STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS.

II. THE DEXTROSE-LAEVULOSE RATIO IN THE MANGOLD

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Brown and Morris [1893] in their well-known experiments on the *Tropæolum* leaf observed that the hexoses of the leaf instead of being present in the proportion corresponding with invert sugar, invariably appeared to consist very largely of laevulose. In several cases dextrose was entirely absent, whilst in others the proportion of laevulose to dextrose varied from about 6 : 1 down to about 2 : 1. As they had concluded on other grounds that the reducing sugars are formed by inversion from cane sugar, they explained the predominance of laevulose as being due to the dextrose being "more readily put under contribution for the respiratory processes of the cell than is laevulose."

Lindet (1900) made a special study of the proportion of the hexoses present in the leaf and leaf-stalks of the sugar beet at different periods of growth. His analyses showed that in normally growing leaves, especially in the earlier stages of growth (July 3rd to 24th), the proportion of dextrose was generally slightly *greater* than that of laevulose, although on several occasions it was slightly *less*; thus on July 3rd and July 24th, for example, it was found that the ratio of laevulose to dextrose was 1·3 and 1·11 respectively. On the other hand and in striking contrast to the leaves, the laevulose in the leaf-stalks was *invariably* found to form only a small proportion of the dextrose present, varying from 0 to 35 per cent. Lindet adopts Brown and Morris' views to explain these results and concludes that the excess of laevulose in the leaves is due to the dextrose being consumed in these tissues by *respiration* more rapidly than the laevulose; on the other hand the laevulose has been

removed from the sap of the stalks in forming new tissue, laevulose being the sugar specially adapted to this purpose. Lindet in a recent paper [1911], inspired by the results of his earlier work, cites experiments made with yeasts which also serve to show that laevulose lends itself to reproductive growth or new tissue formation better than dextrose.

Parkin [1912] in experiments on the snowdrop leaf found that in 47 out of 52 analyses the laevulose was in excess of the dextrose; representing laevulose as unity, in these cases the ratio varied from 1 : 0·4 to 1 : 0·76. In the five remaining cases, the ratio was 1 : 1·01 to 1 : 1·06. The ratio of laevulose to dextrose appeared to rise during the night, that is when photosynthesis is in abeyance; the excess of laevulose was always greatest in the lower (colourless) part of the long snowdrop leaf. To explain his results Parkin also adopted Brown and Morris' view that the dextrose lends itself most readily to the respiratory needs of the plant, whilst the laevulose is used largely in constructive work such as the building up of the plant's framework.

As was pointed out by Brown and Morris in 1893 the correctness of the dextrose and laevulose values depends entirely on the accuracy of the readings of the rotatory power; a slight error in these makes a large difference in the apparent proportion of the two hexoses. The main purpose of the present paper is to show that, whilst it is possible to take the actual readings very accurately (in our case the probable error did not exceed 0·005°), the values are falsified, in the case of most plant material, by the presence of optically active substances other than the sugars, which are not completely precipitated by the basic lead acetate (or other defecating substance) used to purify the solutions. Typical substances of this kind are amino-acids and amides, such as glutamic acid and glutamine, aspartic acid and asparagine. The first three of these have a pronounced positive rotation which is greatly enhanced by acids, whilst asparagine is laevo-rotatory in aqueous solution and dextro-rotatory in acid solution. The influence of these substances in falsifying the results obtained by the method of double polarisation for the cane sugar in the molasses of sugar manufacture has been studied by several chemists, more particularly by H. Pellet (compare *Dosage du Sucre par Inversion*, 1913), but the effect of these and other impurities on the results obtained for the dextrose : laevulose ratio in plant material has not hitherto been taken into account.

We find in the *mangold*, just as Lindet did in the sugar beet, that the dextrose always seems to be in excess of the laevulose, especially in the

mid-ribs and stalks (where the ratio $\frac{D}{L}$ is often extremely high), whereas in the *potato* the reverse holds, the laevulose apparently predominating as in the cases studied by Brown and Morris, and by Parkin. At the same time it can be shown that the apparent excess of dextrose or laevulose is correlated with certain abnormalities in the cane sugar estimations, caused by the presence of optically active impurities. The apparent excess of *dextrose* in the tissues of certain plants (sugar beet and mangold) is indeed due to the presence of a dextro-rotatory impurity (possibly glutamine), whilst the predominance of laevulose in other plants (e.g. tropæolum, snowdrop, potato) is to be attributed to a laevo-rotatory impurity (e.g. asparagine).

In the mangold the difference between the results obtained for saccharose by the reduction method and by the double polarisation method, which we have referred to in the preceding paper (p. 273), is always far greater in the stalks and mid-ribs than in the leaves, a fact which we attribute to the accumulation of optically active impurities in these parts. Side by side with this we have the fact that, whilst in the leaves the ratio of dextrose to laevulose $\left(\frac{D}{L}\right)$ is in general not very far removed from unity, in the stalks and mid-ribs the ratio $\frac{D}{L}$ is very much greater, generally varying from 2·5 to 10. This ratio, too, is far higher in the bottom halves of the stalks than in the top halves, pointing to an accumulation in the lower part of the stalks of the dextro-rotatory impurity. Striking differences are also found between the results for cane sugar in the top and bottom halves, according as they are calculated from the reduction data or from the polarimetric values. Thus in the *top* halves the results obtained by polarisation may be 80 to 90 per cent. *lower* than the values obtained by reduction, whilst in the *bottom* halves they are *high* by 40 per cent. As the day proceeds, the relation of tops and bottoms may be reversed, the impurity which was in the top half passing down to the lower part of the stalks (compare the values at noon, 6 p.m. and midnight given on p. 344, Table VIII).

Independently of any error which may be caused by the improper use of basic lead acetate (see p. 270), a difficulty which makes it impossible to obtain really accurate values of the proportion of dextrose and laevulose lies in the fact that allowance has to be made in the calculation for the reducing power and rotation of the pentoses which are invariably present in the alcoholic extracts prepared by our method of working.

In any particular case, one is entirely ignorant as to the nature of these; if it be assumed that they consist of arabinose and xylose, whilst it is possible to introduce a fairly accurate correction for the reducing power, owing to the fact that the reducing power of arabinose is nearly identical with that of xylose (Daish [1914]), this is not the case for the rotatory power as $[\alpha]_D$ has widely different values for the two pentoses (for arabinose $[\alpha]_D^{20^\circ} = +122^\circ$, for xylose $[\alpha]_D^{20^\circ} = +18{\cdot}78^\circ$). This large disparity in the specific rotatory powers may, in certain cases, involve a difference of 0·1° or more in the rotation from which the dextrose and laevulose are calculated, according as the pentose is assumed to be arabinose or xylose respectively; in the example given, showing our method of calculation (see p. 317), the difference is only 0·041°, but it is frequently much greater and then represents quite a large proportion of the actual angle used in calculating the reducing sugars. On this account, in default of information as to the exact nature of the pentoses present, we have always calculated the dextrose and laevulose on the two assumptions: (1) that the pentose is arabinose, (2) that the pentose is xylose. But it is quite possible that the pentose really present may consist to a greater or less extent of one of the less known pentoses, e.g. *d*-ribose, and if this is the case the results for dextrose and laevulose will be correspondingly at fault.

Dextrose and laevulose are, moreover, calculated from values obtained after allowing for all the other substances present—cane sugar, pentoses, maltose (if present). The degree of accuracy obtained will naturally depend on the accuracy with which the other constituents have been estimated. Even the difference caused by calculating the small proportion of pentose as arabinose or as xylose may, as for example in the mangold leaf, 5 p.m., October 11th, make the ratio $\frac{D}{L}$, which appears to be strictly 1·00 when the pentose is taken as xylose, have a very different value (0·844) when the pentose is assumed to be arabinose.

In putting forward the results given in this paper, I am conscious that the values given as dextrose and laevulose probably do not, in most cases, represent real values; they are therefore designated "apparent dextrose" and "apparent laevulose." Although little confidence can be placed in them as *absolute* values for these sugars, they show a regular variation which is sufficiently striking to justify detailed consideration. This variation may be due either to a real variation of the dextrose and laevulose or, what is more probable, to a regular

variation in the amount of the optically active impurities which are present; if the latter, the fluctuation of these substances during the 24 hours must be quite as great as the fluctuation of the sugars themselves. Until a method has been devised by which it is possible to estimate accurately the true proportions of dextrose and laevulose, when present together, without having recourse to polarimetric data, the facts brought forward in this paper, and that which follows, show that it is impossible to know with any certainty the real proportions of these sugars present in different plant tissues; it is, therefore, equally impossible to draw conclusions as to the function of these two sugars in the plant—whether the one is more suited than the other to build up new tissue or whether one is more easily put under contribution than the other in respiration. The fermentation test, which Parkin and others have used to ascertain whether the solutions they analysed were free from optically active substances other than sugars, is one which is by no means reliable for this purpose. Parkin, whose work in most other respects is valuable, considered that, as the solutions prepared from the snowdrop leaves showed, after fermentation with yeast, a negligible rotatory and reducing power, *no other substances likely to possess these properties were present in the original solutions.* It would be quite possible for large amounts of asparagine to have been present, sufficiently large indeed to explain the apparent preponderance of laevulose in the snowdrop (where the ratio of laevulose to dextrose varied from 1 : 0·4 to 1 : 0·76) and yet to have escaped detection by this method, as the asparagine would be largely, if not entirely, consumed by the yeast in its growth; asparagine indeed is used very largely as a nutrient material for yeasts, for example in Hayduck's solution.

EXPERIMENTAL.

The methods of analysis have been described in the preceding paper; an example is there given of the method of calculating the proportions of dextrose and laevulose according as the pentoses are assumed to be arabinose or xylose (see p. 318). The actual data used are given on pp. 319–325; the results are calculated on the *total vacuum-dried matter* of the leaf.

In the tables which follow, D = per cent. of "apparent dextrose" in the total vacuum-dried matter; L = per cent. of "apparent laevulose" in the total vacuum-dried matter.

TABLE I.

Mangold Leaves. Series I. August 26th–27th, 1913.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose taken as		Hexoses calculated as invert sugar %	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
6 a.m. ...	0·73	Nil	∞	0·73	Nil	∞	0·73	0·73	0·77	
8 a.m. ...	0·64	0·77	0·83	0·87	0·52	1·68	1·41	1·39	1·42	
10 a.m. ...	1·09	1·06	1·02	1·32	0·80	1·65	2·15	2·12	2·16	
12 noon ...	1·11	1·02	1·08	1·33	0·78	1·70	2·13	2·11	2·15	Day
2 p.m. ...	1·01	0·90	1·12	1·24	0·66	1·88	1·91	1·90	1·94	
4 p.m. ...	0·92	0·21	4·34	1·15	0·00	∞	1·13	1·15	1·18	
6 p.m. ...	0·49	0·46	1·07	0·74	0·19	3·87	0·95	0·93	0·96	Sun sets 7 p.m.
8 p.m. ...	0·73	0·14	5·33	0·92	0·00	∞	0·87	0·92	0·90	
10 p.m. ...	0·29	0·45	0·65	0·50	0·23	2·17	0·74	0·73	0·74	
12 night ...	0·32	0·24	1·35	0·55	0·00	∞	0·56	0·55	0·57	Night
2 a.m. ...	0·29	0·08	3·62	0·36	0·00	∞	0·37	0·36	0·38	
4 a.m. ...	0·00	0·20	0·00	0·16	0·02	7·14	0·20	0·19	0·20	Sun rises 5.5 a.m.

TABLE II.

Mangold Leaves. Series II. September 10th–11th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
10 a.m. ...	2·72	3·01	0·904	2·91	2·80	1·039	5·73	5·71	5·72	
1 p.m. ...	3·85	3·62	1·063	4·06	3·37	1·204	7·47	7·43	7·50	Day
4 p.m. ...	3·11	3·87	0·804	3·47	3·46	1·003	6·98	6·93	7·00	
6 p.m. ...	3·59	5·38	0·668	3·83	5·11	0·749	8·97	8·94	8·90	Sun sets 6.26 p.m.
8 p.m. ...	2·56	4·25	0·602	2·95	3·74	0·789	6·81	6·69	6·76	
11 p.m. ...	3·34	3·79	0·881	3·73	3·36	1·110	7·13	7·09	7·10	Night
2 a.m. ...	3·43	4·39	0·781	3·84	3·93	0·977	7·82	7·77	7·81	
4 a.m. ...	3·17	3·73	0·851	3·51	3·36	1·045	6·90	6·87	6·91	Sun rises 5.30 a.m.
6 a.m. ...	2·64	3·68	0·717	2·99	3·30	0·906	6·32	6·29	6·30	Day
8 a.m. ...	2·26	3·15	0·718	2·60	2·78	0·935	5·41	5·38	5·38	

TABLE III.

Mangold Leaves. Series III. October 11th–12th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
9 a.m.	5·62	4·67	1·203	6·07	4·18	1·450	10·29	10·25	10·32	
11 a.m.	5·32	6·35	0·839	5·82	5·81	1·001	11·67	11·63	11·62	
1 p.m.	5·17	7·03	0·735	5·67	6·49	0·874	12·20	12·16	12·12	Day
3 p.m.	4·79	5·43	0·882	5·26	4·91	1·070	10·22	10·17	10·24	
5 p.m.	5·24	6·21	0·844	5·72	5·69	1·005	11·45	11·41	11·46	Sun sets 5.15 p.m
7 p.m.	5·67	5·82	0·975	6·17	5·27	1·171	11·49	11·44	11·47	
9 p.m.	5·92	6·04	0·980	6·38	5·54	1·151	11·96	11·92	11·98	
11 p.m.	5·04	4·32	1·165	5·41	3·91	1·385	9·36	9·32	9·39	
1 a.m.	5·25	5·53	0·950	5·69	5·04	1·129	10·78	10·73	10·78	Night
3 a.m.	6·05	6·37	0·950	6·44	5·95	1·084	12·42	12·39	12·41	
5 a.m.	6·26	5·15	1·215	6·64	4·73	1·404	11·41	11·37	11·49	Sun rises 6.21 a.m.
7 a.m.	4·81	4·80	1·002	5·15	4·44	1·160	9·61	9·59	9·62	Day

TABLE IV.

Mangold Leaf-stalks. Series I. Top and Bottom Halves.
August 26th–27th, 1913.

Time		Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
		D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
6 a.m.	Tops ...	4·36	0·76	5·72	5·09	0·00	∞	5·12	5·09	5·35	
	Bottoms	7·90	0·94	8·37	8·55	0·23	37·2	8·84	8·78	9·11	
12 noon	Tops ...	4·89	5·04	0·97	5·37	4·51	1·19	9·93	9·88	9·97	Day
	Bottoms	10·20	2·70	3·77	11·00	1·83	6·01	12·90	12·83	13·17	
6 p.m.	Tops ...	4·22	3·61	1·17	4·87	2·89	1·68	7·83	7·76	7·89	
	Bottoms	8·45	1·71	4·95	9·10	1·00	9·10	10·16	10·20	10·47	Sun sets 7 p.m.
12 night	Tops ...	5·09	1·29	3·95	5·71	0·61	9·35	6·38	6·32	6·61	Night
	Bottoms	6·80	1·37	4·97	7·38	0·73	10·05	8·17	8·11	8·49	

TABLE V.

Mangold Leaf Mid-ribs. Series II. September 10th–11th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
10 a.m. ...	17·8	5·3	3·36	18·0	5·1	3·53	23·1	23·1	23·6	Day
4 p.m. ...	17·9	4·1	4·37	18·4	3·5	5·26	22·0	21·9	22·6	
11 p.m. ...	15·3	4·9	3·12	15·8	4·3	3·68	20·2	20·1	20·6	Night
4 a.m. ...	12·9	5·8	2·22	13·6	5·1	2·67	18·7	18·7	19·0	
6 a.m. ...	14·4	6·7	2·15	15·2	5·9	2·58	21·1	21·1	21·4	Day

TABLE VI.

Mangold Stalks. Series II. September 10th–11th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
10 a.m. ...	14·8	5·1	2·90	15·3	4·6	3·33	19·9	19·9	20·5	Day
4 p.m. ...	17·5	8·45	2·07	18·1	7·75	2·34	25·9	25·9	26·3	
11 p.m. ...	17·3	4·5	3·84	17·8	3·9	4·56	21·8	21·7	22·4	Night
4 a.m. ...	17·3	6·0	2·88	17·8	5·4	3·30	23·3	23·2	23·7	
6 a.m. ...	19·1	7·6	2·51	19·75	6·95	2·84	26·7	26·7	26·7	Day

TABLE VII.

Mangold Stalks and Mid-ribs. Series III. October 11th–12th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose	
Mid-ribs:									
11 a.m. ...	15·7	7·2	2·18	16·1	6·7	2·40	22·9	22·8	23·4
11 p.m. ...	14·0	4·5	3·11	14·5	3·9	3·73	18·5	18·4	19·0
Stalks:									
11 a.m. ...	19·3	5·8	3·32	19·9	5·2	3·81	25·1	25·1	25·7
11 p.m. ...	16·15	4·85	3·33	16·8	4·2	4·04	21·0	21·1	21·4

DISCUSSION OF RESULTS.

A. *Leaves.*

Series I. In Series I, Table I, the actual percentages of total hexoses are very small, especially at night, during which they range from 0·90 to 0·20 per cent.; consequently, even the small differences in the rotation of the pentoses, according as they are assumed to be arabinose or xylose, lead to considerable differences in the proportions of dextrose and laevulose apparently present. Thus, for example, at 8 a.m., if the pentose is assumed to be arabinose, laevulose appears to be in excess of the dextrose, the ratio $\frac{D}{L}$ being 0·83; if the pentose be taken as xylose, the dextrose appears in excess, the ratio $\frac{D}{L}$ becoming 1·68. In this particular case the rotation of the pentose represented, in a 200 mm. tube at 20°, as arabinose a reading of + 0·049°, as xylose + 0·009°, whilst, after allowing for the saccharose and pentoses present (see method of calculation, p. 317), the rotation left for the hexoses was − 0·042° or − 0·002° in the two cases.

That dextro-rotatory impurities are present in this series to an extent sufficient to invalidate the calculations and so to convey a false idea of the true quantities of dextrose and laevulose, is shown by considering the data obtained at 6 a.m. In this case, if the *whole* of the reducing sugar, obtained from the reduction data, is calculated as dextrose,

a rotation + 0·039° (in 200 mm. tube at 20°) is obtained, whereas the rotation actually observed, after allowing for the cane sugar and pentoses, was + 0·093° (pentoses as arabinose) or + 0·125° (pentoses as xylose). Thus in the two cases a *positive* rotation remains unaccounted for, of + 0·054° or + 0·086° respectively. Similarly at 4 p.m., the whole of the reducing sugars calculated as dextrose would barely account for the actual positive rotation observed if the pentoses be taken as xylose, the excess being + 0·004°; if, however, the pentoses be taken as arabinose, the dextrose would more than account for the rotation observed by + 0·037°, and in this case the dextrose becomes 0·92 per cent. and laevulose 0·21 per cent., the ratio $\frac{D}{L}$ being 4·34. Similarly at 8 p.m. the assumption that the whole of the reducing sugar is dextrose leaves + 0·008° unaccounted for if the pentose is xylose; when it is taken as arabinose, D becomes 0·73 and $L = 0·14$ per cent., the ratio $\frac{D}{L}$ being high, viz. 5·33. Similar observations hold for midnight, 2 a.m. and 4 a.m.; at 4 a.m. the quantity of reducing sugars is so small, that assuming the pentose to be arabinose causes the laevulose to appear in excess, whilst if it is taken as xylose, the dextrose appears largely in excess, $\frac{D}{L}$ being 7·14.

It is clear therefore that little significance can be attached to the values for dextrose and laevulose in Series I owing to the presence of a dextrorotatory impurity[1], the rotation of which is large relatively to that of the small quantity of sugars present. Even though this is the case, between 8 a.m. and 2 p.m. the values of dextrose and laevulose are approximately equal, especially when the pentoses are assumed to be arabinose, the ratio $\frac{D}{L}$ being approximately 1. It should be noted

[1] A determination was made for us by Mr E. Horton of the amino-nitrogen in the Solution *A* used in estimating the sugars in the case of a sample of mangold leaves picked at 2.45 p.m. on October 8th, 1914. 2 c.c. of this solution gave in the van Slyke micro-apparatus 0·225 c.c. of nitrogen at 0 and 760 representing 0·007 grm. of amino-nitrogen per 100 c.c. Calculated as *glutamine* this would represent 1·2 per cent. of glutamine on the total vacuum-dried weight of the leaf at a time of day when, judging by the results of Table III, the proportion of such impurity is at its minimum; in this picking, the cane sugar was 7·5 per cent. and the hexoses 19·1 per cent. of the total vacuum-dried matter, so that the proportion of the optically active amide is in this case only small relatively to the sugars, a fact which would explain that the ratio $\frac{D}{L}$ keeps in the neighbourhood of unity (see Tables II and III).

that at these times the proportion of the sugars is greatest, so that the effect of the rotation of the optically active impurity in falsifying the results is least marked. At night, apparently, an excess of this impurity is liberated, possibly as a waste product of metabolism, owing to degradation changes predominating, so that the laevulose appears to have disappeared entirely at 6 a.m., and a relatively large *positive* rotation remains unaccounted for even when the whole of the reducing sugar is assumed to be dextrose.

Fig. 1 shows the variation of "apparent dextrose" and "apparent laevulose" on the assumption that the pentoses are xylose[1] during the 24 hours, August 26th–27th. Throughout this period the dextrose

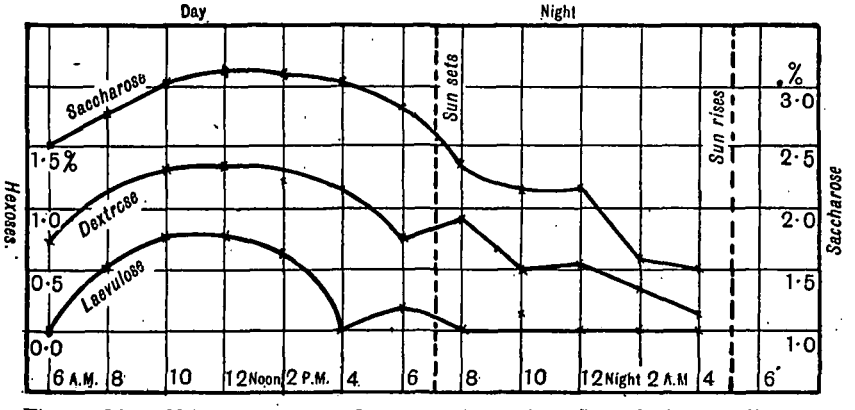

Fig. 1. Mangold leaves, apparent dextrose and laevulose, Series I, Aug. 26–27, 1913 (pentose as xylose).

curve is above the laevulose; during the period of actual insolation the curves are approximately parallel to each other and to the saccharose curve. If it were not for the presence of the dextro-rotatory impurity, the two curves would probably nearly coincide—the dextrose curve being lowered and the laevulose curve being correspondingly raised. On the assumption that the pentose is arabinose, the two curves actually coincide without any such correction being made. The parallelism of the curves of dextrose and laevulose with that of cane sugar is particularly striking when the apparent steepness of the curve of total hexoses is taken into account (see previous paper, Fig. 4), from which it might

[1] Throughout this paper the curves of dextrose and laevulose are drawn only for the case when the pentose is assumed to be xylose. The curves obtained by assuming the pentose to be arabinose are strictly parallel to these curves but slightly higher or lower; the effect of taking the pentose as arabinose instead of xylose is to raise the laevulose curve and lower the dextrose curve.

be inferred that the hexoses are formed more rapidly than the saccharose, and consequently precede it; instead, the quantity of each of the reducing sugars is roughly proportional to the cane sugar present, a fact which points to the formation of the hexoses from this sugar.

Series II. In Series II (Table II) the sugar percentages are far higher than in Series I and the fluctuations in the value $\frac{D}{L}$ are far less marked in consequence. With few exceptions (e.g. at 6 p.m. and 8 p.m.) the value of $\frac{D}{L}$ does not differ much from 1, the percentages of dextrose and laevulose being as nearly equal as one could expect bearing in mind the errors to which the calculations are subject. In general, the values of $\frac{D}{L}$ obtained by assuming the pentose to be arabinose are slightly *lower* than unity, whilst by assuming it to be xylose, they become slightly *higher* than unity. It is probable that $\frac{D}{L}$ would be almost exactly 1 were it not for the presence of small quantities of optically active impurities, which in some cases increase the value, in others lower it. The following table shows that the departure of the value $\frac{D}{L}$ from 1 goes hand in hand with the divergence Δ between the values for cane sugar found by the reduction and double polarisation methods; this divergence is no doubt also caused by the presence of these optically active impurities (see p. 344).

$\frac{D}{L}$ approximately 1			$\frac{D}{L}$ divergent from 1		
Time	$\frac{D}{L}$	Δ*	Time	$\frac{D}{L}$	Δ
10 a.m. ...	1·039	−14·0 %	1 p.m. ...	1·204	+25·9 %
4 p.m. ...	1·003	+ 1·5	6 p.m. ...	0·749	+15·2
11 p.m. ...	1·110	+ 7·2	8 p.m. ...	0·789	+23·2
2 a.m. ...	0·977	+ 7·4			
4 a.m. ...	1·045	+16·1			
6 a.m. ...	0·906	+10·0			
8 a.m. ...	0·935	+10·6			

* Δ represents the difference between the *reduction* and *polarisation* values for *cane sugar*, expressed as a percentage of the *average* value found by reduction by the invertase and citric acid methods; thus, e.g., +10·0 per cent. shows that the average value found by the double polarisation method is 10 per cent. higher than the average value found by reduction. In this particular case (6 a.m.) the cane sugar found by reduction was 4·24 per cent. on T.V.D.M., and by double polarisation 4·65 per cent.

The closer the value $\frac{D}{L}$ approximates to 1, the closer is the agreement between the cane sugar values by the two methods; at 4 p.m. particularly, when the value $\frac{D}{L}$ is practically 1, the divergence Δ is exceedingly small, viz. 1·5 per cent. only. It would appear that at this time the amount of optically active impurities influencing the results is practically *nil*. On the other hand, at 1 p.m., 6 p.m., and 8 p.m., when the value of $\frac{D}{L}$ departs considerably from unity, *in either direction*, there is a correspondingly large difference in the results for cane sugar by the two methods, the polarisation results being from 15 to 25 per cent. high. The figures at 6 p.m. and 8 p.m. are particularly interesting because at these times there is apparently an excess of laevulose[1], pointing to the presence of a *laevo-rotatory*, not a dextro-rotatory, impurity such as was present in the earlier part of the day (e.g. at 1 p.m.). At the same time, however, the polarisation figures are still higher than the reduction figures, showing that the *change* of rotation which accompanies the inversion process involves probably transformation of the laevo-rotatory substance into a compound with a still greater laevo-rotation—such as would happen, for example, in the transformation of asparagine into aspartic acid.

It is interesting to consider the curves in Fig. 2 showing the variation of the "apparent dextrose" and "apparent laevulose" during the 24 hours. Although these curves probably do not show the variation of the true sugars so much as that of the optically active impurities, they exhibit a regularity which points to the latter substances being formed regularly and progressively. In general the apparent dextrose and apparent laevulose increase when the cane sugar increases and fall when this sugar falls. The dextrose curve rises and falls three times in succession during the 24 hours, the rise and fall in the night (8 p.m. to 8 a.m.) being far more gradual and regular than the two abrupt changes during the day. The laevulose curve is less regular, rising gradually till 4 p.m., when D and L are practically equal; from this point the laevulose rises suddenly and follows the abrupt rise in the saccharose curve, which takes place just before sunset. The latter rise is therefore

[1] This apparent excess of laevulose would formerly have been explained by assuming that at these times the respiratory changes (utilising dextrose) predominate over the tissue-building changes. The polarisation data clearly point to abnormal quantities of optically active impurities at these points, which falsify the real values of D and L.

associated with a sudden increase in the amount of a *laevo-rotatory* impurity; at 4 p.m. the laevulose curve rises above the dextrose curve and remains above it until 10 p.m. From 6 p.m. to 8 p.m. $\frac{D}{L}$ has abnormally low values (0·749–0·789), but at 10 p.m. it is again practically unity, and it so remains throughout the night with very little change, although the laevulose curve rises and falls between 11 p.m. and 4 a.m., and falls between 4 a.m. and 8 a.m.

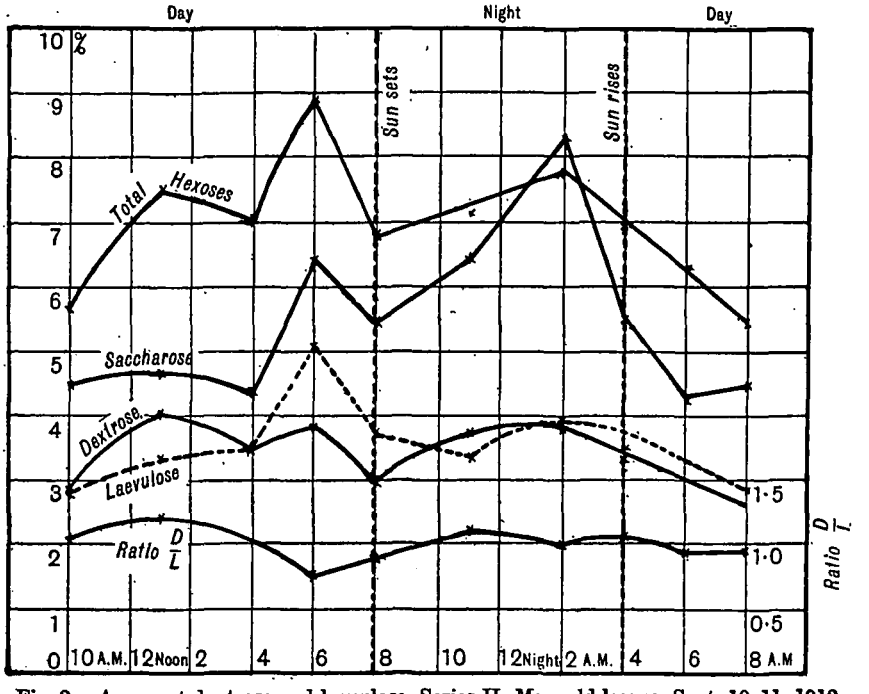

Fig. 2. Apparent dextrose and laevulose, Series II, Mangold leaves, Sept. 10–11, 1912.

The curve showing the variation of the ratio $\frac{D}{L}$ is also given in Fig. 2; it illustrates the marked periodic character of the fluctuations. These occur in two well-defined periods: In the first, a regular rise and fall of the ratio occurs in the eight hours from 10 a.m. to 6 p.m., during which the dextrose appears in excess; in the second period, from 6 p.m. to 10 p.m., the laevulose is in excess, but the ratio $\frac{D}{L}$ increasing. In the remaining 12 hours, from 10 p.m. to 10 a.m. there is little change in the value $\frac{D}{L}$ which remains very nearly unity.

Series III (Table III, Fig. 3). As in Series II, the ratio $\frac{D}{L}$ is, with few exceptions (9 a.m., 11 p.m. and 5 a.m.), approximately unity when the pentoses are taken as xylose; in most cases the ratio is greater than

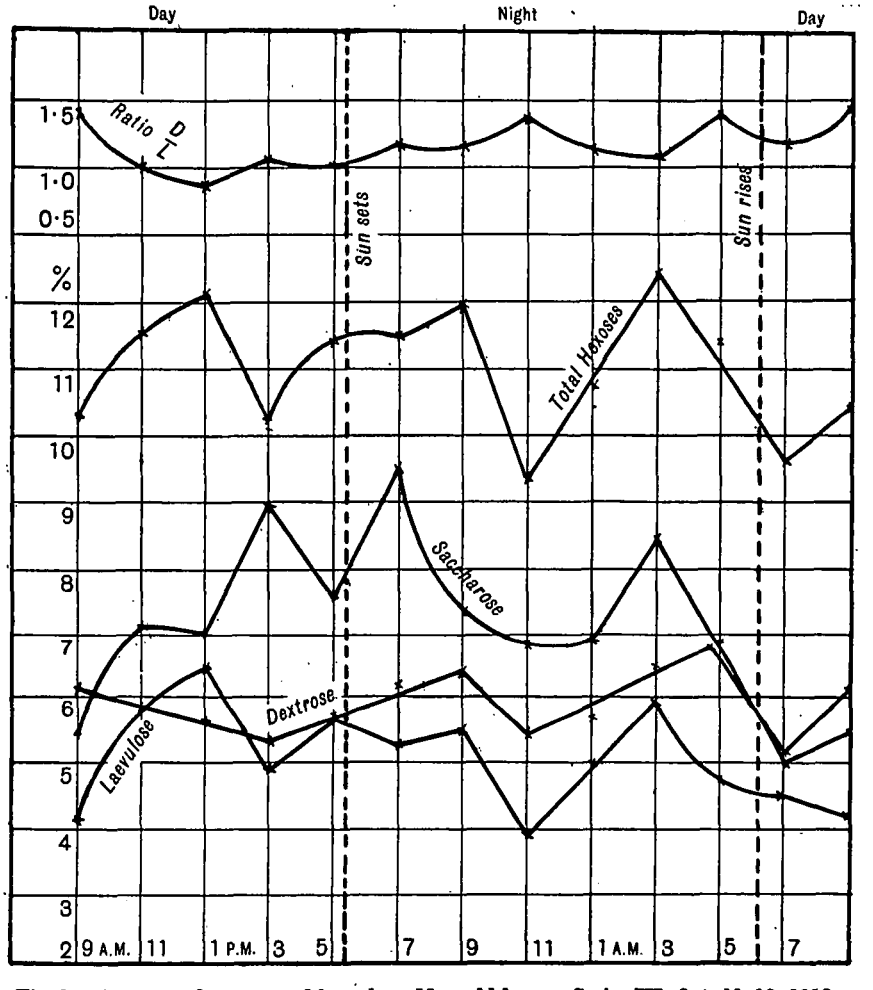

Fig. 3. Apparent dextrose and laevulose, Mangold leaves, Series III, Oct. 11–12, 1912.

1 when the pentoses are assumed to be xylose and less than 1 when they are taken as arabinose. As in Series II it can be shown that when the ratio $\frac{D}{L}$ most departs from unity (5 a.m., 7 a.m., 9 a.m.) the difference

Δ between the values found for saccharose by reduction and polarisation is greatest, the polarisation figures being 20 to 30 per cent. high.

During the greater part of the day (except at 9 a.m., when $\frac{D}{L} = 1{\cdot}45$) the proportions of apparent dextrose and laevulose are very nearly equal, as might be expected if they were formed from saccharose, but at night the dextrose appears to be in excess. A striking difference from Series II is that the dextrose instead of rising during the day and then falling, at first appears to *fall* and then to rise (see Fig. 3); at night, instead of a rise and fall, there is a fall between 9 and 11 p.m., followed by a gradual rise to 5 a.m. But in both series there appears to be a similar periodic character in so far as there are three rises and three falls in the 24 hours. In Series III the variation of the apparent dextrose is most striking as taking place along practically straight lines in a very regular manner. The apparent laevulose curve in Series III is entirely different from the dextrose curve but it follows fairly closely the curve of total hexoses, and less closely the curve of saccharose. The fluctuations of the apparent laevulose are considerably greater and more abrupt than those of the dextrose, as was also the case in Series II.

The curve showing the variation of $\frac{D}{L}$ is also given. $\frac{D}{L}$ falls from 9 a.m. to 1 p.m., then rises slowly and more or less by successive steps to a maximum at 11 p.m., when it again falls and rises twice before 9 a.m.

B. *Stalks and Mid-ribs.*

The most striking fact which appears from the data given in Tables IV to VII is that in the stalks and mid-ribs the apparent dextrose is always in large excess of the laevulose. Whereas in the *leaf* the ratio $\frac{D}{L}$ does not depart much from unity[1], in the mid-ribs and stalks it is rare to find this ratio anywhere in the neighbourhood of 1. Only in the earliest stages of growth (August 26th) and then only in the top half of the stalks, nearest the leaf, at noon and 6 p.m., when freshly formed sugars from the leaf are passing into the stalks, does the ratio

[1] The departure of this ratio from unity in Series I, Table I, is to be attributed to the impossibility, owing to the presence of optically active impurities, of ascertaining the true proportions of dextrose and laevulose in these cases, where the reducing sugars are present in small amounts. In Series II and III, the ratio $\frac{D}{L}$ is nearly unity throughout the whole 24 hours.

$\frac{D}{L}$ become nearly equal to unity. In all other cases the ratio varies from 2·5 to 5, or even higher in the case of the bottom halves of the stalks. In passing from the leaves to mid-ribs, from mid-ribs to the tops of stalks and from the tops of stalks to the bottom the proportion of apparent dextrose steadily and rapidly increases.

Series I. Tops and bottoms of stalks. The analyses given in Table IV show that the proportion of apparent dextrose to laevulose $\left(\frac{D}{L}\right)$ is far higher in the bottom half of the stalks than in the top half at the same time. At 6 a.m. of August 26th at first sight it would appear that during the night the laevulose practically disappears from both top and bottom halves of the stalk just as it appeared to do in the leaf (see Table I) during the night in the same series. Lindet observed a similar phenomenon in the case of the sugar beet and attributed the predominance of dextrose in the stalks to the laevulose being used more rapidly than the dextrose for purposes of tissue building. But that it is quite unsafe to rely upon the polarimetric data as affording any real index of the proportions of dextrose and laevulose actually present in the stalks is shown by the following considerations. The stalks stand out in striking contrast to the leaves as regards the extraordinary divergences between the results obtained for saccharose by the reduction method and by the method of double polarisation. In some cases, for example at 6 p.m., Table IV, the polarisation results for cane sugar in the bottom halves are 40 per cent. *higher* than the values obtained by reduction; and yet *at the same time* the tops give polarisation results which are 85 per cent. *low*. The following table (Table VIII) gives a comparison of the data obtained for cane sugar by the two methods (reduction and polarisation), showing that the divergence is very much greater in the stalks than in the leaves and much more variable in its nature. The fact that the *tops* may give by polarisation a large apparent deficiency of saccharose and the *bottoms* at the same time a large excess as compared with the reduction values (see the data at 12 noon and 6 p.m., Table VIII), or *vice versa* as at midnight when the relations are reversed, points to the presence *in the top and bottom halves of the stalk at different periods of the day of quite different impurities, with different and opposite rotatory powers* (substances as different as *d*- and *l*-glutamine or *d*- and *l*-asparagine).

A careful comparison of Table VIII with the curves showing the variation of apparent dextrose and laevulose in the stalks (Figs. 4

and 5) shows that *it is possible to correlate the wide variations in the differences* (Δ) *between the reduction and polarisation values for cane sugar with the variations in the apparent dextrose and laevulose,* a fact which points to their having a common origin, namely the presence of optically active impurities. Fig. 4 shows the variation of the apparent dextrose and laevulose in the *top* half of the stalks and also the curves for cane sugar and total hexoses. During the whole 24 hours the "dextrose" fluctuates only slightly—there is a slight rise and fall during

TABLE VIII.

Divergence of Results for Saccharose by Reduction and Polarisation in Mangold Stalks—Series I.

		Saccharose found				Polarisation results *high* by % (Δ)	
		Citric inversion		Invertase inversion			
		By reduction	By polarisation	By reduction	By polarisation	Citric inversion	Invertase inversion
6 a.m.	Tops ...	3·36 %	2·85 %	4·14 %	4·56 %	−15·2 %	+10·3 %
	Bottoms	3·47	3·60	3·89	3·60	+ 3·7	− 7·4
12 noon	Tops ...	4·52	0·08	4·26	0·40	−98·0	−90·4
	Bottoms	—	2·99	4·12	4·19	+ 9·14	+ 1·8
6 p.m.	Tops ...	4·14	0·64	3·92	0·61	−84·6	−84·5
	Bottoms	4·03	5·65	4·09	5·77	+40·3	+41·2
12 night	Tops ...	4·12	5·31	3·98	5·10	+29·1	+27·8
	Bottoms	—	—	4·15	3·18	—	−23·3

the day and a slight rise and fall at night. The saccharose also is nearly constant during the 24 hours. But the "apparent laevulose" varies enormously. Between 6 a.m. and noon this sugar increases from *nil* to 4·5 per cent., but from noon onwards falls along almost a straight line until the zero is again reached shortly after midnight. The important fact to be noted is that *while the apparent laevulose increases the differences* (Δ) *between the polarisation and reduction values of cane sugar become more and more negative* (change from + 10 to − 90 for invertase values, Table VIII), whilst when the apparent laevulose falls the values of Δ become more and more positive (− 84 per cent. at 6 p.m., + 28 at midnight). It may be noted that the curve of apparent

laevulose follows more or less closely the general course of the curve of total hexoses (calculated as invert sugar, from reduction values only). But there is no real significance in this because with the dextrose values apparently constant, the laevulose figures, which are also calculated from the same reduction values, necessarily follow the figures for total hexoses (at any moment $D + L =$ total hexoses).

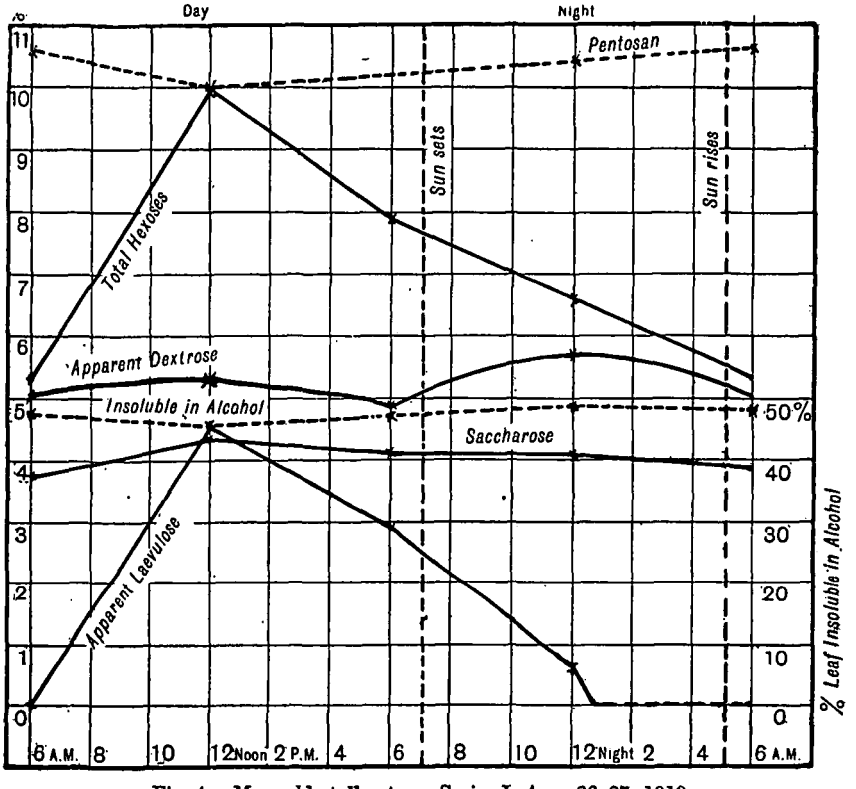

Fig. 4. Mangold stalks, tops, Series I, Aug. 26–27, 1913.

Exactly the same kind of relation can be traced between the fluctuation of the apparent hexoses and the values of Δ in the *bottom* halves of the stalks (Fig. 5). In this case, however, *both* the dextrose and laevulose appear to undergo wide variations. From 6 a.m. to noon the dextrose increases *absolutely* faster than the laevulose (from 8·55 to 11·0 per cent. for dextrose compared with a change from 0·2 to 1·8 per cent. for laevulose) although *relatively* the dextrose does not increase so rapidly as the laevulose, as shown by the fall of $\frac{D}{L}$ from 37·2 to 6·0.

The increase in the proportion of apparent dextrose is accompanied by the rise of Δ from a negative value (− 7·4, invertase figure, Table VIII) to a slightly positive value (+ 1·8). From noon to 6 p.m., although both dextrose and laevulose appear to be falling, the laevulose relatively

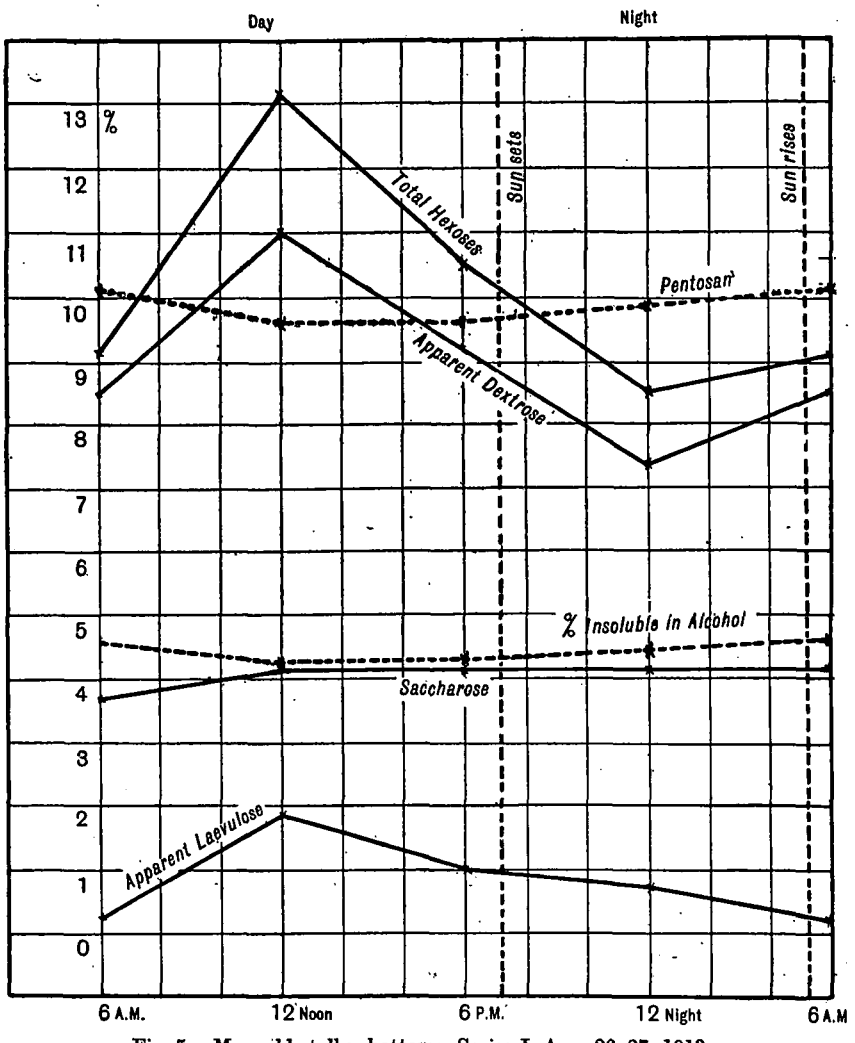

Fig. 5. Mangold stalks, bottoms, Series I, Aug. 26–27, 1913.

falls faster than the dextrose $\left(\frac{D}{L}\ \textit{increases}\ \text{from 6·0 to 9·1}\right)$ and the value of Δ increases to + 41 (Table VIII). Between 6 p.m. and midnight laevulose appears to fall little, but the dextrose rapidly, at the same

time Δ changes from + 41 per cent. to a negative value − 23 per cent. After midnight the apparent dextrose suddenly begins to rise again and the laevulose to fall; as would be expected the value for Δ becomes less negative (changes from − 23 to − 7·4).

As showing the gradual transference of the optically active impurities from the tops to the bottoms of the stalks, it is interesting to compare the values in Table VIII for say 6 a.m. with those for 12 noon. The impurity in the 6 a.m. tops is such as to cause Δ to have a positive value + 10·3 per cent. (invertase); at the same time, however, the bottoms have a negative value − 7·4, but at 12 noon the value of Δ for the bottoms has become positive, viz. + 1·8 (the sum of + 10·3 and − 7·4 is + 2·9). Similarly at 6 p.m. the value of Δ is negative in the tops (− 84·5) but positive in the bottoms, but at midnight the bottoms show a negative value, − 23·3; had the whole of the material causing the negative value at 6 p.m. been transferred to the bottoms, the change expected would be − 84·5 + 41·2 or − 43·3.

In the case of the stalk bottoms (Fig. 5), where the fluctuation of the apparent laevulose is relatively small, it is the dextrose curve which follows most closely the curve of total hexoses, but as pointed out in the case of the laevulose in Fig. 4 this has no real significance and is a result merely of the method of calculation.

If one compares merely the relative position of the apparent dextrose and laevulose curves in Figs. 4 and 5, dextrose seems to accumulate at the bottoms of the stalks far more than the laevulose, the values for dextrose (7·4 to 11·00 per cent.) being higher in Fig. 5 than in Fig. 4 (4·87 to 5·71), whilst the fluctuations of laevulose are smaller (0·23 to 1·8 per cent. in Fig. 5 as compared with 0 to 4·5 per cent. in Fig. 4). But from the considerations already brought forward it is clearly quite unsafe to conclude that it is actually dextrose which accumulates at the bottom of the stalks, as large quantities of other optically active substances are undoubtedly present, which cause the wide divergences between the results for cane sugar by the polarisation and reduction methods. If dextrose were the principal sugar present (in some cases it appears to be, as at 6 a.m., the sole hexose in the stalks) it would point, as assumed by Lindet, to the laevulose being largely consumed on the way from leaf to root for constructive purposes; but it would necessitate also that the saccharose in the root is built up from the dextrose being conveyed to it. This would involve a transformation in the root of dextrose into laevulose, followed by a synthesis of cane sugar from dextrose and laevulose. Whilst this operation is a possible one, it is more

likely that the actual reducing sugars in the stalk reach the root as invert sugar[1] and that the apparent predominance of dextrose in the stalks is due solely to dextro-rotatory impurities; the existence of these is clearly proved by the enormous differences found in the cane sugar estimations by the polarisation method. Until really reliable methods of determining the true proportions of dextrose and laevulose have been devised it is impossible to draw any further conclusions on this point.

Series II. The considerations put forward above, correlating in Series I the apparent dextrose and laevulose values with the divergence between the cane sugar values determined by the reduction and polarisation methods, hold for the stalks and mid-ribs in Series II also[2]. The following table gives the values of Δ, and Figs. 9 and 10 of the preceding paper show the curves for dextrose and laevulose. It will be seen that, as in Series I, when the apparent laevulose increases rapidly as compared with dextrose the values of Δ become less positive; when the apparent laevulose decreases, the values become more and more negative.

Fig. 9 of the preceding paper shows the apparent variation of dextrose and laevulose in the stalks as compared with that of saccharose and the total hexoses and the variation of the ratio $\frac{D}{L}$. As in the tops of stalks in Series I the apparent dextrose rises slightly during the day (10 a.m. to 4 p.m.) but then remains practically constant until 4 a.m. next morning. The laevulose rises considerably more rapidly from 10 a.m.

[1] It is quite possible that the ratio of dextrose to laevulose in the mixture of sugars reaching the root is not strictly 1, owing to one of the sugars being put more under contribution for purposes of growth or respiration in the leaves or stalks than the other. But it is probable that the ratio is very nearly unity as is the case in the leaves (September and October), when the amount of optically active impurities interfering with the determination is a minimum.

[2] The same principle can be applied to the leaves of Series II and III to explain the fluctuations of Δ, i.e. the difference between the results found for cane sugar by double polarisation and by reduction, which are far less marked in the case of the leaves than with mid-ribs and stalks, because the proportion of optically active impurities is relatively less. In practically all cases when the apparent dextrose increases faster than the apparent laevulose, the divergence becomes increasingly positive; when the laevulose increases faster than dextrose the divergence becomes more negative. As pointed out on p. 339, when $\frac{D}{L}$ is unity there is the closest agreement between the results for cane sugar obtained by the two methods, and the departure of the ratio $\frac{D}{L}$ from 1 is probably merely apparent and not real.

to 4 p.m. then falls until 11 p.m., when a second rise occurs. In the mid-ribs (Fig. 10 of preceding paper) the reverse is the case, the dextrose being nearly constant during the day and falling at night, whilst the laevulose falls by day and increases by night.

TABLE IX.

Divergence of Results for Saccharose in Stalks by Polarisation Method. Series II. September 10th–11th, 1912.

Time	% saccharose found				Polarisation results high by % (Δ)		$\frac{D}{L}$ (xylose)
	Citric inversion		Invertase inversion				
	Reduction	Polarisation	Reduction	Polarisation	Citric inversion	Invertase inversion	
10 a.m.	5·25	5·82	4·39	—	+10·7	—	3·33
4 p.m.	5·75	4·70	4·78	6·59	−17·9	+35·0	2·34
11 p.m.	5·18	8·26	—	6·67	+60·0	—	4·56
4 a.m.	5·34	5·38	5·10	6·45	+ 0·8	+26·5	3·30
6 a.m.	5·25	5·68	4·88	4·82	+ 8·4	+ 1·9	2·84

SUMMARY.

1. It is shown that in the extracts of mangold leaves and stalks optically active impurities are always present which are not precipitated by basic lead acetate and hence vitiate the estimation of the dextrose and laevulose. These substances are possibly acid amides (such as glutamine and asparagine) or amino-acids (such as glutamic and aspartic acids) which form soluble lead salts.

2. These impurities occur in the leaves, but are much more abundant in the mid-ribs and stalks.

3. In the leaves the dextrose and laevulose appear to be present in approximately equal amount, as would be expected if they were formed from saccharose by inversion. When the ratio $\frac{D}{L}$ departs from unity it is probably owing to the presence of a dextro-rotatory impurity (glutamine?) which increases the amount of dextrose apparently present; but at certain times of the day a laevo-rotatory impurity seems to predominate so that the ratio $\frac{D}{L}$ becomes less than unity.

4. In the mid-ribs and stalks, especially at the bottoms of the latter, the dextrose always appears to be in very large excess as compared with the laevulose; this is probably due to the proportion of the dextro-rotatory impurity being relatively greater in these parts than in the leaf, as is shown by the divergences between the polarisation and reduction values of saccharose being far greater.

5. The apparent fluctuations in the *ratio* of dextrose to laevulose are probably due to fluctuations in the optically active impurities rather than to variations in the sugars themselves. This is shown by the fact that these fluctuations can be correlated with the differences between the cane sugar values as determined by reduction and polarisation. When the apparent laevulose increases faster than the dextrose the results for cane sugar obtained by polarisation are *lower* than the reduction values; when the apparent dextrose increases faster than the laevulose or the laevulose falls more rapidly than the dextrose, the polarisation results are in excess of the true values.

6. The fluctuations of the apparent dextrose and apparent laevulose take place more or less regularly during the 24 hours; this points to a regular variation in the optically active impurities.

7. In the *leaves* the values of saccharose obtained by the double polarisation method are almost always *higher* than the reduction values; in the stalks, however, they are sometimes very high and sometimes very low. This is probably due to the presence of at least two different optically active substances at different times of the day. The increase of the apparent laevulose corresponds with the increase of the substance causing low results for cane sugar by the double polarisation method; the increase of apparent dextrose corresponds with a falling off of this substance and the formation of the impurity which gives high results.

8. Until more reliable results can be obtained for the true dextrose and laevulose by methods which are independent of the polarimetric data, it seems justifiable, from the results brought forward, to assume that the dextrose and laevulose exist in the leaves and stalks as invert sugar and travel in nearly, if not exactly, equal proportions to the root, where retransformation into saccharose occurs. This assumption best agrees with the regular rise and fall of the total hexoses in the stalks and mid-ribs along almost straight lines during the night, as contrasted with the more irregular fluctuation of the apparent dextrose and laevulose taken separately.

9. It is impossible in the present state of our knowledge to draw any conclusions from the proportion of apparent dextrose or laevulose

in plant tissues as to whether either of these sugars is better adapted than the other to tissue formation or to respiration. All such conclusions in the past are valueless because the analytical methods at present existing do not give true values for these sugars.

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