STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS.

III. THE CARBOHYDRATES OF THE LEAF AND LEAF STALKS OF THE POTATO. THE MECHANISM OF THE DEGRADATION OF STARCH IN THE LEAF.

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In the first two papers of this series (pp. 255–351) we have dealt with the carbohydrates of the mangold, a plant which, like its near relation the sugar beet, stores only saccharose in its root. One of the most striking features of this plant is that it forms no starch in the leaf, except during the very earliest stages of growth when it is a seedling; it is only during this period, when the root is very small and has not developed sufficiently to store the sugars formed, that starch appears in the leaf at all. When the mangold has begun to develop a large storage reservoir in the root and the sugar can be readily translocated away so that all danger is avoided of too high a concentration in the leaf, starch ceases to be produced, and during the whole of the growth in August, September, and until the roots are lifted at the end of October, it is entirely absent from the leaf. Maltose too is entirely absent. In these respects the mangold, although a dicotyledon, resembles monocotyledonous plants such as the onion (*Allium cepa*) and snowdrop (*Galanthus nivalis*), which do not form starch in the leaf although they store both starch and inulin in the bulb; in many respects, as we have shown, the phenomena of formation and translocation of sugars in the mangold are similar to those observed by Parkin [1912] in the

[1] Mr A. J. Daish, who shared our earlier work, would have taken part in this investigation had not his military duties, after the outbreak of war, rendered it impossible. He assisted us during the heavy work of the 24 hours picking of July 16–17th, 1914, and we wish here duly to acknowledge this.

snowdrop. It appears, however, that in the later stages of growth (September and October) certain gummy substances, which were not studied in any detail, are formed as a reserve in the leaf tissue and appear to be broken down to sugars at night, thus playing a similar part to the starch in most foliage leaves.

In view of the fact that we found, by using our method of estimating maltose by maltase-free yeasts, that maltose is entirely absent not only from the leaf and stalks of the mangold, which does not store starch, but also from the leaves of many other plants which form an abundance of starch, we considered it desirable, in order to test Brown and Morris' views [1893] as to the part played by diastase during the night in breaking down the starch to maltose, to study the variation of the carbohydrates in the potato leaf throughout a complete 24 hours period. The potato forms considerable quantities of starch in its leaf and if, as seemed possible, maltose is an intermediate stage in the synthesis of starch, just as it is in its degradation by enzymes, it should appear in the leaf, at least in small quantities, during the day; if the starch is broken down by ordinary diastase in the way suggested by Brown and Morris, maltose should appear in increasing quantities at night during the disappearance of the starch from the leaf. Finally, if Brown and Morris' view [1893, p. 673] is correct that maltose is the translocation form of starch, maltose should be found in the stalks.

Our experiments (see Tables I and II, p. 366) show that maltose is entirely absent from the leaf and stalks of the potato at all periods of the day and night. We have now made nearly 500 analyses by means of maltase-free yeasts of many different kinds of plants, including the nasturtium (*Tropæolum majus*), turnip, carrot, sunflower (*Helianthus annuus*), dahlia, *Arum maculatum* and vine (*Vitis vinifera*); *in no case has maltose been found either in the leaf or stalks*, even in such plants as the turnip or nasturtium which store very large quantities of starch in the leaf[1]. We need here only refer to the data given in a previous paper (Davis and Sawyer [1914]) for the quantitative fermentations carried out with the alcoholic extract of the turnip leaf (starch = 12·77 per cent. of the total vacuum-dried leaf). In order to work with as

[1] In one case (July 9th, 1913) the leaf of the turnip was found to contain 18·73 per cent. of starch calculated on the vacuum-dried matter left after extracting the sugars, etc., with alcohol; this calculated on the total vacuum-dried matter of the leaf, including the sugars, becomes 12·79 per cent. In a sample of *Tropæolum* leaf (July 4th, 1913) the starch formed 26·75 per cent. of the dry leaf after extraction, or 17·6 per cent. of the total vacuum-dried matter of the original leaf.

large a quantity of substance as possible 1 litre of the purified aqueous solution of the sugars (a quantity which represented 44·69 grms. of the total vacuum-dried leaf matter) was evaporated *in vacuo* to 175 cc. and made up to 250 cc. Three portions of 50 cc. each were fermented during 3 weeks to 1 month with *S. marxianus* and *S. exiguus*, and after treatment with alumina cream made up to 100 cc. Two duplicate fermentations were carried out with a pure culture of distillery yeast.

50 cc. of the filtrate (representing 4·469 grms. of T:V.D.M.) gave in the case of the *S. marxianus* and *S. exiguus*, 0·0524 grm. CuO; and gave in the case of the distillery yeast, 0·0500 grm. CuO.

These values are practically identical and well within the range of error of the method. They show that *maltose was entirely absent from the turnip leaf* in question. The reducing power, as pointed out [1914], is to be attributed to unfermentable *pentoses*; it corresponds with 0·51 per cent. of pentose; 0·60 per cent. was found by the ordinary phloroglucinol method when applied directly to the solution containing the sugars, prior to fermentation.

Brown and Morris, in their important memoir, gave what was undoubtedly good evidence of the presence of maltose in their extracts of *Tropæolum* leaf. They were not content merely with the analytical data but endeavoured "in view of the immense importance which must necessarily be attached to this product of starch hydrolysis to obtain more direct evidence of its presence." They succeeded in isolating an osazone, apparently identical with maltosazone, from the solution of the mixed sugars contained in a large quantity of the dry leaves of *Tropæolum*, and analysed it. Finally they showed the presence of maltose by treating a solution of the mixed sugars of the leaf, after completely inverting the saccharose, with a preparation of the enzyme maltase. This enzyme always brought about a large increase in the cupric reducing power, amounting generally to about 75 per cent. of the increase observed on digesting the same solution with dilute acid.

It is impossible in view of these facts to doubt that maltose was present in the material worked with by Brown and Morris. It is, however, possible to reconcile these results with our own by taking into account the difference between the methods of extraction of the sugars adopted by Brown and Morris and by ourselves. We have been led to conclude that plant leaves which store starch contain in addition to the enzymes of ordinary diastase (a fact which was first definitely proved by Brown and Morris) the enzyme *maltase*, which is capable of breaking down maltose to dextrose. We have shown in a previous paper

(Davis and Daish [1914]) that taka-diastase, the mixture of enzymes isolated from *Aspergillus oryzae*, differs from the ordinary diastase of malt extract mainly in containing maltase in addition to the ordinary starch resolving enzymes. Taka-diastase therefore converts starch paste completely into a mixture of maltose and dextrose, the latter rapidly increasing in amount until 80–85 per cent. of the sugar is in this form. We consider that the ordinary foliage leaf contains a mixture of enzymes similar to that elaborated by *Aspergillus oryzae*, and of such a nature that the maltase is always present in relative excess so that the maltose formed by the breaking down of the starch is very rapidly and completely converted into dextrose. Now in our method of preparing the leaf samples for analysis, the material was dropped into boiling alcohol containing a little ammonia, so that the enzymes were destroyed instantly, but Brown and Morris and most other workers in this field *dried their leaf material in an oven before extracting the sugars*. During this drying, owing to the large quantity of moisture in the leaf, the temperature only rises gradually and the enzymes continue to act for a considerable time before they are destroyed. Maltase is the first of the enzymes to be put out of action; it is well known to be one of the most unstable of enzymes. Our experiments (Davis, 1914, 1) with taka-diastase show that it is largely destroyed before a temperature of 55° is reached. When leaves are dried in an oven, after the maltase has been destroyed at say 50° C., the ordinary diastatic enzymes continue to act under optimum conditions as regards temperature and considerable quantities of starch are broken down to dextrin and maltose. This action lasts until the temperature rises to about 80°, when the dextrin and maltose-forming enzymes are also destroyed. As the maltase has been completely killed in the earlier period of drying, the maltose formed in this way will persist as such and be found in the mixture of sugars subsequently extracted from the dried material.

In support of this explanation of the differences between Brown and Morris' results and our own, several facts may be adduced. In Brown and Morris' experiments the proportion of starch in the freshly plucked nasturtium leaf at the end of a sunny day was found to range between 2·9 and 7·4 per cent. of the total dry matter; we have always found the starch in the same leaf to be considerably higher, thus in the example given on p. 353, footnote, the starch was 17·6 per cent. of the total vacuum-dried matter or about two and a half times the highest figure given by Brown and Morris. In one case cited by these workers the starch was found to be 4·59 and the maltose 5·33 per cent., the sum of the two being

9·9 per cent.; this is still considerably lower than the values we have found. Kluyver [1914] recently on repeating Brown and Morris' experiments with *Tropæolum*, but using a new biochemical method of estimating the hexoses, saccharose and maltose[1], based on the differences in the amount of carbon dioxide evolved on fermenting the solution with special *torulae* and with ordinary yeast, also found in all cases relatively very small amounts of maltose. Thus in one case, which is of special interest because the hexoses and cane sugar are nearly identical with values found by Brown and Morris, Kluyver found in leaves plucked at 2.30 in the afternoon, hexoses = 5·2 per cent., saccharose = 4·6 per cent., *maltose* = 0·3 per cent.; this compares with Brown and Morris' analysis, hexoses = 5·6 per cent., saccharose = 4·9 per cent., *maltose* = 1·2 per cent. In the remaining cases which Kluyver cites the results are given merely relatively, without calculating back on the dry matter of the leaf: but, in every instance, the proportion of maltose found was exceedingly small as compared with the saccharose and hexoses. Thus, for example, a sample of *Tropæolum* leaf picked at 4 p.m. on July 28th, and therefore corresponding with an analysis in which Brown and Morris found saccharose 8·02 per cent., maltose 3·62 per cent., and in which maltose formed 27·5 per cent. of the total sugars, gave

Saccharose	...	25·8 mgrms.
Hexoses	...	21·8 ,,
Maltose	...	1·6 ,,

The maltose found here forms only 3·2 per cent. of the total sugars. In Brown and Morris' experiments the maltose always appeared to be at a maximum at the end of the afternoon, that is at the same time as the starch reached its highest values; for example, in one case (p. 669) a leaf picked at 5 p.m. when the starch formed 4·59 per cent. of the dry leaf, maltose was found to be present to the extent of 5·33 per cent., *and to form* 56 *per cent. of the total sugars.*

These results are fundamentally different from Kluyver's obtained at the same time of day and the difference is probably to be explained

[1] *Torula monosa* does not contain the enzymes maltase and invertase, and hence is capable of fermenting the hexoses only, leaving the maltose and saccharose unchanged; *Torula dattila* contains invertase but not maltase, and therefore ferments cane sugar and the hexoses but not maltose. Dr A. J. Kluyver has been kind enough to send us pure cultures of these Torulae, which Dr H. Limbosch has tested for us in our laboratory, according to our own methods of working, on very carefully purified specimens of sugars. We can confirm Kluyver's statements as to the specific nature of these organisms which should prove of considerable service in sugar analysis of the kind we have had to deal with.

by the fact that in Brown and Morris' experiments the heating up of the leaves in drying was much slower and allowed far more diastatic action to occur than in Kluyver's experiments. Kluyver especially points out that his leaves, which were dried in thin layers in a baker's oven heated to 105°, were exposed to the drying process during only 5 to 10 minutes. We have ourselves made several experiments with *Tropæolum* leaves dried *rapidly* in a steam oven and by our own methods have invariably found *no* maltose to be present, just as in the case of the same leaves dropped into boiling alcohol.

From the above facts we have concluded that the maltose which was undoubtedly present in Brown and Morris' experiments in relatively large amounts and in Kluyver's experiments in far smaller proportions owing to the greater rapidity of drying, was not formed in the tissue of the leaf as such during growth, but was produced by the degradation of starch by the diastatic enzymes remaining after the maltase in the leaf had been destroyed in the first stage of the drying process. As regards the mechanism by which starch is utilised in the plant when, at the end of the day, the reserves in the leaf are called upon, *we consider that the starch is hydrolysed completely to dextrose by the leaf enzymes*, which resemble the enzymes of *Aspergillus oryzae* in containing an abundance of maltase. Brown and Morris' main view that the starch is utilised by a purely enzymic process seems to us perfectly correct, but we regard the enzymic degradation as stopping, not at maltose, as supposed by Brown and Morris, but at the stage of dextrose, the final product of starch hydrolysis. One of us has shown (Davis, *Chemical World*, 1914, p. 271) that yeasts which do not contain the enzyme maltase, for example, *S. anomalus* and *S. exiguus*, are quite unable, even when in the throes of starvation, to make use of maltose in the solution in which they are growing; similarly we find that *Torula monosa*, which does not contain invertase, is unable to make use even of cane sugar. Plant tissue, we consider, in exactly the same way before it can utilise starch, maltose or saccharose, for purposes of growth, must break these substances down to the simple hexoses by enzyme action. This view explains the significance of the fact that the sugars in the stalks of all the plants we have examined consist largely of the simple hexoses; these sugars are capable of being directly assimilated by the cambium layer of the stems or by other growing points. The necessity of transformation of saccharose into invert sugar thus explains the almost ubiquitous presence of invertase in the plant, except in such storage reservoirs as the mangold root, where cane sugar is permanently housed.

The views we put forward are in accord with modern views, based largely on the work of Abderhalden and his school, as to food assimilation by animals; in all cases it is necessary for such food, for example, proteins, to be broken down by enzymes into its simplest components or "*Bausteine*," which are then taken up by the different cells or tissues and synthesised afresh.

The theory we have given of the method by which starch is broken down in the leaf would lack justification unless definite evidence of the presence of maltase in leaf tissue could be brought forward. At the suggestion of one of us, Mr A. J. Daish has made a special study of this question. In a series of experiments, details of which will be published later, he has found that maltase is always present in the leaf tissue he has examined when starch is also present. Little doubt therefore can be entertained of the correctness of the view we put forward that starch is broken down in the leaf to dextrose. The fact that maltose can never be detected either in the leaf or stalks of plants points to the amount of maltase always being in relative excess in the cells where the starch degradation actually occurs, so that it is able to deal instantly with the whole of the maltose formed from the starch. The fact that maltose, unlike cane sugar, never occurs in the stalks or conducting vessels is probably due to the fact that maltase is an intracellular enzyme and apparently acts in close collaboration and in the immediate proximity of the ordinary diastase which first attacks the starch in the cells where this substance is stored.

Cane sugar is apparently the first sugar formed in the potato leaf and is transformed into hexoses for translocation.

The most striking point which appears when the analyses of the potato leaves and potato stalks are compared (see Tables I and II) is that whereas *the saccharose is greatly in excess of the hexoses in the leaf, the reverse is true in the stalks.* These results are exactly similar to those obtained with the mangold leaf in the early stages of growth (Series I), a fact which points to the mechanism of formation and translocation being the same in both cases. Saccharose is probably the first sugar formed in the mesophyll of the leaf; it is gradually inverted on its way through the veins, mid-ribs, and stalks, the inversion becoming more and more complete as the root or tuber is approached. In this series of pickings it must be borne in mind that the "stalks" were mainly those bearing the small leaflets and did not include any of the stem

in the neighbourhood of the tuber where, by analogy with the mangold and snowdrop (Parkin [1912]), the hexoses would be found probably to preponderate even more than is shown in Table II. Time has allowed us only to take one series of pickings with the potato, but it seems highly probable that, as in the case of the mangold, sugar beet, and snowdrop, the proportion of hexoses to saccharose becomes greater and greater in both leaf and stalk as the season advances, and the storage function becomes more and more predominant.

As regards the transformation of the hexoses into starch in the tuber, it is interesting to note that in this way the hexoses are as it were imprisoned and held until required for later use, when the appropriate enzymes again degrade the starch to sugars. In the mangold the imprisonment of the hexoses in the root is effected by their transformation into cane sugar.

From data which we have obtained with many other plants, to be published later, it appears that cane sugar is produced, generally in a predominant proportion, in the leaf of *all* plants, whatever be the form in which the sugars are finally stored (cane sugar, starch, inulin or dextrose). Thus, for example, we find that, when proper precautions are taken to prevent enzymic change, contrary to Deleano's [1912] recent statement, cane sugar is the principal sugar of the vine leaf (*Vitis vinifera*). In this plant the storage form is dextrose, and unless the cane sugar is a primary product of the mesophyll tissue it is difficult to see any special reason for its predominance in the leaf. If dextrose and dextrose alone were, according to Strakosch's [1907] views, the direct product of photosynthesis, one would expect to find it the principal if not the sole sugar in the leaf of a plant which stores dextrose as its reserve carbohydrate. In fact, as stated in our previous paper (I), all the data we have obtained with plants of many different kinds best harmonise with the view put forward by Brown and Morris [1893], that saccharose is the first sugar formed in photosynthesis and that the hexoses are formed from it and not *vice versa*. It seems to be the general *function of the mesophyll tissue to elaborate saccharose directly*; this is broken down in the veins, mid-ribs and stalks, and reaches the place of storage in the form of hexoses. Unless saccharose is a primary product it is difficult to see why it should predominate in the leaves of plants of such different types as the potato, the vine, sunflower and snowdrop, in none of which is cane sugar the storage form; there seems, indeed, no useful purpose in its production at all in such cases, as the substances stored are undoubtedly built up from hexoses, which are the

predominating constituents of the sap in the *stalks*, and could very well be translocated directly as such from the leaf. It is possible, and may be argued, that the saccharose in the leaf serves to regulate the osmotic pressure, owing to the ready interconversion of saccharose and hexoses; but in plants which form starch, such as the potato, this regulation could be quite as well effected by the precipitation of the polysaccharide and the function of the cane sugar is not easily understood unless it be regarded as a primary and compulsory product of the mesophyll.

The Dextrose-Laevulose Ratio. As was the case in the mangold leaf, it is shown that it is impossible to obtain accurate values for dextrose and laevulose owing to the presence in the solutions of optically active impurities which are not removed by the ordinary process of defecation by basic lead acetate. These impurities also interfere with the estimation of the saccharose by the double polarisation method and, as in the mangold, the fluctuations of the apparent dextrose and laevulose can be correlated with the divergences between the values found for saccharose by the reduction and by the optical methods (see p. 344). It appears that *two* optically active impurities with rotations of opposite sign are formed at different periods of the 24 hours, and it is the variation of these that causes the apparent fluctuations in the proportion of dextrose and laevulose. In the leaf a substance with a laevo-rotatory power generally predominates, so that the laevulose appears to be greatly in excess of the dextrose; but in the stalks this is no longer the case and dextrose appears to be largely in excess of the laevulose.

Experimental.

The methods of sampling, extraction, and analysis were the same as those described in the case of the mangold (see Paper I). The potatoes (King Edward VII) were grown on a piece of ground at the side of the laboratory; at the date of picking (July 16th–17th, 1914) the plants were just beginning to form flower buds and the tubers were small. Rain had fallen heavily on July 12th, but the days following were dry and sunny. Pickings were taken every two hours. The leaflets were detached from the rachis, but the mid-ribs of these leaflets were not cut out so that the results given for "leaves" refer to the whole leaflets including these mid-ribs; what we have called "stalk" consisted in reality mainly of the rachis of the compound leaves and included only a small portion of the main stalk or stem, namely the portion furthest from the tubers.

Estimation of Starch and "Soluble Starch."

The dried potato leaf obtained after completely extracting the sugars and other substances soluble in 80 per cent. alcohol was found to contain large quantities of a substance readily soluble in water and possessing a high positive rotation. This made it necessary to modify the method of estimating starch which we have employed (Davis and Daish [1914]) by first completely extracting this substance with water from the portion of material used in the analysis. At certain times of the day (4 p.m. to 8 p.m.) the aqueous extract so obtained contained a substance which resembled soluble starch or dextrin in yielding a mixture of maltose and dextrose on treatment with taka-diastase. In all cases the reducing power (if any) and rotatory power of the aqueous extract were determined after diluting to a known volume (250 cc.); an aliquot portion (150 cc.) was then treated with taka-diastase, and, after the conversion, with basic lead acetate (which generally produced a copious precipitate owing to the presence of tannins, etc.), being then diluted to a known volume (200 cc.). The reducing and rotatory powers of the solution were determined and from the change in these brought about by the taka-diastase the "soluble starch" (or dextrin) was calculated. In most cases the "soluble starch" was *nil*, but between 4 p.m. and 8 p.m. considerable quantities could be detected. Even in these cases, however, the amount of soluble starch found in this way did not account for more than 25 to 50 per cent. of the rotation observed in the aqueous extract; in all cases, too, the basic lead acetate added after the conversion produced a heavy, gelatinous precipitate, pointing to the presence of tannins, gums, etc. The aqueous extract before conversion invariably had a slight cupric reducing power (50 cc. of the 250 cc. gave 0·01 to 0·02 grm. CuO) which may perhaps have been due to unextracted sugars; but as in the experiments with mangold leaves, the extraction of sugars was always complete, it is probable that the reduction was due to a substance of the tannin class. For purposes of comparison we give in Table I the actual values calculated for the rotation $(\alpha)_D$ in a 200 mm. tube of the aqueous extract of the leaf material corresponding with 100 grms. of the *total vacuum-dried matter* of the leaf (including the sugars and alcohol soluble substances). The value is also given for the "soluble starch" ($[\alpha]_D = 202°$) that this would correspond with, calculated as a percentage on the total vacuum-dried matter. Thus a comparison can be made of the true soluble

starch found by the taka-diastase and the value found in this way from the rotation.

The true *starch* in the leaf was estimated by treating with taka-diastase the leaf material remaining after extraction with water; this was, of course, first gelatinised by boiling water in the usual way.

The following is an actual example showing the method of analysis and calculation.

Potato Leaf, 8 p.m., July 16th, 1914.

10·7148 grms. of the dried powdered leaf material remaining after the extraction of sugars[1], dried at 110° *in vacuo* until the weight was constant gave 9·8405 grms.; the moisture present therefore = **8·16** per cent.

The vacuum-dried weight of matter soluble in alcohol for this picking was 59·25 grms.

The weight of oven-dried matter (fibre, starch), etc., left after extraction with alcohol was 111·55 grms.; as the moisture in this was 8·16 per cent., the vacuum-dried weight = 102·42 grms.

The total vacuum-dried matter (T.V.D.M.) therefore

$$= 102{\cdot}42 + 59{\cdot}25 \text{ grms.} = \mathbf{161{\cdot}67} \text{ grms.}$$

The 9·8405 grms. of leaf substance was transferred to a beaker flask of 250 cc. capacity and left with about 200 cc. of water and 2 cc. of toluene for 24 hours at 38°, stirring well at intervals. The clear solution was then decanted through a starch-free filter paper as completely as possible and the residue washed by decantation until the volume in the flask was 250 cc.

Aqueous extract. 50 cc. of the 250 cc. gave 0·0217 grm. CuO.

Polarisation in 200 mm. tube = + 0·358° (sodium flame, 20°). This rotation calculated as 100 grms. of vacuum-dried extracted leaf

$$= \frac{0{\cdot}358 \times 2{\cdot}5 \times 100}{9{\cdot}8405} = 9{\cdot}10°.$$

Calculated on 100 grms. of T.V.D.M. $= \dfrac{9{\cdot}10 \times 102{\cdot}42}{161{\cdot}67} = \mathbf{5{\cdot}76°}$.

Calculated as soluble starch ($[\alpha]_D = 202°$) per 100 grms. of T.V.D.M.

$$= \frac{0{\cdot}358 \times 2{\cdot}5 \times 10^4 \times 102{\cdot}42}{2 \times 202 \times 9{\cdot}8405 \times 161{\cdot}67} = \mathbf{1{\cdot}43} \text{ per cent.}$$

[1] This had been dried in an oven at 100°, ground in a mill and kept in a stoppered bottle until the analysis was made. For precautions in sampling this material see Davis and Daish [1914], p. 161.

"Soluble Starch" (or dextrin) in Aqueous Extract.

150 cc. of the 250 cc. were left with 0·1 grm. of taka-diastase and 1 cc. of toluene for 24 hours at 38°; to the solution 5 cc. of basic lead acetate solution were then added, which was *just* sufficient to precipitate the whole of the tannins, gums, etc. The solution was diluted to 200 cc. at 15° and filtered; the slight excess of lead in the filtrate was *exactly* precipitated by adding solid sodium carbonate and the reducing and rotatory powers of the filtrate determined.

50 cc. of the 200 cc. gave 0·0718 grm. CuO.
Rotation in 400 mm. tube at 20° = + 0·202°.

Correcting for 0·1 grm. taka-diastase, under exactly similar conditions (correction for CuO = 0·0360 grm.; for polarisation = + 0·106°), we have

CuO due to sugars present = + 0·0358 grm.
Polarisation due to sugars present = + 0·096°.

It is necessary to correct for the reducing power and polarisation of the original solution; for the reducing power we have

$$\frac{150}{200} \times 0{\cdot}0217 = 0{\cdot}0163 \text{ grm.}$$

As to the rotatory power, measurements made with the various pickings in which "soluble starch" was entirely absent showed that if the reducing substances be assumed to be sugars, with a cupric reducing power 2·5 grms. CuO per grm., they had the specific rotatory power $[\alpha]_D^{20^\circ} = + 25°$. The assumption that this is the case when the soluble starch is present will give no sensible error; we have therefore α_n due to these substances in a 400 mm. tube

$$= \frac{0{\cdot}0163}{2{\cdot}5} \times \frac{25 \times 400 \times 2}{10^4} = + 0{\cdot}013°.$$

We have therefore as final values for maltose and dextrose formed by the diastase conversion:

CuO ex 50 cc. = 0·0358 − 0·0163 = 0·0195 grm.
Polarisation in 400 mm. tube = 0·096 − 0·013 = 0·083°.

If x = dextrose in 50 cc.; y = maltose in 50 cc.,

$$2{\cdot}58x + 1{\cdot}38y = 0{\cdot}0195$$
$$4{\cdot}22x + 11{\cdot}01y = 0{\cdot}0830$$

Solving, $x = 0{\cdot}00443$ grm.; $y = 0{\cdot}00586$ grm.

The total dextrose corresponding with leaf material taken

$$= 0{\cdot}00443 \times \frac{200}{50} \times \frac{250}{150} = 0{\cdot}0296 \text{ grm.}$$

The total maltose corresponding with leaf material taken

$$= 0{\cdot}00586 \times \frac{200}{50} \times \frac{250}{150} = 0{\cdot}0391 \text{ grm.}$$

"Soluble starch" corresponding with dextrose

$$= 0{\cdot}0296 \times 0{\cdot}9 \quad = 0{\cdot}02664 \text{ grm.}$$

"Soluble starch" corresponding with maltose

$$= 0{\cdot}0391 \div 1{\cdot}055 = 0{\cdot}0371 \text{ ,,}$$

$$\text{Total} \quad = 0{\cdot}0637 \text{ ,,}$$

Percentage of "soluble starch" $= \frac{0{\cdot}0637}{9{\cdot}8405} \times 100 = 0{\cdot}65.$

∴ Percentage of soluble starch on T.V.D.M. in leaf

$$= \frac{0{\cdot}65 \times 102{\cdot}42}{161{\cdot}67} = \mathbf{0{\cdot}41}.$$

True starch. The leaf material remaining after the extraction with water was gelatinised by heating with about 200 cc. of water during 30 minutes in boiling water; after cooling, 0·1 grm. taka-diastase was added and 2 cc. of toluene and the mixture left 24 hours at 38°, stirring at intervals. Two drops of concentrated sodium hydroxide were then added to destroy the enzyme and the solution filtered from the leaf material on a Buchner funnel; this was thoroughly washed with water by decantation until the total volume of the filtrate was about 475 cc. Basic lead acetate was then added (2·5 cc. was generally just sufficient) and the volume made up to 500 cc. The slight excess of lead in the filtrate was removed by adding *exactly* the necessary quantity of solid sodium carbonate and after filtering the reducing power and rotation of the solution were determined.

50 cc. gave 0·0908 grm. CuO

Polarisation = 0·264° in 400 mm. tube at 20°.

These values corrected for 0·1 grm. of taka-diastase under exactly similar conditions (CuO correction = 0·0138 grm.; polarisation = + 0·073°) give

Corrected CuO from 50 cc. = 0·0770 grm.,

Corrected polarisation = + 0·191°.

If x = dextrose in 50 cc.; y = maltose in 50 cc.,

$$2{\cdot}58x + 1{\cdot}38y = 0{\cdot}0770$$

$$4{\cdot}22x + 11{\cdot}01y = 0{\cdot}191°.$$

Solving, $x = 0{\cdot}02587$ grm. in 50 cc.

$y = 0{\cdot}00746$ grm. in 50 cc.

Total dextrose in 500 cc. = 0·2587 grm. = 0·2328 grm. starch.

Total maltose in 500 cc. = 0·0746 grm. = 0·0707 „ „

$\therefore$ Total starch = 0·3035 grm.

Percentage of starch in the vacuum-dried extracted leaf

$$= \frac{0{\cdot}3035 \times 100}{9{\cdot}8405} = 3{\cdot}08.$$

Percentage of starch in the total vacuum-dried matter

$$= \frac{3{\cdot}08 \times 102{\cdot}42}{161{\cdot}67} = \mathbf{1{\cdot}95}.$$

SUMMARY OF ANALYSIS[1].

Rotation of aqueous extract calculated on 100 grms. T.V.D.M. in 100 cc.	= 5·76°
This represents as "soluble starch"	= 1·43 %
Actual "soluble starch" found by diastase	= 0·41 %
True starch found by diastase	= 1·95 %

[1] A duplicate analysis of this sample gave: Rotation of aqueous extract per 100 grms. T.V.D.M. = 5·58°; calculated as soluble starch this = 1·38 per cent. "Soluble starch" by diastase = 0·30 per cent.; true starch = 1·24 per cent. The duplicates here given for the true starch are not so close as is usually the case in these analyses; a more typical case (4 a.m.) gave 1·24 and 1·43 per cent. The greatest difficulty is encountered in the sampling, as was pointed out in a former paper; for each analysis the whole of the powdered leaf material, especially that at the bottom of the bottle, where the heavy starch grains tend to collect, should be turned out on a sheet of paper and 10 grms. sampled so as to represent a fair average of the whole of the material.

Results of Analyses.

Table I.

Potato Leaves, July 16th–17th, 1914.

July 16th. Sun rises 4.2 a.m. Sun sets 8.10 p.m. July 17th. Sun rises 4.4 a.m.

Time	Temp. ° F.	% T.V.D.M. soluble in alcohol	Saccharose % on T.V.D.M.				Hexoses as invert sugar %	I.S./C.S.	Pentose %	Pentosan %	Maltose %	Aqueous extract		Soluble starch %	True starch %	Remarks
			Citric acid	Invertase	Δ = C.A. − I.	Average						α_D per 100 grms. T.V.D.M.	α_D calc. as sol. starch %			
6 a.m.	57	37·2	2·16	2·11	+0·05	2·14	0·40	0·19	0·35	5·72	0·00	6·02°	1·49	0·00	1·88	Sunny
8 a.m.	60	39·1	2·47	2·60	−0·13	2·53	1·00	0·39	0·37	5·37	„	8·32	2·06	„	2·00	Sunny
10 a.m.	61	38·5	2·81	2·65	+0·16	2·73	0·37	0·14	0·52	5·30	„	3·36	0·83	„	2·55	Slightly overcast
12 noon	62	39·3	3·39	3·19	+0·20	3·29	1·21	0·37	0·43	5·35	„	5·06	1·24	„	1·40	Shower 1.30
2 p.m.	63	34·1	3·81	3·50	+0·31	3·66	0·67	0·18	0·44	5·40	„	5·30	1·31	„	1·81	Shower 3.30
4 p.m.	63	36·2	3·56	3·34	+0·22	3·45	0·93	0·27	0·42	5·33	„	8·88	2·20	0·58	1·56	Very bright
6 p.m.	64	35·8	3·46	3·22	+0·24	3·34	1·27	0·38	0·46	5·42	„	8·96	2·22	1·00	5·95	Bright
8 p.m.	60	36·6	2·77	2·69	+0·08	2·73	1·22	0·45	0·42	5·51	„	5·67	1·40	0·36	1·61	Dark 9.15 p.m.
10 p.m.	59	34·8	2·76	2·49	+0·27	2·63	0·40	0·15	0·45	5·35	0·00	5·02	1·24	0·00	2·60	
12 night	56	36·6	2·48	2·30	+0·18	2·39	0·73	0·30	0·44	5·60	„	8·32	2·05	„	0·24	
2 a.m.	55	37·5	2·38	2·26	+0·12	2·32	0·68	0·29	0·37	5·74	„	7·59	1·88	„	0·28	
4 a.m.	53	37·7	2·09	1·44 (?)	+0·65 (?)	1·76	0·15	0·08	0·43	5·70	0·00	5·71	1·42	0·00	1·33	1st light 3 a.m.

Table II.

Potato Stalks. July 16th–17th, 1914.

Time	Sugars in leaf %		% of stalk soluble in alcohol	Saccharose % on T.V.D.M.				Hexoses as invert sugar %	I.S./C.S.	Pentose %	Pentosan %	Maltose %	Aqueous extract		Soluble starch %	True starch %	
	Saccharose	Hexoses		Citric acid	Invertase	Δ = C.A. − I.	Average						α_D per 100 grms. T.V.D.M. in 100 cc.	α_D calculated as soluble starch %			
6 a.m.	2·14	0·40	35·9	3·20	3·28	−0·08	3·24	4·94	1·52	0·43	12·45	0·00	8·93°	2·21	0·00	0·10	Day
2 p.m.	3·66	0·67	39·7	3·44	3·41	+0·03	3·42	5·58	1·63	0·50	11·15	„	11·12	2·75	„	0·27	Day
8 p.m.	2·73	1·22	38·2	3·55	3·60	−0·05	3·57	5·63	1·58	0·53	12·15	„	5·73	1·42	„	0·13	Day
2 a.m.	2·32	0·68	35·2	2·61	2·70	−0·09	2·65	4·63	1·75	0·75	12·10	0·00	7·46	1·85	0·00	0·62	Night

Discussion of Results.

A. *The Relation between the Sugars and Starch of the Leaf.*

As in the mangold leaf during the early stages of growth, saccharose is the predominating sugar in the potato leaf—the curve of sac-

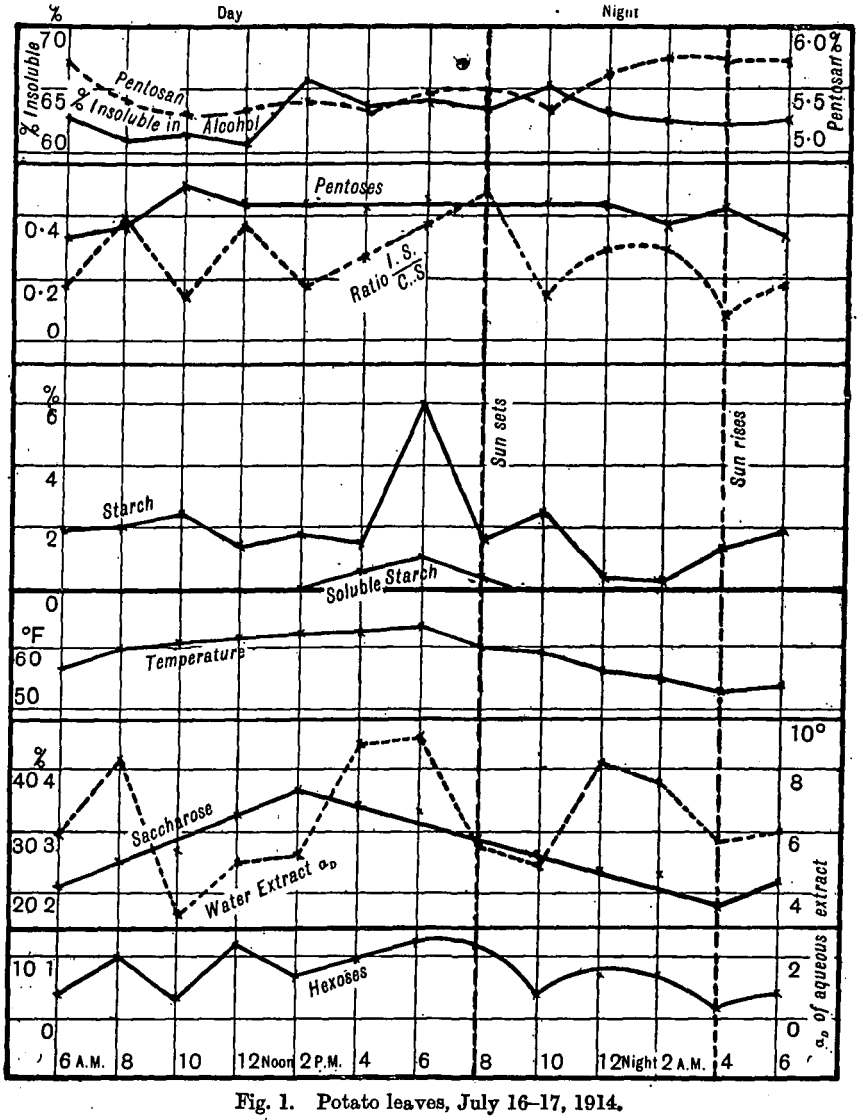

Fig. 1. Potato leaves, July 16–17, 1914.

charose in Fig. 1 is well above the curve of hexoses and is distinguished from it by its regularity. From 6 a.m. to 2 p.m. as the temperature

rises the saccharose increases along practically a straight line, which runs more or less closely parallel to the temperature curve. The maximum of saccharose is, however, reached earlier than the temperature maximum, viz. at 2 p.m.; after this the saccharose falls continuously along nearly a straight line, throughout the rest of the day and night, until sunrise next morning. The range of variation during the 24 hours is from 1·76 to 3·66 per cent.

The hexoses are present in relatively small amount and during the day fluctuate far more, and less regularly, than the cane sugar: the total variation is only from 0·4 to 1·2 per cent. The small changes in the hexoses between 6 a.m. and 2 p.m. synchronise with small changes in the starch present in the leaf, as if interconversion of these substances occurred. As will be seen later, if any reliance can be put upon the dextrose values, it is the dextrose which undergoes the greatest change (see Fig. 3). This sugar appears to fall from 8 a.m. to 10 a.m., whilst the starch increases; from 10 a.m. to noon the starch falls slightly and the dextrose increases. From 12 to 2 p.m. the starch increases and the dextrose falls almost to zero. After 2 p.m. the saccharose steadily falls whilst the hexoses increase, apparently owing to the inversion of the cane sugar, until 8 p.m. At the same time a sudden rise in the starch occurs between 4 p.m. and 6 p.m.; the starch which during the earlier part of the day had changed very little increases from 1·5 to 5·95 per cent. It is a striking fact that directly after the saccharose reaches its maximum at 2 p.m. the "soluble starch" (or dextrin) can be detected in the leaf material. This increases along a straight line until a maximum is reached at 6 p.m. which corresponds with the maximum of the starch. It is probable that the "soluble starch" is formed as an intermediate product between the hexoses (? dextrose) and the true, insoluble starch stored in the leaf. This form of starch is only to be detected in the leaf between 2 p.m. and about 9 p.m., its formation synchronising with the abnormally rapid increase of the starch, which occurs 2 or 3 hours before sunset. In this particular case, the starch stored in the leaf just before sunset is apparently very rapidly put under contribution again, as it falls in amount to about 1·6 per cent. at 8 p.m. The rapid fall of hexoses from 1·2 to 0·4 per cent. between 8 p.m. and 10 p.m. corresponds with a rise of starch from 1·6 to 2·6 per cent., whilst the fall of starch from 10 p.m. to midnight corresponds with a rise of hexoses. Between 12 midnight and 2 a.m. starch has almost disappeared from the leaf (0·2 to 0·3 per cent.), but just before sunrise, apparently in response to the first sign of daylight, the starch increases

to about 1·3 per cent., whilst the hexoses fall correspondingly. It is noteworthy that the starch appears to be formed in early morning considerably before the sugars show any increase. The slight increase of starch after sunset, between 8 p.m. and 10 p.m., at the expense of the hexoses is also very striking; at this time of day the intensity of the light was small.

Hexose-saccharose ratio. The curve showing the variation of this ratio naturally follows in its general outline the hexose curve with its abrupt changes. This is a consequence of the linear character of the saccharose curve.

Pentoses. These show a slight increase in the early part of the day, but from noon onwards are practically constant.

Pentosans and matter insoluble in alcohol. In the mangold leaf one of the most striking features was the absolute parallelism of the curves of pentosans and of matter insoluble in alcohol. This parallelism is almost entirely lost in the case of the potato leaf, apparently owing to the presence of starch and its precursors. At night, in particular, the pentosans appear to increase, whilst the matter insoluble in alcohol (including the starch) diminishes.

Rotation of the aqueous extract of the dried leaf tissue left after extracting the sugars. The curve showing the variation of the rotation of the aqueous extract of the dried leaf tissue from which all alcohol-soluble substances have been removed is probably an index of the variation of synthetical products intermediate between the hexoses and starch. Generally speaking this curve is intermediate in its character between the starch curve and the hexose curve. Table I shows that the rotation of this extract calculated as soluble starch points to the presence of considerable quantities of substances with a high *positive* rotation, which are possibly of the nature of gums but more probably are up-grade or down-grade products of starch, other than dextrin or soluble starch. They are generally not convertible into hexose by taka-diastase; it is only between 4 p.m. and 8 p.m. that a small quantity of a substance which is so convertible appears in the leaf. Even when this is present the rotation of the aqueous extract is from 2½ to 4 times that corresponding with the "soluble starch" actually found.

During the early part of the day up to 12 noon the curve of the rotation, α_D, is more or less parallel with the hexose curve[1]; as the

[1] It must be borne in mind that the two curves (hexose and rotation curves) apply to different portions of the material analysed: the hexoses are estimated in the alcohol-soluble extract, whilst the rotation curve refers to the *aqueous* extract of the material left after all the substances soluble in alcohol have been removed.

hexoses rise so does the rotation of the aqueous extract and when the hexoses fall abruptly, as between 8 and 10 a.m., *when the starch increases*, the rotation also falls very greatly. From 10 a.m. to 2 p.m. the general character of the hexose and rotation curves is similar as regards rise and fall; from 2 p.m. onwards, when the cane sugar falls and the hexoses increase, there is a rapid rise of the rotation curve, which seems to follow more or less the formation of "soluble starch" and starch. The rotation curve reaches a maximum at the same time (6 p.m.) as the hexoses, soluble starch and true starch, and then falls abruptly, just as the starch curves fall, between 6 p.m. and 8 p.m. At night the rotation curve follows, on an exaggerated scale, the curve of hexoses and is the inverse of the starch curve.

The intimate relation existing between the three curves under discussion, which show the variation of the hexoses, starch and rotation of the aqueous extract of the sugar-free leaf, points to the starch and hexoses being readily interconvertible. The substance with high positive rotatory power which appears so intimately related to the starch and hexoses may either be an intermediate product in the synthesis of starch (other than dextrin or soluble starch) or a substance such as a protein or gum, with a high positive rotation, which stands in close causal relationship with this synthesis. In the present state of our knowledge it is useless to offer further conjectures.

B. *The Stalks and the Translocation of the Sugars.*

As in the mangold stalks, the saccharose remains practically constant in the potato stalk throughout the day (3·2 to 3·6 per cent.) in spite of a much larger variation of this sugar in the leaf (see Fig. 2). At night a slight fall occurs followed by an increase after sunrise to nearly the former level. The hexoses vary in somewhat the same way, but the range of variation is greater during the day (4·94 to 5·63) and the fall at night correspondingly larger (5·63 to 4·63). The curve of *apparent dextrose* (for data see Table V) is almost parallel to the saccharose curve and the same is true of the curve of *apparent laevulose*; the dextrose, as in the mangold stalk, always appears to be in large excess as compared with the laevulose, the ratio $\frac{D}{L}$ (pentoses as xylose) varying between 4·5 and 5·5. Although the laevulose and dextrose curves are practically parallel, the *absolute* increases during the day being nearly the same, the value $\frac{D}{L}$

falls considerably from 6 a.m. to 2 p.m., owing to the smallness of the laevulose values.

In the potato stalks, the fluctuations of the "apparent laevulose" are far less than in the mangold stalks (compare Fig. 2 with Figs. 7, 8 and 9 in preceding paper I), whilst the ratio of hexoses to saccharose remains nearly constant (see Table II) throughout the 24 hours. The variation of "apparent" dextrose and "apparent" laevulose is discussed later (see p. 373).

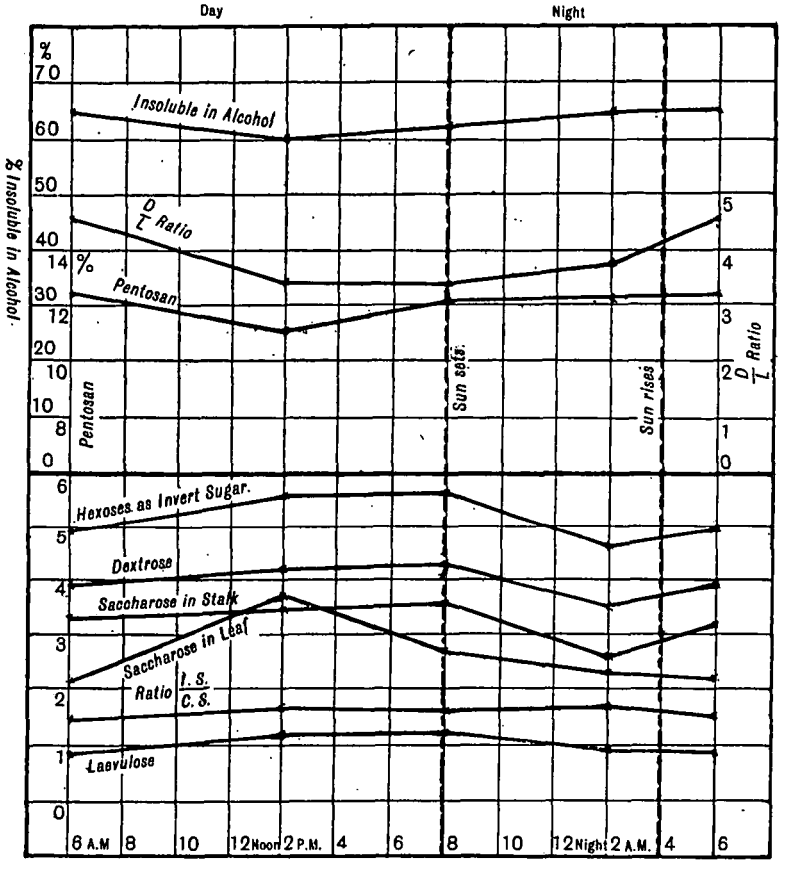

Fig. 2. Potato stalks, July 16–17, 1914.

The fact that in the leaf the saccharose is always greatly in excess of the hexoses (ratio $\frac{\text{I.S.}}{\text{C.S.}}$ varies from 0·08 to 0·45) whereas in the stalks the hexoses are always greatly in excess of the saccharose (ratio $\frac{\text{I.S.}}{\text{C.S.}}$

varies from 1·52 to 1·75) is best explained as in the case of the mangold by the view that in the leaf the saccharose is a primary product and is converted into hexoses for purposes of translocation.

Pentosans and Matter Insoluble in Alcohol.

In the stalks of the potato, unlike the leaves, practically no starch is present to interfere with the parallelism of the curves showing the pentosan content and the total leaf matter insoluble in alcohol (cellulose + lignified tissue) (see Fig. 2). From 6 a.m. to 2 p.m. the sugars and other substances soluble in alcohol are increasing so that the proportion of matter insoluble in alcohol falls. It should be noted that in the potato stalks the sugars form only a small proportion of the increase of total soluble matter; thus between 6 a.m. and 2 p.m. their increase is only 0·8 per cent., whilst the other soluble matters increase by 3·8 per cent.[1] After 2 p.m. the insoluble matter gradually increases to practically its earlier value, and the same is true of the pentosans.

The following table (Table III) gives a comparison between the potato and the mangold as regards the range of variation of the total sugars and of the matter soluble in alcohol in the leaf and stalk.

As regards the *leaf* constituents this table shows that the potato in its early stages of growth closely resembles the mangold at a corresponding stage; the range of variation of the sugars and of the substances soluble in alcohol is nearly the same in both cases. In both cases also the saccharose is greatly in excess of the hexoses. It is probable that, in the potato as in the mangold, during the later period of growth, when storage is the principal function, the relative proportion of saccharose and hexoses would be found to change, the hexoses then predominating in the leaf as well as in the stalks.

In the potato *stalks*, however, the actual proportion of substances soluble in alcohol is considerably less (35·2–39·7) than in the mangold (43·4–46·8), but the range of variation during the day is greater. The

[1] In the mangold stalks during the day the increase of the sugars is considerably greater than the increase of the total substances soluble in alcohol; during this period the soluble substances other than the sugars (amino-acids, tannins, amides) fall off greatly relatively to the sugars. Thus:

Mangold Stalks. Series I. August 26th–27th (average of top and bottom halves).

Increase of total sugars from 6 a.m. to noon	= 4·87 %
Increase of total alcohol-soluble substances	= 3·0 %

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

Increase of total sugars from 10 a.m. to 4 p.m.	= 6·20 %
Increase of total alcohol-soluble substances	= 1·5 %

variation of the sugars (1·93 per cent.) is however far less than in the mangold (4·68). The last two columns show how greatly the proportion of sugars and substances soluble in alcohol increases in the mangold in the later stages of growth.

TABLE III.

Range of Variation of Sugars and Alcohol-soluble Matter in the Mangold and Potato.

	Potato July 16–17, 1914	Mangold Series I, Aug. 26–27, 1913	Mangold Series II, Sept. 10–11, 1912	Mangold Series III, Oct. 11–12, 1912
Leaf:	%	%	%	%
Total sugars	1·91–4·93 Δ=3·02	1·70–5·27 Δ=3·57	9·62–17·17 Δ=7·55	14·5–21·0 Δ=6·5
Alcohol-soluble substances	34·1–39·3 Δ=5·2	37·2–42·5 Δ=5·3	44·2–54·7 Δ=10·5	47·9–54·95 Δ=7·05
Stalks:				
Total sugars	7·28–9·21 Δ=1·93	10·95–15·63 Δ=4·68	25·32–31·76 Δ=6·44	— —
Alcohol-soluble substances	35·2–39·7 Δ=4·5	43·4–46·8 Δ=3·4	64·2–66·9 Δ=2·7	— —

Table III shows that in both mangold and potato *leaves* the daily fluctuation of the substances soluble in alcohol is always far greater than (often nearly double) that of the total sugars. The same is true of the potato *stalk*, but in the mangold stalk the change in the sugars is always much greater than that of the alcohol-soluble constituents.

C. *The Dextrose-Laevulose Ratio.*

The "apparent" dextrose and laevulose have been calculated, as in the case of the mangold, on the assumption that the pentoses are either arabinose or xylose. The values are given in Tables IV and V.

D = percentage of apparent dextrose calculated on the total vacuum-dried matter (T.V.D.M.).

L = percentage of apparent laevulose calculated on the total vacuum-dried matter (T.V.D.M.).

I. *Leaves.*

As was the case in Series I of the mangold pickings (Paper II, p. 335) the results obtained for the "apparent" dextrose and laevulose are of little real value as an index of the true proportions of these sugars present, owing to the presence of optically active impurities which cannot be

removed by the ordinary treatment with basic lead acetate. The quantity of the reducing sugars is so small that the error introduced in this way becomes relatively very great; a large difference too is seen between the results for dextrose and laevulose according as the pentose is assumed to be arabinose or xylose. This is not because the pentose is actually present in large amount (it ranges only from 0·35 to 0·52

TABLE IV.

Apparent Dextrose and Laevulose in Potato Leaves.
July 16th–17th, 1914.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %		Hexoses % calc. as invert sugar	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Pentose as arabinose	Pentose as xylose		
6 a.m.	Nil	0·42	0·00	Nil	0·42	0·00	0·42	0·42	0·40	
8 a.m.	0·26	0·76	0·34	0·46	0·53	0·85	1·01	1·00	1·00	
10 a.m.	Nil	0·38	0·00	Nil	0·38	0·00	0·38	0·38	0·37	
12 noon	0·25	0·95	0·26	0·48	0·73	0·66	1·23	1·21	1·21	Day
2 p.m.	Nil	0·69	0·00	Nil	0·69	0·00	0·69	0·69	0·67	
4 p.m.	Nil	0·97	0·00	Nil	0·97	0·00	0·97	0·97	0·93	
6 p.m.	0·19	1·11	0·17	0·45	0·83	0·54	1·30	1·28	1·27	
8 p.m.	0·17	1·08	0·16	0·39	0·83	0·47	1·25	1·22	1·22	
10 p.m.	Nil	0·42	0·00	Nil	0·42	0·00	0·42	0·42	0·40	
12 night	Nil	0·76	0·00	0·18	0·57	0·32	0·76	0·75	0·73	Night
2 a.m.	Nil	0·71	0·00	0·19	0·50	0·38	0·71	0·69	0·68	
4 a.m.	0·01	0·15	0·06	0·15	Nil	∞	0·16	0·15	0·15	

TABLE V.

Apparent Dextrose and Laevulose in Potato Stalks.
July 16th–17th, 1914.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %		Hexoses % calc. as invert sugar	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Pentose as arabinose	Pentose as xylose		
6 a.m.	3·68	1·11	3·32	3·91	0·86	4·58	4·79	4·77	4·94	
2 p.m.	3·90	1·53	2·55	4·17	1·23	3·39	5·43	5·40	5·40	Day
8 p.m.	3·95	1·55	2·55	4·23	1·24	3·41	5·50	5·47	5·63	
2 a.m.	3·25	1·25	2·60	3·53	0·94	3·75	4·50	4·47	4·63	Night

per cent. on the T.V.D.M.), but because the quantity and rotatory power of the hexoses is exceedingly small (0·15 to 1·27 per cent.). At 8 a.m., for instance, if the pentoses are taken as arabinose, $\frac{D}{L} = 0{\cdot}34$, but if they are assumed to be xylose $\frac{D}{L}$ becomes 0·85.

That the results are vitiated by the presence of a *laevo*-rotatory impurity appears clearly in the data for 6 a.m., 10 a.m., 2 p.m., 4 p.m. and 10 p.m., in all of which cases the amount of dextrose appears to be *nil*. If in these cases the whole of the reducing sugar is assumed to be laevulose, the negative rotation calculated does not account, on the assumption that the pentose is xylose, for the negative rotation actually observed. The following table shows the differences:

Time	Actually observed for hexoses in 200 mm. tube at 20°*	Calculated for hexose = laevulose	Rotation not accounted for
6 a.m.	−0·143°	−0·060°	−0·083°
10 a.m.	−0·076	−0·036	−0·040
2 p.m.	−0·075	−0·055	−0·020
4 p.m.	−0·146	−0·102	−0·044
10 p.m.	−0·063	−0·039	−0·024

* After allowing for the pentoses (as xylose) and saccharose present.

If the results are calculated on the assumption that the pentose is arabinose, the negative rotation not accounted for becomes even greater; thus at 6 a.m. it becomes − 0·138° instead of − 0·083°. The number of cases in which dextrose appears to be entirely absent is increased on this assumption.

It is clear therefore that the apparent predominance of laevulose in the potato *leaf* is due to the presence of relatively large quantities of a laevo-rotatory impurity (? asparagine), and it is probable that the dextrose and laevulose, as in the mangold leaf, are really present in equal proportions, that is as invert sugar, and are formed from saccharose. It is interesting that the dextrose appears in largest amount at 6 p.m. and 8 p.m., that is at the time when the starch content reaches a maximum and is subsequently put under contribution. As we show on p. 357, the starch is broken down by the leaf enzymes completely to dextrose. Fig. 3 shows the variation of the apparent dextrose and laevulose during the 24 hours (pentoses assumed to be xylose); as an index of the real fluctuation of the hexoses the curves have, of course, no value, but they are interesting as showing that the laevo-rotatory impurity varies regularly during the 24 hours. The laevulose always

appears in considerable excess except just about sunrise (4 a.m.) when the whole of the very small amount of hexose present (0·15 per cent.) appears as *dextrose* not laevulose, and a *positive* rotation of + 0·015 remains unaccounted for. A somewhat similar abnormality was found just before sunrise in the case of the mangold leaves, Series I; whereas during the greater part of the 24 hours *dextrose* appeared to be in excess (see Table I, Paper II, p. 332, arabinose values) in the mangold leaf, at 4 a.m., when the total hexose was exceedingly small (0·2 per cent.), laevulose suddenly appeared to predominate; at the same time, the

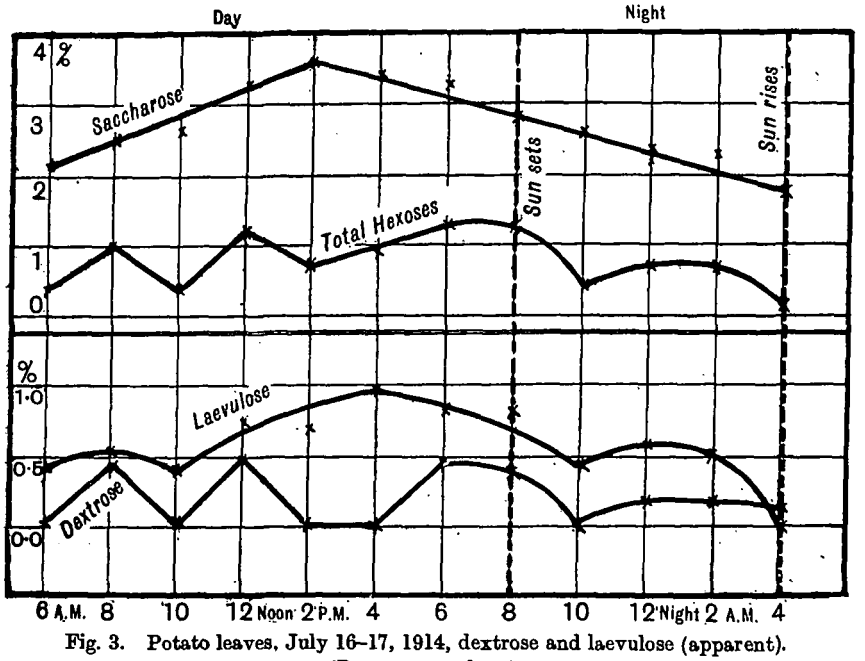

Fig. 3. Potato leaves, July 16–17, 1914, dextrose and laevulose (apparent). (Pentose as xylose.)

polarisation results for cane sugar, which in general throughout the day were *higher* than the reduction values, suddenly became *lower*. But 2 hours later, at 6 a.m., the values were again much higher. The following data illustrate this:

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

	D %	L %	$\frac{D}{L}$	Δ* in saccharose values
2 a.m. ...	0·29	0·08	3·62	+ 7·3
4 a.m. ...	0·00	0·20	0	− 29·2
6 a.m. ...	0·73	0·00	∞	+42·6

Sunrise = 5.5 a.m

* Δ = difference between values found for saccharose by polarisation and by reduction.

It would appear, therefore, that in the potato as in the mangold leaf *two* oppositely active impurities are present at different times of the day. During the greater part of the day laevulose appears to be in excess owing to a laevo-rotatory impurity predominating, but *at night* the amount of this impurity diminishes until it is replaced just before sunrise (4 a.m.) by an excess of *dextro*-impurity. The variation is well seen by considering the data obtained by assuming the pentoses to be arabinose; the amount of laevo-rotation left unaccounted for when the whole of the hexose is assumed to be laevulose gradually drops from 10 p.m. to 2 a.m., whilst at 4 a.m. dextrose appears to be present.

At 10 p.m. negative rotation unaccounted for = −0·062° (200 mm. tube)
12 midnight negative rotation unaccounted for = −0·010°
2 a.m. negative rotation unaccounted for = −0·002°
4 a.m. positive rotation unaccounted for = +0·015°

Between 6 a.m. and 8 a.m., that is just after sunrise, the quantity of *negative* impurity suddenly increases very largely, the negative reading unaccounted for at 6 a.m. being greater than at any other period of the 24 hours (− 0·138° if pentose is arabinose, − 0·083° if xylose).

The following table (Table VI) shows how the presence of the optically active impurities causes abnormally large differences in the results found for saccharose by the reduction and by the polarisation methods. This table should be compared with the similar table obtained in the case of the mangold leaf (see p. 338, preceding paper).

TABLE VI.

Divergence of Results for Saccharose by the Reduction and Polarisation Methods—Potato Leaves. July 16th–17th, 1914.

	Citric acid inversion			Invertase inversion			$\frac{D}{L}$
Time	% saccharose reduction	% saccharose polarisation	Δ%	% saccharose reduction	% saccharose polarisation	Δ%	(pentose xylose)
6 a.m.	2·16	2·48	+14·8	2·11	2·70	+17·1	0·00
8 a.m.	2·47	2·37	− 4·0	2·60	2·67	+ 2·7	0·85
10 a.m.	2·81	2·71	− 3·5	2·65	3·10	+17·0	0·00
12 noon	3·39	3·63	+ 7·1	3·19	3·41	+ 6·9	0·66
2 p.m.	3·81	4·46	+17·0	3·50	4·54	+29·7	0·00
4 p.m.	3·56	3·75	+ 5·3	3·34	3·83	+14·6	0·00
6 p.m.	3·46	4·34	+25·4	3·22	4·48	+39·2	0·54
8 p.m.	2·77	2·21	−20·2	2·69	2·98	+10·8	0·47
10 p.m.	2·76	2·31	−16·2	2·49	2·77	+11·2	0·00
12 night	2·48	2·46	− 0·8	2·30	2·39	+ 3·9	0·32
2 a.m.	2·38	1·91	−19·8	2·26	1·98	−12·4	0·38
4 a.m.	2·09	2·43	+16·2	1·44	2·51	+74·4	∞

As in the case of the mangold leaves, the polarisation results for saccharose are generally considerably *higher*—often 20 to 30 per cent. higher—than the reduction values[1]. The two methods only give approximately the same results when $\frac{D}{L}$ approximates to 1 (e.g. at 8 a.m. and 12 noon, when Δ (invertase) is only 2·7 and 6·9 per cent.). When the dextrose appears to disappear $\left(\frac{D}{L}=0\right)$ the divergences (Δ) are greatest (e.g. 6 a.m., 10 a.m., 2 p.m., 4 p.m., 10 p.m.). As showing the presence of *two* distinct and oppositely active impurities, it is interesting that between 6 a.m. and 4 p.m. when the laevulose on the whole appears to increase (see Fig. 3) a rise in the apparent dextrose corresponds with a diminution in the difference between polarisation and reduction values for saccharose, and *vice versa*; but from 4 p.m. to 10 p.m. (laevulose falling) an increase in the apparent dextrose carries with it an increase in the divergence, whilst a fall in the dextrose is accompanied by an opposite result (compare the figures in Table VI with the curves in Fig. 3). Between 10 p.m. and 2 a.m., when the laevulose again appears to rise and fall, the difference between the two sets of values becomes less and less (invertase figures) and finally *negative*. It is interesting to compare the following figures:

				Sunrise	
	10 p.m.	Midnight	2 a.m.	4 a.m.	6 a.m
Rotation not accounted for by the hexoses ...	−0·062°	−0·010°	−0·002°	+0·015°	−0·081°
Δ (invertase) between saccharose values ...	+11·0 %	+ 3·8 %	−12·5 %	+74 %	+17·2 %

The abrupt change between 2 a.m. and 4 a.m. (corresponding with the sudden fall to zero of the apparent laevulose) from a negative rotation not accounted for to a high positive value, and a negative difference − 12·5 per cent. to a high positive value + 74 per cent., is followed, *immediately after sunrise*, by equally great changes in the

[1] If the discrepancy between the results for saccharose were due solely to an amino-acid or amide such as asparagine, one would expect the divergence to be diminished by taking the first (direct) polarisation after saturating the solution with sulphur dioxide so as to make it strongly acid (see Pellet, *Dosage du Sucre par Inversion*, 1913). As a matter of fact in the case of the potato whether the direct reading was taken (as has been usual in our experiments) in practically neutral solution or whether it was taken in acid solution (SO_2) made very little difference in the majority of cases, the figures usually being very high as compared with the reduction values.

opposite direction. The fluctuations, whatever be their cause, show throughout evidences of periodicity; this appears most clearly in the shape of the curve of apparent laevulose.

II. *Stalks.*

The results for the potato stalks closely resemble those found for the mangold stalks in the fact that the dextrose present appears always to be in large excess as compared with the laevulose; the ratio $\frac{D}{L}$ varies from 3·39 to 4·58 (pentose as xylose). But there is this striking difference: in the mangold, dextrose appeared to be the predominant sugar in both leaf and stalks, but in the potato it is in excess *only in the stalks*, whilst in the *leaf*, as pointed out above, laevulose predominates. It is also very striking, that whereas in the mangold the greatest fluctuations and the greatest divergences between the reduction and polarisation values for saccharose were found in the stalks and mid-ribs (Δ varied from + 40 per cent. to − 90 per cent., see Table VIII, preceding paper), caused no doubt by large fluctuations in the optically active impurities present, in the potato stalks *the differences are as a rule relatively small, and, in general, less than in the leaves.*

The following table (Table VII) shows this:

TABLE VII.

Divergence of Values of Saccharose by Polarisation and Reduction Methods—Potato Stalks. July 16th–17th, 1914.

	Citric acid inversion			Invertase inversion			
Time	Saccharose by reduction %	Saccharose by polarisation %	Δ %	Saccharose by reduction %	Saccharose by polarisation %	Δ %	$\frac{D}{L}$ (xylose
6 a.m.	3·20	3·70	+15·6	3·28	3·62	+10·4	4·58
2 p.m.	3·44	3·84	+11·6	3·41	3·92	+14·9	3·39
8 p.m.	3·55	3·35	− 5·6	3·60	3·85	+ 7·0	3·41
2 a.m.	2·61	2·82	+ 8·0	2·70	2·88	+ 6·7	3·75

The extreme divergence here is only 15 per cent., whilst in general the divergence (Δ) is less than 10 per cent. There are no such abrupt changes from positive values to negative values as were met with in the mangold stalks (see p. 344) and had their counterpart in the sudden variation in the values for apparent laevulose (see Figs. 7 and 8, Paper I). One of the most striking differences between the potato stalks and the

mangold stalks is that in the former the curves *of apparent dextrose and apparent laevulose run almost parallel to one another* throughout the 24 hours, and at the same time about parallel to the saccharose curve (see Fig. 2, p. 371). Each sugar increases slightly and continuously during the day and then falls at night.

It seems probable from the parallelism of the curves of saccharose and total hexoses that the dextrose and laevulose are actually present in the stalks as invert sugar, being formed from the saccharose by inversion; the large apparent excess of dextrose would then be due to the presence of a *dextro*-rotatory impurity which accumulates in the stalks (whereas in the leaf a *laevo*-rotatory substance is generally in excess). The divergence Δ between the reduction and polarisation values is relatively small in the case of the potato because the substance is of such a nature that the *change* of rotation brought about by the processes of inversion is relatively small; but the existence of this divergence (up to 15 per cent.) is a proof that some such compound is present. On the other hand the practical parallelism of the curves of apparent dextrose and laevulose, which is in striking contrast with the *mangold* stalks, suggests that whatever be the impurity which is present, its amount remains relatively constant throughout the 24 hours.

On the other hand the relatively small divergence between the polarisation and reduction values for saccharose in the potato stalks, in contrast with the large divergences found in the mangold stalks, may be taken to mean that only small amounts of the optically active impurity are present in the potato stalks and that the values of dextrose and laevulose in the stalks (*not* in the leaves) nearly represent the true values for these sugars. If this is the case the dextrose has accumulated in the stalks far more than the laevulose, possibly owing to the latter sugar being used up for tissue building[1], and to the fact that the starch formed in the leaf gives dextrose as sole product when hydrolysed by the leaf enzymes (see p. 357). One would naturally expect the starch in the tuber to be built up from dextrose as it yields dextrose exclusively when hydrolysed by either acids or taka-diastase and the predominance of dextrose in the stalks conveying sugars to the tuber would be quite natural if this were the case. The question, however, whether the dextrose is in actual excess over the laevulose in the stalks or whether

[1] It is interesting to recall Meyer's observation in 1886 that almost all leaves capable of forming starch at all produce it abundantly from a 10 per cent. solution of laevulose and a relatively small number only from dextrose. On general grounds, considering the relationship of starch and dextrose, one would have expected the reverse to be the case.

the two sugars are present mainly in the form of invert sugar can only be decided definitely when methods have been devised of estimating the two sugars, in presence of each other, which are free from the errors caused by optically active impurities.

SUMMARY.

1. In the potato leaf when the tubers are beginning to develop the principal sugar present is saccharose; its amount increases from sunrise up to 2 p.m., following approximately the curve of temperature. It then falls during the rest of the day and night. The rise and fall are both linear.

2. The hexoses are present in the leaf in very small amounts—generally less than 1 per cent. of the total dry weight of the leaf. They fluctuate considerably during the early part of the day, the fluctuations being apparently determined by conversion into or formation from starch.

3. During the early part of the day up to 2 p.m. the proportion of starch changes very little, the small fluctuations which occur being related to changes in the starch. The starch is apparently formed from the hexoses.

4. Directly the amount of saccharose has reached its maximum at 2 p.m. the hexoses begin to increase in the leaf, owing apparently to hydrolysis of the saccharose to invert sugar; at the same time "soluble starch" (or dextrin) is first detected in the leaf and its amount increases regularly up to 6 p.m. At 6 p.m., 2 hours before sunset, the true starch in the leaf reaches a maximum value, far greater than any previous value during the day. The starch and "soluble starch" subsequently fall rapidly until between midnight and 2 a.m. the amount left is exceedingly small (0·2 per cent.). The starch is apparently converted directly into hexose (dextrose), the amount of which increases in the leaf.

5. In the stalks reducing sugars predominate greatly over the saccharose in spite of the fact that in the leaf the latter is in excess. As in the mangold it is probable that cane sugar is the first sugar formed in the leaf and that it is hydrolysed by invertase in the veins, mid-ribs and stalks, for the purpose of translocation.

6. As in the mangold, the true proportions of dextrose and laevulose cannot be determined in the leaves and stalks owing to the presence of soluble optically-active impurities, which vitiate the polarimetric data.

It is shown that the presence of these impurities also falsifies the results obtained for saccharose by the double polarisation method. The fluctuations of the "apparent dextrose" and "apparent laevulose" in the leaf really indicate variations in these impurities rather than variations in the hexoses, which are perhaps present mainly as invert sugar. In the stalks, where the amount of optically active impurity appears to be less than in the leaves, it is possible that the dextrose is actually in excess as it appears to be, and that the starch in the tuber is built up from this sugar.

7. Maltose is invariably absent from the potato leaf and also from the leaves of other plants which form much starch in the leaf. The degradation of starch in the leaves is probably effected by a mixture of enzymes similar to the enzymes of *Aspergillus oryzae* (taka-diastase); maltase is always in relative excess, so that the starch is degraded completely to dextrose. The series of changes is therefore:

Starch → dextrins → maltose → dextrose.

Appendix. Experimental Data.

Potato Leaves. July 16th–17th, 1914.

In the first two analyses (6 a.m. and 8 a.m.), after the treatment with basic lead acetate, the precipitate was washed to 2 litres, but the first and second litre of washings were analysed separately so as to obtain an idea of the amount of sugars left behind when the washing was continued only to 1 litre. As it was found that this was quite appreciable, in all the later analyses the lead precipitate was washed to nearly 2 litres, and after precipitation with sodium carbonate the solution was made to 2000 cc. ($= A$).

The extract of the leaf material was evaporated *in vacuo* and made up to 500 cc. 440 cc. of the 500 were treated with basic lead acetate and the precipitate washed to 1 litre; the filtrate was treated with solid sodium carbonate and made to 1000 cc. $= A$.

The second litre of washings was also treated with solid sodium carbonate and made up to 1000 cc. $= A'$.

Time	Vacuum-dried matter soluble in alcohol, grms.	Vacuum-dried matter insoluble in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *A* used for reduction (*x*)	Polarisation of *A* in 200 mm. tube*, $a_D^{20°}$	Reduction of solution *A*, grms. CuO	Invertase inversion: Reduction of *x* cc. after inversion, grms. CuO	Invertase inversion: a_D after inversion (200 mm.)	Citric inversion: Reduction of *x* cc. after inversion, grms. CuO	Citric inversion: a_D after inversion (200 mm.)	Phloroglucide from 50 cc. *A*	Remarks
6 a.m.	34·32	57·91	92·23	25 cc. *A*	+0·115°	0·0426	0·1445	−0·104°	0·1470	−0·108°	0·0169	1st litre (*A*)
	,,	,,	,,	25 cc. *A′*	±0·000	0·0032	—	—	0·0125	−0·000	—	2nd litre (*A′*)
8 a.m.	43·30	67·55	110·85	25 cc. *A*	+0·261	0·0835	0·2300	−0·075	0·2223	−0·050	0·0127	1st litre (*A*)
	,,	,,	,,	25 cc. *A′*	−0·002	0·0024	—	—	0·0190	−0·022	—	2nd litre (*A′*)
10 a.m.	44·15	70·58	114·73	25 cc. *A*	+0·117	0·0387	0·1247	−0·078	0·1298	−0·061	0·0077	*A* = 2000 cc.
12 noon	44·75	69·24	113·99	25 cc. *A*	+0·183	0·0513	0·1548	−0·056	0·1612	−0·066	0·0054	,,
2 p.m.	34·57	66·72	101·29	25 cc. *A*	+0·149	0·0312	0·1318	−0·051	0·1408	−0·050	0·0045	,,
4 p.m.	49·38	87·17	136·55	25 cc. *A*	+0·139	0·0505	0·1800	−0·096	0·1887	−0·100	0·0071	,,
6 p.m.	43·22	77·50	120·72	25 cc. *A*	+0·189	0·0578	0·1684	−0·107	0·1767	−0·101	0·0069	,,
8 p.m.	59·25	102·42	161·67	25 cc. *A*	+0·190	0·0725	0·1960	−0·090	0·1998	−0·042	0·0094	,,
10 p.m.	39·74	74·30	114·04	25 cc. *A*	+0·120	0·0272	0·1073	−0·061	0·1161	−0·041	0·0059	,,
Midnight	43·87	76·07	119·94	25 cc. *A*	+0·133	0·0390	0·1168	−0·044	0·1232	−0·047	0·0063	,,
2 a.m.	53·22	88·90	142·12	25 cc. *A*	+0·168	0·0415	0·1328	−0·024	0·1375	−0·020	0·0063	,,
4 a.m.	42·58	70·36	112·94	25 cc. *A*	+0·147	0·0188	0·0648	−0·035	0·0857	−0·032	0·0053	,,

* In most cases the solution was sufficiently colourless to allow the reading to be taken in a 400 mm. tube. The data given are, however, all reduced to the 200 mm. standard

Potato Stalks. July 16th–17th, 1914.

Distilled *in vacuo* and made up to 250 cc. 190 cc. of the 250 treated with basic lead acetate, filtered and washed to .1 litre, solid sodium carbonate added and made up to 1000 cc. = Solution *A*.

Time	Vacuum-dried matter soluble in alcohol, grms.	Vacuum-dried matter insoluble in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *A* used for reduction (*x*)	Polarisation of *A* in 200 mm. tube, $a_D^{20°}$	Reduction of solution *A*, grms. CuO	Invertase inversion: Reduction of *x* cc. after inversion, grms. CuO	Invertase inversion: a_D after inversion (200 mm.)	Citric inversion: Reduction of *x* cc. after inversion, grms. CuO	Citric inversion: a_D after inversion (200 mm.)	Phloroglucide from 50 cc. *A*
6 a.m.	31·47	56·24	87·71	25 cc. *A*	+0·468°	0·2200	0·3563	+0·023°	0·3525	+0·019°	0·0090
2 p.m.	29·27	44·45	73·72	25 cc. *A*	+0·385	0·2094	0·3292	+0·001	0·3304	+0·005	0·0085
8 p.m.	29·79	48·14	77·93	25 cc. *A*	+0·422	0·2242	0·3568	+0·012	0·3550	+0·038	0·0102
2 a.m.*	25·10	46·14	71·24	25 cc. *A*	+0·345	0·1914	0·2950	+0·021	0·2918	+0·023	0·0170

* In this analysis the extract was made to 200 cc. (not 250) and 170 cc. of the 200 treated with basic lead acetate.

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