Campbell, A. V. 1912. Carbohydrates of the mangold leaf. *The Journal of Agricultural Science.* 4 (3), pp. 248-259.

CARBOHYDRATES OF THE MANGOLD LEAF.

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Introduction.

The chemistry of the sugars of foliage leaves and the successive transformation undergone by the various carbohydrates has been the subject of considerable speculation. Reducing substances have been known in foliage leaves for a number of years.

Müller-Thurgau (1), R. Kayser (2) in 1883 were probably the first to discuss the nature of the substances undergoing translocation in green leaves. Sachs (3) concluded that these were derivatives of the starches. A. Meyer (4) in 1885 attempted to ascertain the amounts and nature of these products, and was probably the first to show the existence of true sugars in situ. Brown and Morris in 1893 (*Trans. Chem. Soc.* LXIII. 604) demonstrated the presence of sucrose in foliage leaves and gave strong reasons for regarding it as the immediate product of assimilation and the source of the starch afterwards accumulated in the leaf.

Experimental. During the summers of 1909 and 1910 experiments have been carried out in this Laboratory in order to find out if any light might be thrown on these complex metabolic changes which are taking place in green leaves.

Brown and Morris (5) in 1893 attacked the problem, using the leaves of *Helianthus tuberosus* and *Tropaeolum majus.* Their experiments were commenced in order to try and "discover the relation which each sugar bears to the primary assimilation products on the one hand, and to the leaf-starch on the other; to determine, in fact, which are the 'up-grade' sugars towards starch, and to see if any indications were forthcoming of the existence in the leaf of 'down-grade' sugars proceeding from the hydrolysis of starch, a fact of very great importance as bearing upon the physiological mechanism involved in the dissolution of starch in the living cell. It seemed probable also that the observations on the 'down-grade' sugars might serve to support or not, as the case might be, the supposition of Sachs that the starch is in a continual and rapid state of flux, that, in fact, all the products of assimilation necessarily pass through the form of starch."

The conclusions arrived at by Brown were that:—

(1) Neither dextrose nor levulose were the primary products of assimilation, but rather that cane sugar seemed to be the first sugar synthesised by the assimilatory process.

(2) Cane sugar performed the functions of a temporary reserve material accumulating in the leaf when assimilation is proceeding vigorously.

(3) When the cane sugar reaches a certain amount it is changed into a more permanent reserve material, viz. starch.

(4) The form in which the carbohydrates wander from cell to cell in the leaf are threefold, cane sugar being translocated as dextrose and levulose and starch as maltose.

Although much has been written on the variation in composition and fluctuation of carbohydrates in foliage leaves, no attempts have been made to trace any cycle of events which probably occur in the leaf throughout a period of light and dark, thus leaving us to draw conclusions from a few unconnected determinations. In the hopes of gaining more information as to this cycle the following experiments were carried out.

The work was conducted on the leaves of Mangold (*Beta maritima*). The leaves selected were grown at Rothamsted Experimental Station on a plot giving normal growth. In the preliminary stages a number of difficulties arose. It was found that drying at 100 C. or killing by chloroform ether or toluene before drying, greatly influenced the sugar content.

In the end it was found necessary to kill by immersing the chopped up leaves into boiling alcohol and commencing the extraction at once. Leaves of average size were selected and chopped up after removing the midrib. 50 grs. were taken for the extraction; the percentage of dry matter being determined in another portion. Extraction of the sugars was effected by boiling with 92 per cent. alcohol, the extraction being continued until all the colouring matter was removed from the leaves. In the preliminary experiments it was found that this was sufficient and that no sugars remained in the leaf-stuff after treatment. The alcohol was then distilled off and the extract finally taken down to about 20 c.c. on a water bath. A little water was then added and the whole again evaporated until no alcohol remained. The chlorophyll then came out of solution. The solution, after the addition of a little alumina cream or *kieselguhr*, was heated for a few seconds, after which it was made up to 50 c.c. and filtered through a clean dry funnel, the first 40 c.c. being taken for analysis. They were made up to 100 c.c. The cupric reducing power of this extract was then determined, using Pavy's solution. The sugar solution was then inverted by boiling for 7 mins. with 2 per cent. citric acid, and the reducing power again determined. Further hydrolysis was brought about by refluxing a diluted portion with 10 c.c. strong hydrochloric acid for an hour, the acid being then neutralised and a further estimation of the total sugars made. From these determinations it was possible to calculate the percentage of

(*a*) Reducing substances (glucose and levulose, &c.).
(*b*) Cane sugar.
(*c*) Maltose.

These determinations are open to criticism on the ground that they may not be "absolute results." The errors, however, if any, occur in each determination, and will not affect the results for purposes of comparison.

The starch was estimated in the leaf residue by O'Sullivan's diastase method, the conversion being brought about at 62° C. after gelatinisation. Preliminary difficulties having been got over, and a series of duplicate analyses having been done to test the reliability of the methods employed, various types of leaves were examined to contrast colour and also youth and age. One or two determinations were also made to find if the leaves on the several plots showed any marked differences in sugar content owing to the methods of manuring.

Normal leaves were also examined at stated periods to ascertain if there was any difference in sugar content owing to variation of illumination.

Finally an attempt was made to trace the diurnal fluctuations of the sugar.

Fluctuations. For this determination leaves were collected every two hours of the day and night during Friday night and Saturday, Sept. 17th, 1910.

Determinations were made in duplicate, the mean results being shown in Table I. and also tabulated in graphical form in Graph I., the percentage of sugars being plotted against the hour of collecting.

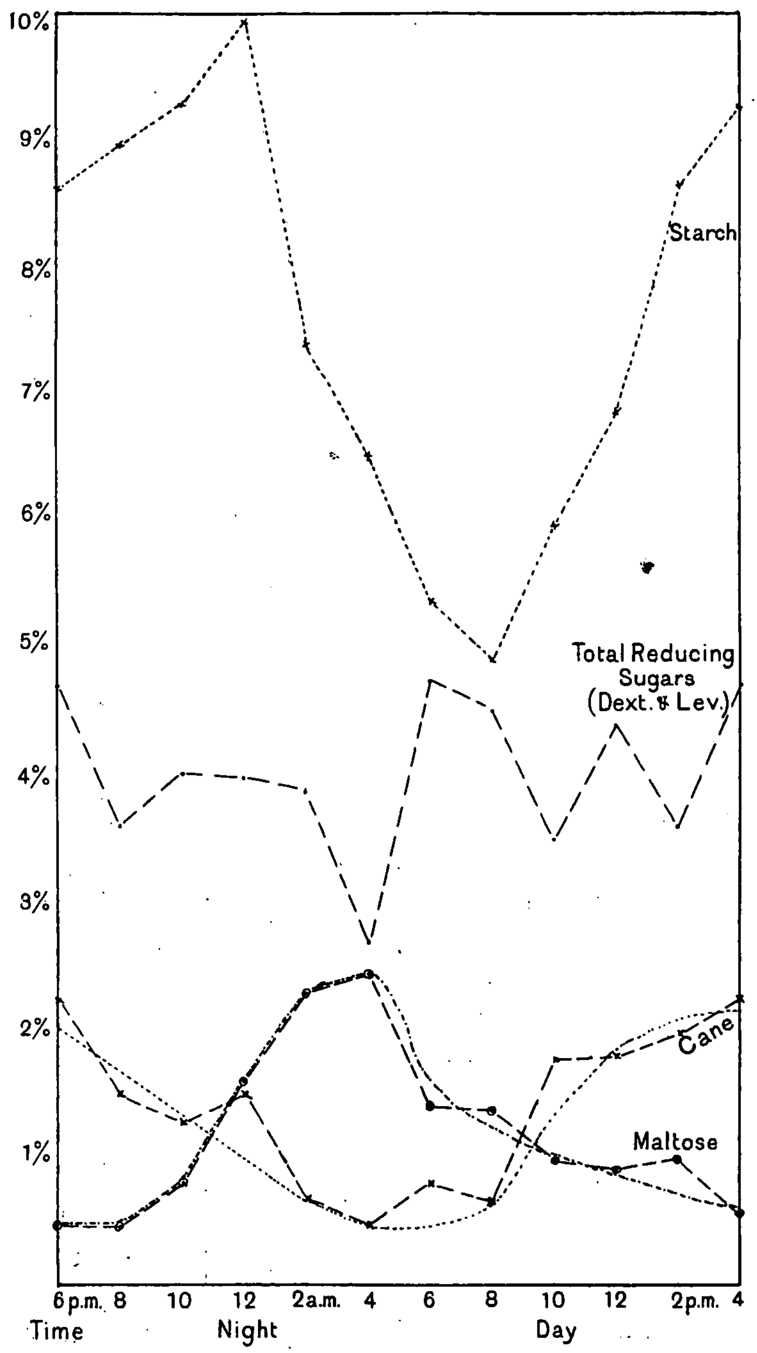
TABLE I. *Carbohydrates in Mangold Leaf. Percentages at 2 hour intervals.*

Time	6 P.M.	8 P.M.	10 P.M.	Midnight	2 A.M.	4 A.M.	6 A.M.	8 A.M.	10 A.M.	Midday	2 P.M.	4 P.M.
Dry matter actual wts.	4·04	3·56	3·62	3·3	3·78	3·63	3·70	4·35	4·12	4·26	4·52	4·84
Dextrose and Levulose	4·67	3·58	3·98	3·92	3·81	2·64	4·70	4·43	3·44	4·33	3·57	4·65
Cane sugar	2·20	1·46	1·23	1·47	·68	·45	·79	·63	1·72	1·76	1·92	2·20
Maltose	·48	·46	·79	1·57	2·23	2·40	1·37	1·32	95	·88	·97	·53
Starch	8·60	8·95	9·30	9·95	7·36	6·44	5·31	4·86	5·90	6·80	8·66	9·24

TABLE II. *Carbohydrates in Mangold Leaf recalculated as Carbon.*

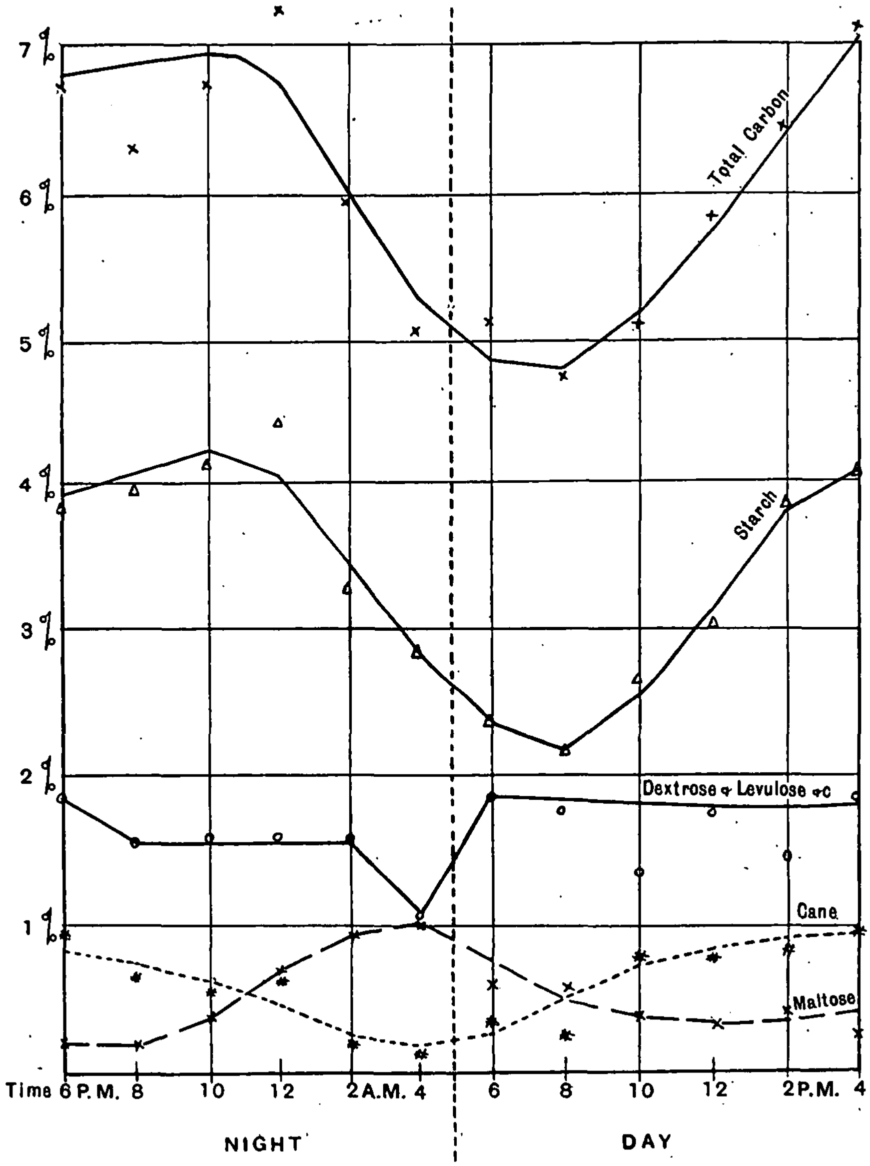
Time	6 P.M.	8 P.M.	10 P.M.	Midnight	2 A.M.	4 A.M.	6 A.M.	8 A.M.	10 A.M.	Midday	2 P.M.	4 P.M.
Dextrose and Levulose	1·87	1·52	1·59	1·57	1·52	1·03	1·88	1·77	1·37	1·73	1 43	1·86
Cane sugar	·92	·61	·52	·62	·23	·19	·33	·26	·72	·74	·81	·92
Maltose	·20	·19	·33	·66	·93	1·01	·57	·55	·39	·37	·40	·22
Starch	3·82	3·98	4·14	4·41	3·26	2·86	2·36	2·16	2·62	3·02	3·85	4·11
Total	6·81	6·30	6·78	7·26	5·94	5·09	5·14	4·74	5·10	5·86	6·49	7·11

GRAPH I. *Carbohydrates in Mangold Leaf. Percentages at 2 hour intervals.*



The same determinations calculated in terms of carbon are given in Table II. and Graph II. in order that any conversions may be the more easily noticed.

Graph II. *Carbohydrates in Mangold Leaf calculated as Carbon.*



Discussion of Results. Before attempting to discuss the significance of the curves it should be noted that the figures are expressed as percentages of the dry matter of the leaf. To follow the actual migration of the carbohydrates it would be necessary to know the absolute weight of a typical leaf, or at least of a unit leaf area. Considerable difficulties are likely to be attached to this determination, which was not carried out in this preliminary work. However, the percentage results will approximate to the truth, since the total weight of the migratory carbohydrates never exceeds 15 per cent. of the dry matter of the leaf and the total fluctuations are within 5 per cent.

Dextrose and levulose, together with the other reducing substances, do not appear to fluctuate very much. There is evidence that these reach two levels, the leaf containing an approximately constant percentage in the night-time, and a higher percentage in the day. The fact that the curve jumps up suddenly from 4 to 6 a.m. suggests that the leaf responds very quickly to light. This is also shown by the downward movement from 4 to 6 p.m. Again, this constant low level in the evening when no assimilation is taking place would tend to show that there was not much translocation of dextrose and levulose as such in the night period, or we should expect a gradual depletion of the reducing sugars. It may be, however, that these are formed side by side with maltose by the destruction of starch.

Cane sugar varies between ·5 to 2·5 per cent. This is apparently produced in the leaf-matter during periods of illumination. On the days on which the experiment was conducted (Sept. 17) the sun rose at 5.30 a.m., which time synchronises with the beginning of the upward movement of the cane sugar curve. This continues throughout the daytime and begins to drop in the afternoon. This lags behind the glucose curve, beginning its upward movement about an hour later.

In the case of *starch* the fluctuations resemble those of cane sugar, the only differences being (*a*) the lagging behind (whether this lag is sufficient to justify the hypothesis that starch is formed after cane sugar is a very doubtful point), (*b*) the continued upward movement from 6 p.m. till 11 p.m. It is suggested that this is due probably to continued elaboration of other carbohydrates, possibly glucose.

Maltose, as will be seen from the graphs, fluctuates considerably, but in the opposite sense to the cane sugar, commencing its up-grade movement at 8 p.m. and continuing until the first dawn. This seems to give us an important indication of the sequence of changes in the leaf. The maltose appears to be the last carbohydrate to be formed and therefore the one most intimately connected with the translocation process. That it is not formed directly may be assumed from the low proportions observed during the light period, unless it is being (*a*) translocated as fast as formed, which is unlikely, or (*b*) immediately changed into starch. It is more likely that the maltose is not a direct up-grade product of assimilation, during which period it is also highly probable that little or no translocation is taking place because of the great increase in total carbohydrates.

A few determinations of hemi-cellulose were made in the starch from leaf material by estimating the sugars after hydrolysis for 2½ hours with strong HCl. The results obtained seemed to show that this was probably constant, although they were not at all satisfactory.

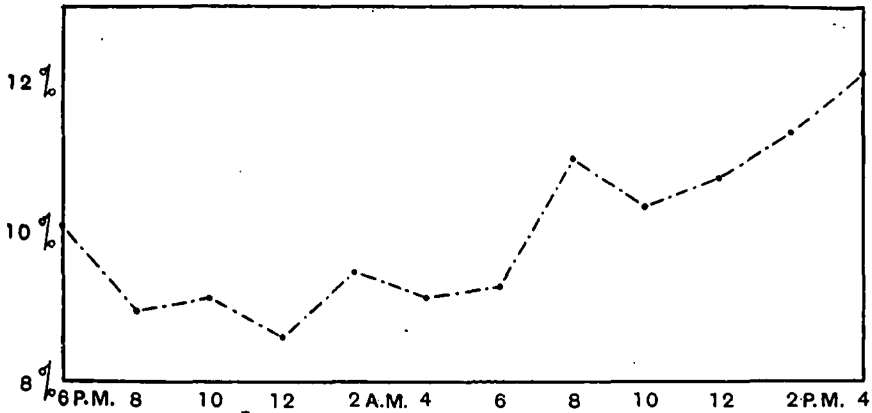
The fluctuation of total carbohydrate may be seen from the total carbon curve. That this curve is influenced greatly by sunlight is clearly shown, the upward movement representing a storing up of assimilated products and the downward gradient translocation, though we must bear in mind the fact that these are not absolute results because they are not calculated on to a basis of unit leaf area. Nevertheless we can test their accuracy by comparing the gains and losses with those obtained by other workers. Table III. and Graph III.

TABLE III.

September 17*th*, 1910.

Time	6	8	10	12	2	4	6	8	10	12	2	4
°/₀ D. M.	10·05	8·90	9·06	8·60	9·45	9·09	9·25	10·89	10·30	10·05	11·30	12·10

GRAPH III.



show the percentage of dry matter in mangold leaves taken at the same time as those used for the sugar determinations. The maximum fluctuation in the case of mangold leaves is shown to be about 5 per cent. of the dry weight of the leaf.

That the nett results of assimilation and translocation are of this order may be seen if we compare the results of Brown and Escombe and the later results of Thoday (6). The old method of Sachs is now looked upon as being untrustworthy, since the rates of assimilation measured under his method by direct weighing always give a rate of assimilation about three times greater than that deduced from the intake of CO_2. In an actual result by Brown and Escombe, working with *Catalpa bignonioides*, they found:—

1. Increased weight per sq. dec. per hour (Sachs half-leaf method) = ·00669 grs.
2. „ „ „ „ „ from CO_2 method = ·00235 grs.

In 1909 Thoday seriously criticised the Sachs method, attributing these higher results to shrinkage, to eliminate which he introduced a stamping method of taking samples. By this method results were obtained which compared favourably with the CO_2 intake method. The results were as under:—

Helianthus tuberosus. Leaves attached to plant. Intermittent sun. Time 7½ hours. 10 A.M. *to* 5.30 P.M.

Leaf	Dry wt.	Gain in dry wt.	Gain CO_2	Gain dry wt.	% gain dry wt., average
1	·417	·039	·061	·041	9·75
2	·365	·023	·047	·032	7·40
3	·393	– ·006	– ·007	– ·006	—
4	·470	·024	·033	·021	4·90

From this we see that the average percentage increase during seven hours' sunshine was 7·35, which is quite comparable with the results put forward, considering that the day on which the experiment was performed was almost without sun, although being quite bright.

The determinations are neither exact nor numerous enough to enable conclusions to be drawn with any great confidence as to the steps by which assimilation and translocation are effected, but taking the results as they are they seem to suggest that the reducing sugars are the first carbohydrates to be formed as soon as daylight begins. A little later the cane sugar curve begins to rise, and later still the starch curve. It would also appear that the cane sugar curve does not rise until the reducing sugars have reached the maximum which they maintain throughout the period of illumination. Similarly the starch curve does not rise in its turn until the cane sugar has reached its maximum. It is impossible as yet to lay much stress upon this sequence, but it would seem to suggest that the more elaborate carbohydrate does not begin to form until the simpler one has reached a certain concentration in the cell. The fact that the maltose curve reaches its maximum during the night, and does not rise until the starch curve begins to fall, would indicate that maltose is a down-grade product from the starch, and therefore a form in which the starch is being translocated. If however the carbohydrates leave the leaf in the form of maltose, it is difficult to conceive how this maltose can be transformed into cane sugar, in which form storage in the root takes place.

VARIATIONS DUE TO MANURING.

The produce of two plots only were analysed in order to find if the sugars in the leaf-stuff varied according to the manures applied. The plots selected were those known as 5 *AC* and 6 *AC*.

Each of these gets dressings of artificial manures, superphosphate at the rate of 3·5 cwt. per acre and nitrogenous dressings of 2000 lbs. rape cake and 400 lbs. ammonium sulphate per acre. In addition to this 6 *AC* gets K_2SO_4 at the rate of 500 lbs. per acre. The crop on 5 *AC* is much less than that on 6 *AC* and is always badly attacked with *Uromyces betae*, the fungus establishing itself early and causing the leaves to become very dark coloured and unhealthy looking. Samples were taken from these plots at 8 a.m. on October 8th. As the results were so very peculiar, the analyses were repeated some days later with the same result.

	Oct 8th		Oct. 14th	
	6 *AC*, 8 A.M.	5 *AC*, 8 A.M.	6 *AC*, 8 A.M.	5 *AC*, 8 A.M.
Dex. and Lev....	5·75	11·70	5·53	10·91
Cane sugar	1·06	·35	1·13	·81
Maltose	2·86	1·78	3·65	1·94

It will be seen from these results that the sugars are for the greater part in the form of glucose and levulose. The meaning of this from the point of view of metabolism is very obscure. Enzymic action and the processes operative in translocation are in some way hindered by the lack of potash, which is probably causing abortive cell tissue which will not allow the free passage of the elaborated carbohydrates. It is suggested, however, that these abnormal conditions that exist are instrumental in providing a "feeding ground" for the fungoid disease with which the plot is affected, the attack being due to the abnormal percentage of reducing substances. Previous work by Massee (8) and also by Miyoshi (9) has shown that it is highly probable that this is the case, parasitism being due to the presence of some positively chemiotactic substance within the leaf-stuff, and, moreover, evidence is forthcoming which shows that grape sugar and cane sugar act in a very positive way. Owing to the lateness of the season this work could not be carried further.

Variations due to Age.

A few determinations were made in order to find if there was any difference in the carbohydrate content of young and old leaves. The young leaves seemed to contain rather more dextrose and levulose than the older leaves on the same plant. At the same time determinations were made to see if the carbohydrates were in the same proportion in opposing half leaf samples in order to get some insight into experimental error.

The results were as follows:—

Date	Sept. 6, 11 A.M.		Sept. 8, 6 A.M.		Sept. 8, 7.30 P.M.		
Description...	Right	Left	Right	Left	Old leaves, Left	Old, Right	Young, Right
Dex. and Lev....	3·35	3·30	3·99	4·07	4·91	4·02	7·05
Cane sugar	1·39	1·43	1·26	1·54	1·80	1·01	1·80
Maltose	1·80	1·99	1·23	2·27	·24	·24	·66

Abnormally dark green leaves were also contrasted with normal leaves, the collections being made on Sept. 6th at 11 a.m. and 5.30 p.m. after a dull day. In this case the maltose in the dark green leaves is rather high. The determination of carbohydrates in the normal leaf at 11 a.m. also shows an exceptionally high percentage of maltose.

Two other determinations were made on similar leaf samples collected at the same time: (*a*) was extracted at once and analysed, (*b*) was killed by boiling in alcohol for a short time, and then allowing to stand for 36 hours in order to see if any hydrolysis was brought about by the acids of the leaf. Apparently no change takes place.

	Sept. 6, 11 A.M.	5.30 P.M., dark green leaf	Sept. 6, 5.15 P.M.	Sept. 6, midnight		Aug. 31, 4.30 P.M.	
						a	*b*
Dex. and Lev....	3·35	4·25	4·53	2·75	2·64	3·52	3·62
Cane sugar......	1·39	1·37	1·93	·49	·86	2·42	2·45
Maltose	1·80	1·34	·66	1·06	1·23	·30	·01

The author is well aware how tentative and preliminary the work described above must be considered. It is only possible to carry it on during a limited period of the year and so far most of the time has been spent in elaborating the method of work, and in making a first series of determinations to ascertain the nature of the problem and order of the quantities to be measured. It is clear that further determinations will have to be made on larger quantities of material and by more refined methods of analysis. Unfortunately the author is precluded from continuing the investigation and therefore desires to put on record the results that have been already obtained.

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