaves is of too dark a colour to allow its angle of rotation to be read at all clearly in the saccharimeter. Basic lead acetate, in addition to precipitating tannin matters, removes the colour. Besides this reagent animal charcoal will also abstract the pigment. On boiling with this substance nearly all the colour is absorbed, leaving the solution almost colourless.

Unfortunately animal charcoal also removes some of the sugar. Its absorbent capacity for saccharine matter has first to be satisfied before it can be used in quantitative work. Hence it is a tedious substance to employ in estimations.

We have tested the absorptive capacity of animal charcoal for the three sugars, glucose, fructose and sucrose, separately, using the charcoal at the rate of 10 grams for every 100 c.c. of the one per cent. sugar solutions.

The sugar solution was first boiled with the requisite amount of charcoal, filtered, and its rotation taken when cool. Another quantity of the sugar solution was then boiled with the same charcoal, filtered and rotated, and the operation was repeated twice more, with the same charcoal.

The angle of rotation of each sugar solution after the first treatment was considerably less than before, showing that some sugar had been absorbed by the animal charcoal.

					Rotation of sugar solution previous to treatment	after first treatment with animal charcoal
Glucose	...	...	...	...	+ 2.84	+ 2.36
Fructose	...	...	...	...	— 4.88	— 4.01
Sucrose	...	...	...	...	+ 3.8	+ 2.59

After the second treatment the charcoal is about satisfied as regards the two hexose sugars respectively, but not quite for the sucrose. This latter sugar seems to be absorbed somewhat more greedily than the former two. Thus in abstracting colour from a solution of a mixture of these three sugars, the animal charcoal must be boiled successively with two

separate portions of the sugar solution, and these must be rejected. The third portion after decolourising with the same charcoal may be regarded as unaltered in respect to its sugar percentages.

This is borne out by our experiments with the Snowdrop leaf extract—the same charcoal, 5 grams being used consecutively with six different portions (50 c.c. each) of the original leaf extract.

				Grams of copper reduced per 100 c.c.	Rotation
Before Treatment	...	...	...	0.883	too dark to read
1st	„	...	...	not taken	+ 0.88
2nd	„	...	...	0.753	+ 1.05
3rd	„	...	...	0.865	+ 1.5
4th	„	...	...	0.883	+ 1.45
5th	„	...	...	0.900	+ 1.55
6th	„	...	...	0.879	+ 1.34

The rotation is practically constant after the third treatment, and the cupric reduction nearly so too. An interesting point also is the fact that the amount of copper reduced after decoloration (provided the animal charcoal has previously been satisfied as regards its capacity for absorbing sugar) is the same as that in the original coloured extract, showing that no substances which reduce Fehling's reagent have been abstracted with the colouring matter. As regards the opticity it is impossible to state, as before decoloration the rotation could not be read with any accuracy.

Analysis of the Extract after decoloration with animal charcoal

In the first place, as might be expected, estimations of cane sugar in the original coloured extract and in a portion of the same extract decolourised by 'satisfied' charcoal agree closely, as revealed by the increase in cupric reducing power brought about either by Clerget's method of inversion or by invertase. Also the quantity of sucrose agrees fairly closely with the amount found in the extract treated in the usual way with basic lead acetate and hydrolysed by Clerget's method.

Coloured extract gave	...	...	0.567 grams sucrose	} From 5 grams dry leaf
Decolourised extract gave	...	...	0.574 „	
Treated with basic lead acetate in the usual way gave	...	...	0.555 „	

Some of the decolourised extract was treated with yeast, and after fermentation its cupric reduction taken. The weight of copper obtained amounted to 0.126 grams per 100 c.c. of extract, i.e., per 5 grams of dry leaf. Before fermentation this came to 0.883 grams of copper. The

difference, 0.757 grams, represents the weight of copper due to fermentable sugars.

Not only has the decolourised extract after fermentation still considerable cupric reducing power as shown above, but also a slight levorotation. Particular attention has been paid to this latter point, and there seems little doubt that the leaf extract, when untreated with basic lead acetate, has after fermentation a slight left-handed rotation, due probably to the substance or substances which cause the residual cupric reduction. An experiment carried out very carefully afforded the following result:—

	Before fermentation	After fermentation
Rotation in the 200 mm. tube	... + 1.3	... — 0.2

Thus we have gleaned the following experimental facts:—

	Extract decolourised by 'satisfied' animal charcoal	Extract treated with the requisite amount of the basic lead acetate solution
After yeast fermentation	... Some cupric reduction Slight levo-rotation	No cupric reduction No rotation

Hence before fermentation in the case of decoloration with animal charcoal a small part of the cupric reduction is due to other causes than fermentable sugars, and the rotation is in a small measure likewise influenced. Whereas in the case of colour-removal by basic lead acetate all the cupric reduction and rotation may be taken as arising from fermentable sugars alone (presumably from glucose, fructose and sucrose).

When the fermented 'unleaded' extract is boiled for some time with a little mineral acid, the cupric reduction is clearly raised, indicating the presence of a glucoside. Further investigation on this point has not, however, been pursued.

The cupric reducing power of sucrose in the presence of hexoses

Sucrose alone has no reducing power on Fehling's reagent, but if a little hexose sugar be present, then it does reduce slightly. The cuprous oxide produced is greater than can be accounted for by the hexose solely. This, then, is a disturbing influence which must be taken into account when using Fehling for the estimation of sugar mixtures containing sucrose.

We have tested various mixtures of the three sugars, glucose, fructose and sucrose, to see what influence the various proportions of cane sugar may have on the cupric reducing power, and we can confirm previous

work in finding that the increase due to sucrose is very small, unless it be present in a preponderating ratio. Our leaf-analyses strengthen this conclusion also, since the amount of sucrose calculated from the increase of copper due to inversion was found to differ little from the sucrose obtained from the loss of rotation after inversion. Our results, however, show with very few exceptions, that the cane sugar calculated from the loss of rotation is slightly greater than that obtained from the increase in copper—a result to be expected.

The error arising from the slight cupric reducing power of the sucrose can be easily avoided by first estimating the sucrose from the loss of rotation through hydrolysis. The amount of copper reduced by this quantity of sucrose on inversion can then be calculated and deducted from the total copper obtained after hydrolysis, the remainder will represent the copper due to the glucose and fructose jointly. The rotation exerted by the sucrose present in the extract before hydrolysis, can be calculated and deducted from the original rotation of the extract. Two equations can then be formed, one from the copper and the other from the opticity. By solving these the separate amounts of glucose and fructose can be ascertained.

Below is given, by way of illustration, a typical example of how the percentages of sugars in a leaf-analysis are calculated.

Data obtained from an examination of the extract prepared from 5 grams of dried leaf.

- (1) Angle of rotation before inversion in 200 mm. tube at 19° C.
= +1.37 divisions.
- (2) Angle of rotation after inversion in 200 mm. tube at 19° C.
= -1.84 divisions.
- (3) Total copper reduced after inversion = 2.302 grams.

From (1) and (2) we obtain the total fall in the rotation due to the inversion of the sucrose. This comes to -3.21 divisions, which is equivalent to 0.6331 grams of sucrose. [In the polarimeter used it was calculated that a 1 per cent. solution of this sugar loses -5.07 divisions at 19° C.]

Thus we find that 5 grams of this leaf sample contain 0.6331 grams of cane sugar.

Further data can now be obtained for calculating the respective amounts of glucose and fructose.

- (i.) 0.6331 grams of sucrose will give a rotation of +2.44 divisions.

Therefore the rotation due to glucose and fructose jointly originally present in the extract $= +1.37 - 2.44 = -1.07$ divisions.

(ii.) 0.6331 grams of sucrose when inverted will reduce 1.283 grams of copper.

Therefore the cupric reduction due to the glucose and fructose $= 2.302 - 1.283 = 1.019$ grams of copper.

From (i.) and (ii.) two equations can be framed, the solution of which will give the separate quantities of glucose and fructose originally present.

Let x = glucose.

y = fructose.

Then (1) $3.03x - 5.34y = -1.07$ (from the rotation).

(2) $2.011x + 1.842y = 1.019$ (from the cupric reduction).

The constants used in the first equation are the angles of rotation given respectively by one per cent. solutions of these sugars in the 200 mm. tube at the temperature observed.

Those used in the second are the grams of copper reduced by one gram respectively of these sugars under the conditions observed, the constants being calculated from Brown, Morris and Miller's Table¹.

From the two equations we obtain respectively 0.2128 and 0.3209 grams of glucose and fructose.

Therefore we arrive at the following analysis of the sugar-mixture in the 5 grams of leaf taken—

Sucrose	...	...	...	=	0.6331 grams.
Glucose	...	...	...	=	0.2128 „
Fructose	...	...	...	=	0.3209 „
Total sugar	...	...	...	=	1.1668 „

Multiply by twenty and we get the quantities in 100 grams of this sample of dried leaf, the manner in which results are stated in this paper.

Sucrose	...	...	...	=	12.662 grams.
Glucose	...	...	...	=	4.256 „
Fructose	...	...	...	=	6.418 „
Total sugar	...	...	...	=	23.336 „

The duplicate analysis gave the following percentages:

Sucrose	...	...	...	=	12.618 grams.
Glucose	...	...	...	=	4.498 „
Fructose	...	...	...	=	6.508 „
Total sugar	...	...	...	=	23.624 „

The mean of these two analyses gives the final result

1. *Journ. Chem. Soc.*, LXXI, p. 281, 1897.

Evidence from the Osazone. The aqueous extract of the Snowdrop leaf on heating with phenyl hydrazine and acetic acid produces an abundance of yellow crystals characteristic of glucosazone. A further deposit of crystals suggesting those of maltosazone were never obtained on further heating and allowing the liquid afterwards to stand for some time.

The whole of the osazone resulting from representative samples of the leaf material was put together and the melting point and percentage of nitrogen of the mixture ascertained. The melting point was taken after re-crystallising the osazone from pyridine according to the method described by Tutin¹. This came out between 216-217°, the same figure practically as he obtained for glucosazone.

The percentage of nitrogen found in this mixed sample of osazone was 15.7, which agrees very closely with the theoretical number, 15.64, required for glucosazone. If an appreciable quantity of maltosazone had been present in it, then the nitrogen would have been lower, for it contains only 10.77 per cent. of this element.

No evidence of maltose could therefore be obtained by this means, nor of other sugars such as mannose or galactose. The osazone resulting is in agreement with what might be expected from an extract containing only the sugars glucose, fructose and sucrose.

A Summary of Errors likely to occur in the Leaf-analyses

- (1) The total sugar estimated is probably a little low—approximately about 0.5 per cent—owing to incomplete extraction.
- (2) The figures for the cane sugar err rather on the low side on account of some slight inversion during the process of extraction. The amounts found in the various analyses of the leaf should perhaps be raised by nearly 0.5 per cent. and a proportional quantity deducted from the total hexose sugar.
- (3) The fructose is probably somewhat underestimated owing to the action of the basic lead acetate on this sugar, though precautions were taken to avoid this as far as possible.

Fortunately the errors likely to arise in the estimations would not favour but rather oppose the main conclusions arrived at in this paper.

1. *Proc. Chem. Soc.*, Vol. XXIII, p. 250, 1907.

EXPERIMENTS AND RESULTS

Having dealt at some length with the methods employed for the estimation of the sugars in the foliage leaf of the Snowdrop, we now turn to their application and to the results thereby gained. These are set forth under six headings. Under each are given the character of the experiment and the results of the analyses, concluding with remarks.

I. *The Quantity of Sugar in the Leaf is Considerable.*

Twenty to thirty per cent. of the weight of the dried leaf consists of sugar. The amount varies naturally according to the time of day the leaves are picked for analysis. Allowing 80 per cent. for moisture, an average quantity, the fresh leaf will therefore contain 4 to 6 per cent. of mixed sugars.

Brown and Morris's figures¹ for sugars in the leaves of *Tropaeolum* vary from about 10 to 14 per cent. in the dry material, just about half the quantity. Even on adding the starch, which is absent, as we have seen, in the Snowdrop leaf, the total carbohydrate-content of the *Tropaeolum* leaf is considerably less than that of the Snowdrop, being in round numbers 14 to 18 per cent.

It is fortunate that the Snowdrop leaf is so rich in sugar, as it has allowed us to select five instead of ten grams (the amount taken by Brown and Morris) for each analysis—a great saving of material and so of labour.

The abundance of sugar may partly be explained by the fact that the Snowdrop is a spring plant, performing its assimilation when nights are cold, which will retard translocation and thus tend to carbohydrate accumulation in the leaf. The ratio, therefore, of the rate of photosynthesis to that of translocation is probably greater in a plant like the Snowdrop than in a summer-growing one like *Tropaeolum*; and so the carbohydrate formed in assimilation will tend to accumulate in the leaf more in the one than in the other.

Further, a bulbous plant like the Snowdrop has little available tissue other than the mesophyll for temporarily storing the carbohydrate produced in carbon assimilation, hence its leaf will be apt to contain a higher percentage than that found in a plant with a considerable extent of stem.

Whatever reasons may be adduced, the Snowdrop leaf contains a surprising amount of sugar under ordinary conditions.

1. Brown and Morris, *loc. cit.*, pp. 669 and 671.

II. *The amount of Sugar increases from above downwards in a Single Leaf, and at the same time the ratio of the Sucrose to the Hexoses diminishes.*

By comparing the amount of sugar in the upper green parts of the leaves with that in the lower green parts, it soon became evident that care must be taken to pluck the same relative length of leaf, in order that the results may be strictly comparable.

The foliage leaves of bulbous Monocotyledons commence to assimilate in their apical portions long before they attain their full length. As soon as the uppermost part of the Snowdrop leaf has emerged from the basal protective sheath, it is in a state of maturity and doubtless capable of full functional activity provided the external conditions are favourable. The upper green assimilating part of the leaf is continually being added to by basal growth. From personal observation the leaves of the Snowdrop continue to grow in length till nearly the close of their functional period, the increment of growth in a given time gradually decreasing as the spring advances. The increase in length ceases about the third week in April,¹ and early in May the leaves begin to turn yellow and then gradually die away. Thus most of the carbon assimilation in the case of the Snowdrop occurs in the months of March and April.

The Snowdrop plant is a simple or rather simplified structure. Disregarding the floral part, it consists of an outer sheath enclosing two foliage leaves, joined to a short axis bearing below a tangle of rootlets. The upper part of the sheath remains membranous and is protective in function; the lower part acts as a storage organ and forms the outermost fleshy scale of the 'resting' bulb. Each foliage leaf is sharply divided morphologically into two regions, a basal sheathing part functioning as an organ of storage and an upper assimilating and conducting part—the foliage leaf proper. After the plant has died down, the basal parts of the leaves persist as the middle and inner fleshy scales of the bulb. The leaf proper is further physiologically divided into two regions, an upper and a lower, through the embrace of the external sheath. The upper membranous part of this covers the lower part of the foliage leaf and prevents this portion turning green. The leaf thus consists of an upper green part and a lower colourless part, in addition to its sheathing base (the immature bulb scale). The accompanying diagrams will help to explain the morphology of the plant, as it bears upon this research.

1. From observations made in the vicinity of Carlisle.

DIAGRAM TO EXPLAIN THE MORPHOLOGY OF THE SNOWDROP PLANT
AS IT AFFECTS THIS PAPER.

FIG. 1. A single plant (somewhat reduced in size):—*f.l.*, the two foliage leaves (shaded). *sh.* protective sheath covering the lower parts ("stalks") of the foliage leaves. *b.*, bulb. *r.*, rootlets. *fl.s.*, flower stalk terminating in the solitary flower.

FIG. 2. A complete leaf detached from the plant:—The upper shaded portion (*f*) represents the green part (the foliage leaf proper): the middle narrow unshaded portion (*s*) the colourless stalk part, which in the intact plant is covered by the protective sheath: the basal portion (*b*) one of the new (immature) bulb-scales, which gradually become filled with starch and inulin, elaborated from the sugar formed in the chlorophyll cells. The line, *U—L*, arbitrarily separates the foliar part into an upper and lower portion. Leaves were so divided for the analyses in this section of the paper. Otherwise, as a rule, the whole of the green part of the leaf was taken for analytical purposes.

The sugars, therefore, formed in the upper green part of the leaf through photosynthesis, will have to travel through the colourless region enclosed by the protective sheath, then across the meristematic zone into the young bulb-scale, to be transformed there into starch and inulin—the two reserve carbohydrates of the ‘resting’ bulb.

The colourless part of the leaf enclosed by the membranous, external covering is very rich in sugar—30 to 40 per cent. of its dry weight consisting of such. It evidently functions as a temporary storing place for the sugars formed above in the green part through photosynthesis. The sugar is most likely produced in the green tissue at a quicker rate than it can be stored away as starch and inulin in the cells of the growing bulb-scale, and so accumulates in the non-functional, colourless cells.

In this paper the sugar contents of this lower colourless part of the leaf will not be considered further. Attention will be focussed only on the sugars of the green part, that is the portion of the leaf which appears above the protective sheath.

The leaves for this series of experiments were first cut from the plants just above the protective sheath, and then each one was divided either at its middle or two-thirds of the way up or otherwise, according to the analytical comparison desired.

The sugar was always more abundant in the lower than the upper regions. The difference, however, was by no means uniform, and seemed to depend on whether the plants used for the experiment were growing in close clumps or separately. The difference was more marked in the former than in the latter.

The following are the details of these experiments:—

GROWING IN CLOSE CLUMPS

	Portion of leaf taken		Total sugar	Ratio of sucrose to hexose	Date and time of picking
Experiment I.	Upper two-thirds	...	25.95	1:0.6	Mar. 30, 1905
	Lower „	...	28.72	1:1.1	5.30 p.m.
Experiment II.	Upper two-thirds	...	21.98	1:1.05	Mar. 31, 1905
	Lower „	...	26.48	1:1.38	7.30 a.m.
Experiment III.	Upper halves	...	22.48	1:1.39	Mar. 31, 1905
	Lower „	...	30.59	1:4.0	3.30 p.m.

GROWING SEPARATELY

Experiment I.	Upper halves	...	22.93	1:0.57	April 11, 1906
	Lower „	...	24.46	1:0.6	7 p.m.
Experiment II.	Upper halves	...	19.26	1:0.5	April 12, 1907
	Lower „	...	21.40	1:0.75	noon
Experiment III.	Upper halves	...	24.03	1:0.65	Mar. 30, 1910
	Lower „	...	24.36	1:0.82	2.30 p.m.

In the above six experiments the total sugar, which is calculated for 100 grams of dry leaf material, is invariably greater in the lower part of the foliage leaves as compared with the upper; but this difference is very much greater in the leaves taken from plants growing thickly together than in those growing separately. In the one case the mean difference of the three analyses works out to 5.13 grams, and in the other to 1.33 grams.

Secondly, it is to be noted that in every case the proportion of hexose sugar (glucose and fructose) to sucrose is greater in the lower portions of the leaves; and similarly this difference in the ratio is much more marked in the leaves taken from close clumps than in those from plants growing separately, the mean differences being respectively 1.15 and 0.15.

The leaves of Snowdrops growing in clumps tend to shade one another. The upper portions may be fairly well illuminated, but the lower green parts of many will be much in the shade, and so capable of performing little assimilation. It will be otherwise in plants growing separately. The light will be able to reach in the main the whole length of the green part of the leaf, and so the full extent will share in the assimilation. The results of the analyses rather suggest that if the leaves were similarly illuminated for their whole length, the disparity in sugar contents between the upper and lower parts would disappear. Special care was taken in No. III of the second series of experiments to gather only leaves well exposed to the light for their full length, and the figures show a very close approximation between the two different halves. Further research on the point would seem desirable.

It looks as if the lower green part of the leaf when obscured functions somewhat as a storage-tissue accumulating a larger amount of sugar than it would otherwise have done if exposed to good light and assimilating on its own account. An actively assimilating chlorophyll cell perhaps resists a high percentage of sugar arising within it. The act of assimilating may be antagonistic to that of storing.

For most of the investigations, therefore, the full length of the green leaf has been taken from plants grown in special plots. The bulbs were placed about two inches apart, so as to keep the individual leaves from shading each other for the most part; consequently the whole length of the green part of the leaf was fairly well exposed to the light.

III. *The proportion of Sucrose to Hexose Sugar decreases as the season advances.*

If, for example, a batch of leaves be taken about the beginning of March and compared with a similar quantity plucked at the commencement of April, then, judging from the results here obtained, it will be found that the *proportion* of sucrose to the hexoses (glucose and fructose) will be considerably greater in the first set of leaves than it is in the second. That is to say, there is in the dried leaf material more cane sugar in proportion to reducing sugar during the early part of the season than later. Thus it seems that as the spring advances the hexose sugars increase at the expense of the sucrose. A comparison, of course, must be made between leaves picked about the same period of the day. Leaves gathered in the morning must not, for instance, be compared with those taken in the afternoon; for if this be done, then the effect of photosynthesis will mask the seasonal change occurring in the ratio of these two classes of sugars.

The following analyses supply the data upon which the above deduction is based:—

Date and time of plucking		Maximum shade temperature, in degrees Fahrenheit	In 100 grams. dry leaf.	
			Sucrose, grams.	Hexose, grams.
February 16, 1906, 3 p.m.	...	48·9	19·8	3·56
February 26, 1907, 4—5 p.m.	...	45	15·07	2·53
March 7, 1906, 3.30—4 p.m.	...	66·9	14·55	5·69
March 30, 1905, 5—6 p.m.	...	49·6	15·5	11·4
April 5, 1906, 4—4.30 p.m.	...	60	14·64	11·17
April 5, 1907, 4—4.30 p.m.	...	58	14·64	11·61
April 24, 1905, 4—4.30 p.m.	...	51	14·84	17·29
May 4, 1905, 3—3.30 p.m.	...	53	10·3	12·78

From the above table the following approximate ratios are obtained for the two classes of sugars. The sucrose in each case is represented by unity and the hexose calculated in proportion to this.

				1905	1906	1907
February 16th	...	...	...	—	1:0·2	—
February 26th	...	...	...	—	—	1:0·2
March 7th	...	...	...	—	1:0·4	—
March 30th	...	...	...	1:0·7	—	—
April 4th	...	...	...	—	1:0·8	1:0·8
April 24th	...	...	...	1:1·2	—	—
May 4th	...	...	...	1:1·2	—	—

It is thus seen that the proportion of the two hexose sugars in the foliage leaf gradually rises from February to May, when the leaves begin

to change and wither. With respect to leaves taken in the late afternoon, in February the hexose only represents a sixth of the total sugar; in early March rather less than a third; in late March and the beginning of April nearly a half; and towards the end of the assimilating season more than a half.

Another striking instance of this feature in the ratios of the two classes of sugars is afforded by a couple of analyses carried out in the spring of last year. The leaves for this comparison were picked in the morning about 10 o'clock—one set on March 11th and the other on April 30th, thus seven weeks apart.

				10 a.m., March 11th	10 a.m., April 30th
Sucrose	...	...	...	12.74 grams.	10.42 grams.
Hexose	...	...	...	5.67 „	12.38 „
Ratio	...	...	...	1:0.44	1:1.19

Thus early in the season the quantity of cane sugar present in the leaves was more than double that of the reducing sugars; whereas seven weeks later the two types of sugars approximate closely in quantity.

These two analyses are especially interesting, as the leaves were primarily collected for another purpose, viz., the comparison of the sugars in air-dried leaves with those killed by immersion in liquid air. On glancing back to page 6 it will be seen that both the quantities and ratios of the two kinds of sugars, sucrose and hexose, were nearly the same in the two parallel cases. The liquid air method of preparing the extracts for analysis was so carried out that there was no time for any appreciable invertive action taking place. Consequently it seems very unlikely that the gradual rise noticed in the above analyses in the proportion of hexose sugars as the spring advances is due to any variation in the action of the invertase during the extraction of the leaf powder. The analytical results, then, in all probability represent fairly closely the actual proportions of the two kinds of sugars occurring in the living leaf at the various dates of examination. Thus this gradual proportional increase in the hexose sugars as the season advances appears a fact requiring consideration and, if possible, an explanation.

The mean daily temperature between February and May gradually rises, and so a possible explanation may be sought here. A higher temperature would favour greater enzymic action. More cane sugar might therefore be inverted, and so the reducing sugars would tend to increase at the expense of the sucrose.

However, the seasonal variation observed in the relative proportions of the two classes of sugars can hardly be due to the direct effect of temperature. For example, the maximum shade temperature, as shown in the Table, on March 7th was 66.9° F.,¹ while on March 30th it was only 49.6° F., yet the proportion of hexose to sucrose in the first case is much lower than in the second. Again on April 4th the maximum shade temperature was 60° F., while on April 24th it was only 51° F., yet this disparity in temperature has not influenced the seasonal change in the ratios of the sugars. Further the analyses of February 16th and April 24th, when the maximum shade temperatures were fairly similar, viz., 48.9° F. and 51° F. respectively, show the proportions of the sugars very different.

In some previous work on the carbohydrates of Monocotyledons² certain observations suggested the probability that, as the leaf increases in age, the starch in the mesophyll diminishes. Now sucrose, as a rule, favours the formation of starch in the chloroplasts more than any other sugar. If its percentage falls, then starch will most likely appear less readily and in smaller quantity. Consequently the reason why starch-forming Monocotyledons produce less of this carbohydrate in their foliage leaves, as the season advances, may be due to a general disposition on the part of such leaves as they grow older to accumulate less sucrose and more reducing sugar; at any rate, the behaviour of the Snowdrop leaf in this respect suggests this explanation.

IV. *During any single day of the spring the percentage of hexose sugars in the leaf remains fairly constant, no matter what hour out of the twenty-four the leaves may be examined. The sucrose, on the other hand, fluctuates greatly. It increases during the day and diminishes during the night. Further, leaves detached and insulated contain decidedly more sucrose than their controls.*

The above deduction must not be confused with preceding one. Here we are dealing with the daily variations in the *percentage* of the two classes of sugars; not with the seasonal change in the *relative proportions* observed in leaves taken at different dates throughout the spring, and at the same period of the day in each case. The experiments leading to the above generalization arrange themselves under four sub-headings.

1. This was an exceptionally warm day for the time of the year.

2. Parkin, *loc. cit.*, p. 46.

1. *The comparison of leaves picked in the early morning with those gathered in the late afternoon.*

This series of experiments will show the increase in the total sugar in the leaf due to a certain number of hours of photosynthesis, and also reveal what kind of sugar is especially affected. For the sake of brevity the glucose and fructose are put together as hexose. The ratio of these two sugars to one another is dealt with in a separate section of this paper. The results are stated in grams of sugar per 100 grams of dry leaf—the mode adopted throughout this paper.

Experiment I. March 7th, 1906. Cambridge.

Maximum shade temperature 66·9° F., and minimum temperature previous night 43° F.

			9 a.m.	3.30 p.m.
Sucrose	...	...	11·22	14·65
Hexose	...	...	6·35	5·48
Total sugar	...		<u>17·57</u>	<u>20·13</u>

Remarks. The sucrose has increased during the 6½ hours of sunlight by about 3·4 grams, while the hexose shows somewhat of a decrease.

This was an exceptionally warm day for the time of year. The total increase in sugar is not as marked as in the two following experiments. Perhaps the temperature, or rather strength of sunlight, retarded assimilation in such a cold-season plant as the Snowdrop; or the small increase may be explained on the grounds that increased temperature favours translocation and so less temporary storing of the sugar formed in photosynthesis.

Experiment II.—April 5th, 1907. Carlisle.

Maximum shade temperature 58° F.

			9 a.m.	4.30 p.m.
Sucrose	...	...	9·80	14·65
Hexose	...	...	12·38	11·61
Total sugar	...		<u>22·18</u>	<u>26·26</u>

Remarks. The total increase in sugar is here about 4 grams, and is due solely to the sucrose, the hexose having decreased somewhat.

Experiment III.—April 8th, 1908. Carlisle.

Maximum shade temperature 58° F., and minimum temperature previous night 32° F.

			8.15 a.m.	4.15 p.m.
Sucrose	...	...	8·88	12·92
Hexose	...	...	9·4	10·74
Total sugar	...		<u>18·28</u>	<u>23·66</u>

Remarks. The total increase is nearly $5\frac{1}{2}$ grams, and most of it is due to cane sugar.

The leaves for the above three experiments were picked on fine days, which might be considered favourable for photosynthesis. A considerable increase in the total sugar is noticeable.

The following experiment shows the lack of sugar-increase in leaves gathered on an exceptionally cold day for the time of year:—

Experiment IV.—April 26th, 1908. Carlisle.

COMPARISON OF MORNING AND AFTERNOON LEAVES PICKED ON A COLD SPRING DAY

Maximum shade temperature 46° F., and minimum temperature previous night 32° F.

		8.30 a.m.	4.30 p.m.
Sucrose		9.74	10.10
Hexose		11.61	11.94
Total sugar	...	<u>21.35</u>	<u>22.04</u>

Remarks. The increase is here very small, only 0.7 of a gram. The temperature has most likely been too low for much assimilation. The sucrose has augmented slightly more than the hexose.

Experiment V.—A fifth investigation was carried out to see the effect on the sugar contents of a day's illumination on leaves of plants kept previously in the dark for four days.

The plants were uncovered and exposed to the light at 8.30 a.m., April 12th, 1908; part of the leaves being taken then and dried, and the rest at 4.30 p.m. after 8 hours' illumination. The day was a favourable one for assimilation, the temperature being about normal for the time of the year.

		8.30 a.m.	4.30 p.m.
Sucrose		5.09	12.55
Hexose		2.77	3.61
Total sugar	...	<u>7.86</u>	<u>16.16</u>

Remarks. This is an interesting experiment. It shows in the first place the great fall in the percentage of sugar in the leaf, as might be expected from four days' obscurity. The effect of darkening on the diminution of the sugar is, however, referred to in detail in a special section of this paper. Eight hours of sunlight has caused a great increase in sugar, over 8 grams, and most of this is due to sucrose.

This experiment seems to be strongly in favour of the view that sucrose arises in the leaf before free hexose sugar, such as glucose. If the sucrose results from condensation of hexose in order to lower the osmotic pressure, it would be rather expected in the foregoing experiment that the hexose would be increased more than the sucrose by the exposure of the leaves to light, as the total sugar percentage in the leaves is so low and presumably also the osmotic pressure; but the reverse is seen to be the case.

2. *The sugars in leaves picked in the evening compared with those gathered the following morning.*

This investigation gives some indication of the quantity of sugar which passes out of the green leaf towards the bulb during the night. The total loss of sugar sustained can, however, hardly be due solely to translocation. The respiration of the leaf will consume a small amount most likely.

Experiment I. April 2nd and 3rd, 1905. Carlisle.

The upper halves only of the leaves were used in this experiment. One batch was gathered at 4.30 p.m. and the other at 8 a.m., the next morning. The plants in this instance were not covered, so there might be a little assimilation proceeding after 4.30 p.m., and also before 8 a.m. The minimum temperature during the night was 32·9° F., and the maximum shade temperature the previous day 52·6° F.

			4.30 p.m.	8 a.m. next morning
Sucrose	...	...	14·01	10·36
Hexose	...	...	10·76	10·45
Total sugar	...		24·77	20·81

Remarks. The sugar has diminished by about 4 grams, and this is due almost wholly to the loss in the sucrose. The hexose has remained nearly constant.

Experiment II. March 30th and 31st, 1905. Carlisle.

PLANTS NOT COVERED

Maximum shade temperature 49·5° F., and minimum temperature 38° F.

			5.30 p.m.	8 a.m. next morning
Sucrose	...	...	15·46	10·84
Hexose	...	...	11·41	12·64
Total sugar	...		26·87	23·48

Remarks. The sugar has diminished during the night by 3·4 grams, and this is due solely to a fall in the sucrose, the hexose having increased somewhat.

Experiment III. March 7th and 8th, 1906. Cambridge.

The plants were covered, when the first lot of leaves were gathered from them, in the late afternoon, and kept in darkness till the time the second batch of leaves were taken the following morning; thus no assimilation could take place in the interval, even if the light and temperature were sufficient.

Maximum shade temperature 66·9° F., and minimum temperature during the night 43° F.

			4 p.m.	9.30 a.m. next morning
Sucrose	...	...	14·65	9·64
Hexose	...	...	5·48	5·67
Total sugar	...		20·13	15·31

Remarks. Over 5 grams of sugar have disappeared, due solely to a reduction in the sucrose. The hexose has remained almost constant in amount. The conditions were distinctly favourable for the translocation of sugar.

Experiment IV.—April 5th and 6th, 1907. Carlisle.

The plants were covered when the late afternoon batch of leaves were taken and kept in darkness till noon the following day—a longer period. Hence a greater loss of sugar might be expected, which was the case.

Maximum shade temperature previous day, 58° F.; minimum temperature during the night, 39° F.; maximum shade temperature following day, 52° F.

			4.30 p.m.	Noon next day
Sucrose	...	...	14.65	7.80
Hexose	...	...	13.66	13.29
Total sugar	...		<u>28.31</u>	<u>21.09</u>

Remarks. The sucrose has been nearly halved, while the hexose has kept about constant. The total decrease is over 7 grams in 17½ hours of darkness.

3. *The comparison of sugar contents of leaves detached from the plants with those attached, both being kept in the dark overnight.*

This investigation is supplementary to the foregoing. An indication will thus be given as to what amount of sugar disappears from the leaf otherwise than by translocation. In the late afternoon or early evening a batch of leaves was cut from a plot of plants and their ends placed in water. These detached leaves were put alongside of the remainder of the plants with their leaves intact, and the whole covered till some hour the following morning, when both sets of leaves were taken and dried for analysis. Also at the time when the frame was covered the previous evening, a batch of leaves was picked and dried at once.

Experiment I.—March 7th and 8th, 1906. Cambridge.

Minimum temperature in the frame during the night, 46° F.

			4 p.m., March 7th	9.30 a.m., March 8th	
				Attached	Detached leaves
Sucrose	...	...	14.65	9.64	12.51
Hexose	...	...	5.48	5.67	5.65
Total sugar	...		<u>20.13</u>	<u>15.31</u>	<u>18.16</u>

Remarks. The percentage of hexose has practically remained constant in the three sets of leaves. The fluctuation, as usual, is in the amount of sucrose. This has diminished by 5 grams in the attached

leaves, and by 2 grams in the detached. The difference, 3 grams, must be due to translocation. Whether as much as 2 grams of sucrose has been consumed in respiratory processes or not, cannot be definitely asserted. At any rate, this amount has disappeared. Some may have diffused into the water from the cut ends of the leaves, but any loss in this way must be very small. The injury due to severance of the leaf from the plant may lead to pathological complications so that more sugar may be consumed than normally would be utilised in metabolic activity, hence we cannot conclude for certain that in this experiment the 3 out of the 5 grams of sucrose which have disappeared from the attached leaves during $17\frac{1}{2}$ hours of darkness are due to translocation, and the remainder, 2 grams, to consumption in respiration.

Experiment II.—April 5th and 6th, 1907. Carlisle.

Minimum temperature in the frame during the night, 39° F.

The plants and the detached leaves were kept covered till noon.

6-30 p.m., April 5th				Noon, April 6th	
				Attached	Detached
Sucrose	...	...	14.65	7.80	12.12
Hexose	...	...	13.66	13.29	13.39
Total sugar ...				21.09	25.51

Remarks. The hexose, as in the previous experiment, has kept remarkably constant throughout. The extra $2\frac{1}{2}$ hours of darkness has resulted in the additional loss of 2 grams of sucrose. Of course the two experiments are not strictly comparable, as both the time of year and temperature differ. The detached leaves have lost much less sugar than the attached ones, and the relative proportions of the losses in the two sets of leaves are much the same for the two experiments.

4. *The comparison of sugar contents in leaves detached and insulated with those left attached to the plants.*

The leaves were severed from the plants in the morning and their cut ends placed in water contained in a large flat tray. The individual leaves were both kept free from one another, and in a position approximating to the vertical by means of a device formed of wire netting. The tray containing the severed leaves was placed alongside of the control plants. Thus care was taken to submit the two sets of leaves to similar conditions of illumination and temperature.

The object of this experiment was to see to what extent the sugar

accumulated in the leaf when translocation was prevented, and which sugar was most affected.

Experiment I.—April 5th, 1906. Carlisle.

Maximum shade temperature, 60° F.; a fairly bright day, but hardly any direct sunshine.

Leaves were cut from the plants at 9.30 a.m. Both sets of leaves were taken and dried at 4.30 p.m.

			Attached	Detached leaves
Sucrose	...	...	15.83	19.02
Hexose	...	...	9.65	8.85
Total Sugar	...		<u>25.48</u>	<u>27.87</u>

Remarks. Owing to the translocation of the carbohydrates formed in assimilation being stopped by detaching the leaves from the plants, the total sugar has increased by about 2.4 grams, and this increment is wholly due to the sucrose, the hexose having diminished by 0.8 gram.

Experiment II.—April 5th, 1907. Carlisle.

Maximum shade temperature, 58° F. Sunny morning and dull afternoon.

Leaves treated as for Experiment I.

			Attached	Detached leaves
Sucrose	...	...	14.65	20.42
Hexose	...	...	11.66	10.5
Total sugar	...		<u>26.31</u>	<u>30.92</u>

Remarks. These results correspond with those of Experiment I, except that the total increase in sugar due to prevention of translocation is considerably greater—nearly twice as much. The sucrose, as usual, is the sugar chiefly affected.

Experiment III.—April 21st, 1910. Carlisle.

Maximum shade temperature, 56° F.; minimum temperature previous night, 45° F. Fairly bright, windy day.

Leaves treated as in the two foregoing experiments. Insolated 9-9.30 a.m., cut 4-4.20 p.m.

			Attached	Detached
Sucrose	...	...	18.73	20.66
Hexose	...	...	7.78	7.41
Total sugar	...		<u>26.51</u>	<u>28.07</u>

Remarks. The differences are here less marked than in the two foregoing experiments, but of the same nature. Detachment has caused an increase in total sugar of about 1½ grams, wholly due to sucrose, the hexoses having slightly diminished. The windy day, with direct sunshine, may have been less favourable for assimilation in the detached leaves, as these are apt to flag somewhat under such conditions.

V. *The fructose, as a rule, is in excess of the glucose.*

In the great majority of the analyses, not only have the amounts of sucrose and hexose been estimated separately, but also those of the two sugars composing the latter, viz., the glucose and fructose.

With very few exceptions the fructose has been found to be present in greater quantity than the glucose. In these exceptions the two sugars were in nearly equal proportions, the difference being slightly in favour of the glucose. We are inclined to trace these deviations to errors in analysis, arising most likely from using an excess of basic lead acetate. It has already been pointed out (p. 11) that this reagent is a dangerous one to use, without proper precautions, with sugars, and especially with fructose. If the presence of this acetate does affect the sugars, then the fructose will be influenced much more and come out relatively lower in the calculations than the other two. The tendency in our mode of estimation, as already shown (p. 21), is for the fructose to be lower in proportion than it actually should be, hence the value of the above deduction—viz., that the fructose is in excess of the glucose—is enhanced.

Out of fifty-two duplicate leaf analyses made, forty-seven had the fructose in excess of the glucose, and only seven the reverse. Representing fructose as unity, in the former cases the ratio varied from 1:0·4 to 1:0·76, and in the latter from 1:1·01 to 1:1·06. The average ratio for the whole number of the analyses was 1:0·76. This compares very favourably with the ratio 1:0·7, obtained for fructose and glucose in a miscellaneous sample¹ of leaf, in which the sugars were estimated in such a way as to dispense with the use of basic lead acetate.

The calculation of the separate amounts of glucose and fructose depend chiefly upon one observation, viz., the optical angle of rotation. A slight error in the reading of this will affect the results considerably; consequently it might hardly be expected that any further conclusion, beyond the bare fact of the excess of one hexose sugar over the other, could be reached.

However, the results do suggest the following two probabilities, viz., that the proportion of fructose to glucose tends to rise during the night, i.e., when photosynthesis is in abeyance; and secondly, that this ratio appears to increase from above downwards in the leaf, the excess of

1. This quantity of dried leaf material was accumulated by putting together the small portions that remained from all the other samples of leaves used. Hence its analysis gives a good indication of the average ratio of the sugars.

fructose being especially noticeable in the lowest (colourless) part of the leaf. These two tentative conclusions practically resolve themselves into one, viz., that, as the total hexose (reducing) sugar increases in proportion to the sucrose, so does the fructose rise in ratio to the glucose.

On the assumption that the two hexoses arise from the inversion of part of the sucrose, it would seem that the glucose disappears in some fashion at a quicker rate than the fructose.

The preponderance of fructose over glucose in the Snowdrop leaf is a condition somewhat similar to what Brown and Morris¹ discovered in the case of the leaf of *Tropaeolum*. In their researches on this plant they found the fructose, as a rule,² in much greater abundance than the glucose. In fact, in leaves gathered after several hours of sunshine, no glucose at all could be recognised, though fructose was present in considerable amount.

These authors account for the difference in the amounts of these two hexoses by considering that 'dextrose is more readily put under contribution for the respiratory processes of the cell than is levulose.'³

Further discussion on this point is reserved for the concluding section of this paper.

VI. *Leaves darkened for some days still contain a moderate quantity of sugar. The percentage falls rapidly during the first forty-eight of obscurity and then remains nearly constant.*

The experiments to be described under this heading were begun with the purpose of seeing to what extent the green leaf could be depleted of its sugar by prolonged darkening. Is there a limit to the reduction, or does the percentage of sugar fall to nearly zero? And further, how are the relative proportions of the individual sugars affected?

This part of the research is the least satisfactory and complete. The results obtained for different years are not as concordant as one would have liked. This defect may be partly attributed to the imperfect exclusion of light in the first two experiments. Greater precautions were taken later to have the plot containing the plants completely darkened.

The chief feature shown by these darkening experiments is that the sugar depletion rapidly reaches a point at which it stops; the percentage

1. Brown and Morris, *loc. cit.*, pp. 669 and 671, 1893.

2. On page 666 of their memoir an analysis is given showing dextrose in excess of the levulose. This is an exception to the other estimations they record.

3. Brown and Morris, *loc. cit.*, p. 672.

then remains nearly constant, though the darkening may be continued for some time.

For the four experiments the time of year in each instance was very similar. The full length of the green part of the leaf was taken, except in the case of Experiment II, in which only the upper three-quarters of the length was gathered. The leaves appeared to the outward eye quite fresh and green, even after two weeks in obscurity.

Experiment I.—Plants darkened at 6 p.m., April 6th, 1906.

Time of plucking of leaves for analysis	Sucrose	Hexose	Total sugar in 100 grams of dry leaf
At the time of darkening ...	15.83	9.65	25.48
After 24 hours ...	8.13	9.83	17.96
After 48 hours ...	6.39	7.76	14.15
After 4 days ...	4.79	9.15	13.94
After one week ...	3.41	11.39	14.80

Remarks. The total percentage of sugar has fallen in two days from about 25.5 to 14. On further darkening for another two days, the sugar-contents of the leaf hardly show any additional diminution, and after a full week of obscurity the percentage has, in fact, risen slightly instead of fallen. The hexose during the period that the total sugar remains nearly constant, i.e., from the second day onwards, appears to rise in quantity at the expense of the sucrose. This latter, then, would seem to undergo considerable inversion during prolonged darkness.

Experiment II.—Plants darkened on April 5th, 1907.

Time of plucking of leaves	Sucrose	Hexose	Total sugar in 100 grams of dry leaf
At the time of darkening ...	14.65	11.66	26.31
After eleven days ...	2.27	9.54	11.81
After two weeks ...	5.05	6.54	11.59

Remarks. This experiment was tried with the view of seeing what effect darkening beyond one week (the limit in Experiment I) would have on the sugar-contents. The percentage, instead of remaining about 14, has dropped below 12, and apparently stays nearly constant at this lower figure, even though the darkening be continued for a fortnight. The relative proportions of the sucrose and hexose have, however, changed considerably, but in the opposite direction to what took place in the first experiment.

The two experiments are only in agreement in the fact that the sugar percentage falls to a certain point and then remains constant.

At this stage it was observed that the method of darkening the plants was somewhat imperfect. Here and there it seemed possible that

light sufficient for photosynthesis might have penetrated. Hence for the next two experiments precautions were taken to ensure complete darkness. This has resulted in the percentage of sugar dropping considerably lower.

Experiment III.—Plants darkened at 5 p.m., April 8th, 1908.

Time of plucking of leaves	Sucrose	Hexose	Total sugar in 100 grams of dry leaf
At the time of darkening ...	12.92	10.74	23.66
After 3½ days	5.09	3.35	8.44

Remarks. The total percentage of sugar has dropped from over 23 grams to below 8 grams during the darkening period.

Experiment IV.—Plants darkened at 8 a.m., April 11th, 1910.

In this instance a sample of the leaves was not taken for analysis at the time of covering, but presumably the amount of sugar then would be somewhere near 20 per cent.

Time of plucking of leaves	Sucrose	Hexose	Total sugar in 100 grams of dry leaf
After 3½ days darkening ...	3.66	5.54	9.2

Remarks. The fall in total sugar for the same period of darkening as in Experiment III is not so great, but the difference between the two analyses comes almost within the limit of experimental error. The ratio, however, between the sucrose and hexose is the reverse of what it is in the preceding experiment.

SUMMARY OF RESULTS

1. Only three carbohydrates appear to be present in recognisable quantities in the Snowdrop leaf, viz., the sugars sucrose (cane sugar), glucose (dextrose), and fructose (levulose). Starch is always absent, except for a small amount in the guard cells of the stomata, nor has inulin been found, though both these polysaccharides are stored plentifully in the bulb-scales. Maltose has been searched for, but in vain.

(2) The quantity of total sugars in the leaf is considerable—20 to 30 per cent. of the dry weight, as a rule, in leaves actively assimilating.

(3) The amount of sugar increases from above downwards in a single leaf, and at the same time the ratio of the sucrose to the hexoses (glucose and fructose) diminishes. These differences are much more marked in leaves taken from plants growing in close clumps than in those from plants growing separately.

(4) The *proportion* of sucrose to the hexoses decreases as the season advances. That is to say, there is in the leaf more cane sugar in proportion to reducing sugar during the early part of the season than

later. Thus it seems that as the spring advances the hexose sugars increase at the expense of the sucrose. The comparisons were naturally made between leaves gathered about the same period of the day.

(5) During any single day of the spring the *percentage* of hexose sugars in the leaf remains fairly constant, no matter at what hour out of the twenty-four the leaves may be examined. That of the sucrose, on the other hand, fluctuates greatly. It increases during the day and diminishes during the night. Further, leaves detached and insulated contain decidedly more sucrose than their controls, but the quantity of hexose sugar remains much the same.

(6) The fructose, as a rule, is in excess of the glucose, irrespective of the period of the spring or time of day the leaves are picked for analysis. The results further suggest that, as the total hexose sugar increases in proportion to the sucrose, so does the fructose rise in ratio to the glucose.

(7) Leaves darkened for some days still contain a moderate quantity of sugar. The percentage falls rapidly during the first forty-eight hours of obscurity, and then remains fairly constant.

GENERAL DISCUSSION

Our knowledge of the carbohydrates of foliage leaves dates from the year 1862, when Sachs published the first of his classical memoirs on the significance of starch in carbon-assimilation. By his many and beautiful researches, covering a period of twenty years, he proved beyond doubt that the appearance of starch in the chloroplast is the direct outcome of the fixation of carbon under the influence of sunlight. This carbohydrate was therefore regarded by Sachs as 'the first visible product of assimilation,' though at the same time he was quite open to the view that the formation of sugar preceded that of starch. He, however, considered that all the carbohydrate synthesised in the leaf passed through the starch stage. Respecting the disappearance of the starch from the leaf he was strongly of the opinion that it was conducted away in the form of sugar.

The connection between starch and sugar received considerable attention from Schimper, who, in his paper of 1885,¹ held that starch is not only converted into sugar (glucose) for the purpose of translocation, but that it also arises in assimilation from this sugar. According to

1. Schimper, *Bot. Zeit.*, p. 738, 1885.

Schimper's work, then, glucose rather than starch should be looked upon as the first recognisable product of photosynthesis.

Taking into account the formaldehyde hypothesis of Baeyer advanced in 1870, a general theory of carbohydrate synthesis in the green leaf could now be framed. The carbonic acid (CH_2O_3) is reduced by the light and chlorophyll to formaldehyde (CH_2O) with the evolution of oxygen. The aldehyde as soon as formed is polymerised to glucose ($\text{C}_6\text{H}_{12}\text{O}_6$); this sugar is then condensed to starch, and temporarily stored as such in the chloroplasts. The starch, when required to be moved from the leaf, is re-converted into glucose, and travels in this form. Some such theory of carbohydrate photosynthesis may be said to have been the general one held up to the year 1893.

Practically no attempt had been made to discriminate between glucose (dextrose) and fructose (levulose). Maltose, a sugar then known in connection with the germination of the barley grain, had not even been suggested as a possible leaf-carbohydrate. Even cane-sugar had received scant attention, and its presence in the leaf had not been taken into serious account with regard to photosynthesis. It was looked upon more as a reserve than as a circulating carbohydrate.

A fact known at this period, namely, that several Monocotyledons never under ordinary conditions form starch in their foliage leaves, may be regarded as a feature not wholly in accordance with the above simple theory of carbohydrate assimilation. Arthur Meyer,¹ paying especial attention to such plants, comes to the conclusion that the reason why such leaves fail to form starch is not due to a too rapid translocation. He favours (erroneously, as it would appear now) the view that the synthesised carbohydrate is stored in a different form, and cites *Yucca filamentosa* as a probable case in point. Sinistrin (inulin) he discovered in its leaf, and this he considers may be in place of starch. By estimating the amounts of soluble carbohydrates, he discloses also the interesting fact that the leaves of non-starch formers contain much more sugar than those which normally produce starch.

Meyer's work, though distinctly valuable and suggestive, just failed to advance the subject of carbohydrate assimilation a real step beyond Sachs. This was reserved for Brown and Morris. Their work published in 1893² marks the commencement of the second stage in our knowledge of the carbohydrates of foliage leaves.

1. Meyer, *Bot. Zeit.*, Nos. 27-32, 1885,
2. Brown and Morris, *loc. cit.*

In their study of the *Tropaeolum* leaf, they show strong reasons for rejecting Sachs' supposition that all the carbohydrate formed in photosynthesis passes through the starch stage. It is only the excess of sugar produced that is so transformed and temporarily stored. Further, they prove beyond doubt that the leaf starch is dissolved in a somewhat similar fashion as in the germinating barley grain. The enzyme, diastase, acts upon it and converts it into maltose. Thus this sugar is demonstrated in the foliage leaf for the first time.

But their most novel conclusion is with respect to the cane-sugar. This they found more abundantly present than even starch, and the fluctuations in its amount suggested strongly to them that it is the first sugar to be formed in carbon-assimilation. The presence of the two hexoses are more readily accounted for as the products of the hydrolysis of some of the cane sugar than as originally preceding the sucrose. Since levulose was, as a rule, in greater abundance than the dextrose, the authors conclude that the latter contributes more readily to the respiratory needs of the leaf.

The general theory respecting the carbohydrates of the foliage leaf to be deduced from their experiments and conclusions thus differs considerably from, and is more complicated than, that resulting from the work of Sachs and his immediate successors.

At this point it may be well to bring into line the results of the work on the *Snowdrop* leaf described in this paper. Only three carbohydrates (sugars), viz., sucrose, glucose and fructose, require consideration. Starch is absent, and so is its hydrolytic product maltose. From the analyses the main daily fluctuation is shown to occur in the amount of the cane sugar, the quantity of the hexoses remaining remarkably constant, both under natural and experimental conditions. Even in leaves previously depleted largely of their sugars through remaining in darkness, the glucose or total hexose is not increased by exposure to sunlight, the augmentation occurring almost solely in the sucrose.

Thus our analyses suggest strongly that sucrose is the first recognisable sugar to appear in the *Snowdrop*, and that the two hexoses arise from it through inversion. Our results further support those of Brown and Morris in the fact that the quantity of the fructose is almost invariably in excess of that of the glucose, pointing to the latter sugar contributing more readily to the needs of the leaf.

Let us now turn to the consideration of a piece of research which

supports the older view that glucose (hexose) is the first sugar to arise in photosynthesis. Strakosch¹ in 1907 published an important paper on the carbohydrates of the sugar-beet leaf, employing a new microchemical method for sugar identification based on the production of osazones in the tissues. By this means of localisation dextrose was found to be the only sugar recognisable in the mesophyll, levulose and sucrose appearing first in the lateral veins of the lamina and increasing in the midrib and petiole. These results were confirmed by direct estimations of the sugars in extracts made from the mesophyll (ground tissue) and the veins of the leaf-lamina, respectively.

Strakosch considers, then, that dextrose is the first sugar to arise in photosynthesis. It travels from the mesophyll to the small veins, and a part is there transformed into levulose. The two hexoses then combine to produce sucrose, and the carbohydrate in this form travels to the root to be stored. In leaves previously darkened and then exposed to sunlight, the formation of starch in the chloroplasts commences later than the increase in the sucrose. Maltose seems only to have been detected in the petiole.

A striking point of agreement in this research with the work on the Snowdrop is the constancy observed in the amount of the hexose sugar. There was no appreciable diminution in its quantity during prolonged darkening, and no increase during many hours of illumination. The author, however, has noticed a slight diminution in the hexose, with a corresponding increase in the sucrose, on the first exposure of a darkened leaf to light. He concludes, therefore, that the transformation of hexose into sucrose is dependent upon light, and ceases when the leaf is darkened.

Though Strakosch's theory regarding the sequence of sugars apparently fits the facts, yet his interpretation fails to carry complete conviction. The rapid rise of sucrose in the leaf on its first exposure to light suggests its very early appearance in photosynthesis. Then sucrose, as a rule, seems to immediately precede, that is, gives rise to starch in plant tissues, but here in the chloroplasts of the beet leaf, according to Strakosch's results, it would appear that glucose was the precursor of starch, as cane-sugar was not discovered in the mesophyll.

Robertson, Irvine and Dobson,² in a paper published in 1909 on the

1. Strakosch, *Sitz. K. Akad. d. Wissen. Math.-Nat. Cl.*, Bd. CXVI, p. 855, 1907.

2. *Bio-Chemical Journal*, IV, p. 258, 1909.

sucroclastic enzymes in *Beta vulgaris*, favour the view of Strakosch in regarding both the formation of glucose and fructose as preceding that of the sucrose in the leaf. These two hexoses they consider are condensed to cane-sugar by enzymic means either through the reversible action of the invertase or by a special enzyme.

The problem affecting the first sugar to arise in a free state in photosynthesis thus centres around the origin of the sucrose in the foliage leaf.

Three different views may be presented. The first, that glucose appears primarily. Part of this is converted into fructose; then the two hexoses are condensed to cane sugar. This theory harmonises with the formaldehyde hypothesis. A weak point is the transformation of glucose into fructose. This can be brought about *in vitro* by the action of weak alkali, but then mannose appears also. There is no evidence that this third hexose occurs in the foliage leaf.

The second view, viz., that the cane sugar is derived from the maltose, and so indirectly from the leaf starch, is ruled out of court by the fact that the Snowdrop leaf contains neither starch nor maltose, and yet holds an abundance of sucrose.

The third view, first advanced by Brown and Morris, states that sucrose is the first sugar to arise in photosynthesis, and that the two hexoses, glucose and fructose, as well as the starch, are derived from it. The results with the Snowdrop leaf seem more in accordance with this theory, though at the same time they cannot be considered wholly at variance with the view first put forward.

Granting that sucrose is the first sugar to arise in the free state, how, it may be asked, is this to be reconciled with the aldehyde hypothesis? In the first place this theory still awaits complete proof. As crudely put, the hypothesis may be incorrect. Free formaldehyde, as well as free hexose directly polymerised from it, may never occur normally in the chloroplast. The photosynthetical process connected with the reduction of the carbonic acid may be more complicated than has generally been supposed. The carbohydrates resulting from it may none of them be truly up-grade products. The earliest to appear in the free state may be split off from some complex molecule, and might quite easily be sucrose in many, if not most, of the higher plants.

There is much evidence to show that this sugar is very widely if not universally distributed in the Flowering Plants.¹ It occurs also in

1. Schulze and Frankfurt, *Zeitschrift für physiol. Chemie*, XX, p. 511, 1895, and XXVII, p. 267, 1899; Bourquelot, *J. Pharm. et Chimie*, Sér. 6, XVIII, p. 241, 1903.

the Vascular Cryptogams,¹ but has been less studied in these. Its presence in the Mosses has been asserted. As yet it has not been recognised in the Algae. It is apparently absent in the Fungi, its place being taken most likely by the disaccharide sugar, trehalose.

Though it can function as a reserve carbohydrate, yet there are considerable grounds for believing that it is largely the form in which sugar travels in the tissues. The plant apparently converts indirectly its reserve carbohydrate into this form of sugar for purposes of circulation. This has been observed in several germinating seeds, tubers and bulbs. Though in each case the sugar immediately arising from the stored carbohydrate through enzymic action is different from sucrose, yet on its way to the growing parts of the seedling or young sprout it seems to have been transformed into sucrose, as this latter sugar now appears. To give a few instances. Brown and Morris² showed that the maltose arising from the starch in the endosperm of the sprouting barley grain becomes changed into cane-sugar on its passage into the growing embryo. Further, excised embryos nourished with a solution of maltose accumulate not this sugar but sucrose. Grüss³ has shown, in addition, that such embryos, when fed with glucose, can change this sugar into sucrose. This author⁴ has also found that in the germination of the date palm seed, the mannose and galactose resulting from the reserve cellulose appear as cane-sugar in the embryo. Schultze and Frankfurt⁵ find sucrose quickly following the dissolution (i) of starch in the germinating seed of *Vicia sativa*, (ii) of galactan in that of the lupine, and (iii) of oil in those of *Helianthus* and *Ricinus*. Also in the sprouting potato, where the carbohydrate is stored as starch, sucrose soon makes its appearance. We have found, likewise, in the bulb-scales of the growing Snowdrop, sucrose the most abundant of the sugars instead of maltose and fructose, which might have been expected from the nature of the reserve materials, starch and inulin. As far as the writer is aware, in no case has it been definitely shown that the sucrose arises directly from the hydrolysis of any reserve carbohydrate. It thus bears an analogy to asparagine in nitrogenous metabolism. This amide is of common occurrence in the higher plants, and is probably the form in

1. Anderssen, *Zeitsch. f. physiol. Chemie*, XXIX, p. 423, 1900.

2. Brown and Morris, *J. Chem. Soc.*, LVII, p. 458, 1890.

3. Grüss, *Ber. deut. Bot. Ges.*, XVI, p. 17, 1898.

4. Grüss, *Ber. deut. Bot. Ges.*, XX, p. 36, 1902.

5. Schultze and Frankfurt, *loc. cit.*

which nitrogen largely travels; yet in germinating seeds, on a parallel with cane-sugar, it does not appear directly as a result of protein hydrolysis, but arises somewhat later in an indirect fashion.¹ It may thus be regarded as the ultimate product of protein dissolution, just as sucrose is in the hydrolysis of reserve carbohydrates.

Sucrose has been found in many cases to be the sugar of most nutritive value to the plant. It is also the one which in most cases favours starch deposition. It can therefore be regarded as the precursor of starch.

Its special physical and chemical properties are also of interest. It is very soluble, and readily crystallises—more so than the other sugars occurring in plants. It is very easily hydrolysed by acids and by invertase. It shares with trehalose alone among the disaccharides in having no reducing properties. Maltose, lactose, etc., do reduce, and so may be said to have the aldehyde group in their molecule functional.

Sucrose may thus have been selected by the higher plants as the chief circulating sugar, partly on account of its non-reducing properties and soluble (mobile) nature, and partly on account of the ease with which it can be hydrolysed into its two components, glucose and fructose. These hexoses may, as a rule, play distinct parts in metabolism—the glucose more readily lending itself to the respiratory needs, and the fructose to constructive work, such as the building up of the plant's framework.² It is also within the bounds of probability that cane-sugar itself may take a direct part in the formation of cell-walls. Just as it appears able to be condensed to starch without previous inversion, so it may be transformed directly to cellulose in the construction of cell-walls. Fenton's work³ is interesting in this connection. He has shown that various kinds of cellulose respond markedly to a special ketose test; and thus concludes that this substance may contain one or more groups identical with that present in fructose.

To sum up, sucrose then seems to be of paramount importance to the higher plants. It probably functions largely as a circulating sugar, travelling as such to the growing regions, to be there partly inverted to supply hexose sugar for respiration. To draw a rough analogy, it may be

1. Brown, H. T., *Trans. Guinness Research Laboratory*, Vol. I, pt. 2, p. 296, 1906; Schulze, *Landw. Jahrb.*, XXXV, p. 621, 1906; Scurti and Parrozzani, *Gazzetta*, XXXVIII, i, p. 216, 1908; Wassilieff, *Ber. deut. Bot. Ges.*, XXVIa, p. 454, 1909.

2. See Lindet's recent paper, *C.R. Acad. Sc. Paris*, CLII, p. 775, 1911.

3. Fenton and Gostling, *J. Chem. Soc.*, LXXIX, p. 361, 1901. See also Cross and Bevan, *J. Chem. Soc.*, LXXIX, p. 366, 1901.

likened to the sovereign in our currency. This coin can circulate over the whole world, and at any time or place be changed for local purposes, so sucrose in the plant may be able to travel anywhere in the tissues, and is perhaps continually being called upon to change itself into hexose sugar for local needs.

Is sucrose, then, not intimately bound up with the photosynthetical process? If this sugar be of so much importance to the flowering plant, has not the chloroplast devised a means of rapidly forming it without the previous production of glucose and fructose in quantity? Much further investigation with this possibility in view seems desirable.