On the germination of the tuber of the Jerusalem Artichoke (Helianthus tuberosus).

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A GROUP of plants, prominent among the Compositae, store their carbohydrate reserve-materials in their tubers or tuberous roots in the form of inulin. Of these plants the dahlia (*Dahlia variabilis*) and the Jerusalem artichoke (*Helianthus tuberosus*) are most frequently met with. In the somewhat fleshy, though not tuberous, roots of the common species of *Inula* (*I. Helenium* and *I. Conyza*) a similar accumulation may be found.

Inulin can be prepared from these plants by boiling the tubers, or roots, with large quantities of water, concentrating the decoction so obtained, and allowing it to stand till it deposits a sediment. This is to be redissolved in a small quantity of hot water, decolourised by boiling with animal charcoal, and again concentrated, when it gradually deposits fairly pure inulin. This can be purified by redissolving and evaporating again till the inulin is precipitated, when it should be well washed with cold water containing a little alcohol. In the tuber the presence of inulin can be detected by soaking pieces in alcohol for two or three days and cutting sections. These, dipped in water and examined, show large sphaero-crystals of inulin deposited in the tissue, which often embrace several cells within their area.

Inulin, prepared as described above, is a white powder which is readily soluble in warm water, dissolves only slightly in cold water, and is insoluble in alcohol. It is not thrown down

[Annals of Botany, Vol. I. Nos. III and IV. February 1888.]

from its watery solution on cooling, so that the latter can be diluted to any desired degree. It is precipitated on adding alcohol in sufficient quantity to the solution.

Its relation to sugar is very much the same as that of starch, for it is readily converted into sugar by boiling with acids, or by heating its watery solution under pressure[1], the change being probably one of hydration, just as is the case with starch. Its formula, according to Watts' Dictionary of Chemistry, is $C_{12}\ H_{20}\ O_{10}\ 2H_2O$. It differs from starch in not occurring in the form of grains of definite shape, but being crystalline when isolated, and occurring in solution in the sap of the cells which contain it. Further, it differs from starch in resisting to a very large extent the action of saliva[2], and in being capable of dialysing through a moist membrane. This power however is very feeble. Like starch, it has an action on polarised light.

In the plants spoken of, inulin does not replace starch altogether, for the latter is found in the sub-aerial parts, but it is the only form of carbohydrate reserve-material.

The chemical changes in the reserve-materials accompanying germination have in many cases been shown to be due to the action of different unorganised ferments. There is no doubt that starch is changed into sugar by a body of this description, to which the name of *diastase* has been given, and which has been shown by different writers to occur in almost every growing part of green plants. The changes brought about in the different proteid reserve-materials have by several observers been shown to be due to a similar cause[3], and cellulose is demonstrated to give rise to sugar by the same agency[4]. Prantl[5] and others have shown that sugar is formed from inulin, and in investigating the peculiarities of this change the first question that suggests itself is,—Is the conversion due,

[1] Poulsen, Bot. Microchem. p. 88. [2] Cf. infra.

[3] V. Gorup-Besanez, in Ber. Deutsch. Chem. Gesell. 1874, p. 1478. Green, in Phil. Trans. vol. 178 (1887) B, p. 39. [4] Green, op. cit.

[5] Prantl, Das Inulin, 1870.—Sachs, Lectures on the Physiology of Plants, Engl. ed. p. 343.

as in so many other cases, to an unorganised ferment? Sachs concludes that this is the case, and that the ferment resembles invertin. He does not, however, quote any experiments on the point.

The material used for the investigation was the tubers of the Jerusalem artichoke (*Helianthus tuberosus*). Examination of the young shoots and roots arising from the germinating tuber showed that, while inulin was present in them, there was a far larger proportion of sugar there also than was present in the tissue of the resting artichoke. Inulin, as has been mentioned above, is known to be capable of conversion into sugar, and therefore the abundance of the sugar in these parts suggests that it has arisen at the expense of the inulin, and that consequently in the germinating tuber something may be looked for capable of converting the one into the other. The sap of the tuber when expressed was nearly neutral; with very sensitive litmus paper a trace of acidity could be detected, but so little as to negative the idea that the conversion was brought about by the instrumentality of an acid. The probability of the conversion being due to a ferment-action was at once apparent.

Tubers of the artichoke were planted and allowed to germinate till the young plants arising from them had attained a height of about six inches above ground. The tissue of the tubers was now found to have become much altered, the interior having become spongy and the cells almost empty, while on the outside and for some distance inwards it was firm and succulent. Microscopic examination showed the cells of this outer part to be filled with colourless cell-sap, from which by appropriate treatment the well-known sphaero-crystals of inulin could be separated out. There was a considerable quantity of sugar present, but no starch, neither extract nor sections giving any reaction with iodine. The tubers were minced finely and extracted with glycerine, in which inulin is insoluble. After twenty-four hours the glycerine was strained off and the extract dialysed till the dialysate failed to reduce Fehling's solution.

The subsequent experiments with such a glycerine extract

were carried out, some in test-tubes, and others in parchment dialysers. In the latter cases the dialysates were tested for sugar as the action went on, its presence being taken to indicate a formation of it, as the solution of inulin which was used to test the power of the glycerine-extract was itself dialysed carefully before use, so as to prevent the possibility of the introduction of any sugar during the preliminary operations. The dialysates were changed at intervals of twenty-four hours. In all cases careful control experiments were made with boiled extract, in order to confirm the view that any changes occurring were due to the action of a ferment. The weather being extremely sultry, precautions were taken against the appearance of bacteria by using thymol in all the experiments.

Particulars of two typical experiments are subjoined.

Experiment 1, carried out in test-tubes :—

A contained 10 cc. dialysed extract + 15 cc. inulin solution.

B contained 10 cc. dialysed extract boiled + 15 cc. inulin solution.

A trace of sugar was present in the extract, but not much. Action began gradually, and proceeded slowly and regularly for several days, the difference between the contents of the tubes when tested with Fehling's solution becoming more and more marked as the digestion proceeded. After six days the contents of B gave no more reduction than at first when boiled with Fehling's fluid, while those of A gave a vivid red under the same conditions.

Experiment 2, carried out in dialysers :—

A contained 40 cc. of inulin-solution (1 per cent.) + 5 cc. extract of tubers.

A_1 contained 40 cc. of inulin-solution + 5 cc. extract boiled.

Again there was a small amount of sugar present in the extract.

In two days the dialysate of A had a greater reducing

power than that of A_1, the latter only showing what was due to the sugar in the extract. The dialysates were changed and the dialysis continued for twenty-four hours longer, when the difference in favour of A was very marked, the control showing the presence of a mere trace of sugar.

The same results were arrived at in other cases, the details of the experiments, as to the relative proportions of inulin and extract used, being varied in many ways.

Coincidently with the appearance of the sugar in the dialysates, the amount of inulin in the parchment tubes underwent diminution. The amount of precipitate thrown down by alcohol from the fluid containing the unboiled extract of the tubers became less and less as time went on, measured quantities being taken for that purpose. Similar quantities taken from the controls showed no such diminution.

These results establish the presence of a ferment in the germinating artichoke, by whose instrumentality inulin is transformed ultimately into some form of sugar. The time taken up in the experiments is to be accounted for possibly by the very small quantity of the ferment present, and its dilution in the process of extraction. In the artichoke it is probable that it only exists at any particular time in the cells whose contents are being changed into sugar, and as it takes several weeks for this conversion to be brought about in any tuber, there must be but little ferment to be found at any one moment. Some experiments bearing on this point will be detailed later on.

Like so many of the digestive ferments, both animal and vegetable, the ferment brings about a change which is quite easily caused by other means. The action of acids at a boiling temperature has already been referred to. Besides this I found that prolonged exposure to dilute acids at the ordinary temperature, and still better at about 40°C, is capable of bringing about the same conversion. Alkalis on the other hand have no action on it. Prolonged suspension in cold water will also transform some inulin into sugar, though the energy of water is very feeble compared with that of acid.

Still I always found that any solution or suspension of inulin that had been standing for some weeks contained a trace of sugar. This fact at first was very disturbing, but its importance was minimised by having very careful control experiments always carried out side by side with the others during the whole investigation.

The effect of the ferment is therefore in this, as in so many other cases, to bring about more quickly an effect that can be caused by other agencies.

The ferment so demonstrated is distinct from the ordinary diastase which transforms starch into sugar. An experiment on this point is subjoined.

Large test-tubes were taken and treated as under :—

A contained 10 cc. glycerine-extract of tubers + 15 cc. inulin-solution.

B contained 10 cc. glycerine-extract of tubers boiled + 15 cc. inulin-solution.

C contained 10 cc. glycerine-extract of tubers + 15 cc. one per cent. starch-paste.

D contained 10 cc. glycerine-extract of tubers boiled + 15 cc. one per cent. starch-paste.

The tubes were then put in a water bath at 35°C. A gradually and regularly showed an increase in the amount of reduction noticeable on boiling with Fehling's fluid, while B, C, and D remained exactly as at first for four days. The ferment is therefore not diastase.

It is interesting to note here that while the inulin-ferment is not able to act upon starch, saliva, which is so energetic with the latter, has little or no power to convert inulin. In two experiments upon this point I subjected inulin to the action of saliva for twenty minutes and for twenty-four hours respectively, and got no perceptible amount of sugar formed in either case. That the saliva was active I proved by having control tubes containing starch, which were treated exactly like those containing inulin, and these showed conversion proceeding at the normal rate.

The conditions of the activity of this inulin-ferment are similar to those which govern the digestive ferments of the animal organism. Like saliva it works best in a neutral medium. The presence of a very slight trace of acid does it no harm; in fact it is rather advantageous. The sap expressed from growing tubers is very faintly acid, the acidity being equal to about ·001 per cent. of HCl. Stronger acids than this are prejudicial, and exposure for an hour to an acidity equal to ·2 per cent. of HCl at a temperature of 40° C destroys it altogether. Alkalis are similarly hurtful, no ferment-power surviving an exposure for an hour to a strength equal to 1·5 per cent. of $Na_2 Co_3$ solution. The rapidity with which the destruction of the ferment by acid takes place is dependent on the temperature at which it is kept during the time the two are in contact. At a low temperature it is much less affected than at 40° C, but after an hour's exposure at 10–15° C its working power is very much impaired.

The energy of the ferment shows the same variation with the temperature, being much greater at 40° C than at the ordinary temperature of the soil in which its normal action takes place. The same thing I have noted elsewhere[1] is the case with the proteolytic ferment occurring in the lupin. It is destroyed by boiling.

The products of the action of the ferment on inulin are a sugar and an intermediate body possessing properties which resemble those of inulin on the one hand and sugar on the other. In the first stages of this investigation, formation of sugar was, as already indicated, taken as the sign of the activity of the ferment extracts. The products of the digestion were collected later for more exact enquiry into their composition. To obtain them digestions were conducted for some days in dialysers; the first three days' dialysates were rejected, to be sure that whatever was examined was really the product of the ferment's action and not any dialysable matter possibly mixed with the inulin; the later dialysates were

[1] Phil. Trans., vol. 178 B, p. 46.

collected and concentrated over water baths till of very small bulk, when they had a syrupy appearance and consistency.

These concentrated dialysates were found to contain three bodies that could be separated from each other by treatment with alcohol. The first of these was a sugar, and was separated by extracting the syrupy residue with absolute alcohol, when about half of it dissolved. On decantation from the undissolved residue, and concentration over a water bath, this again became syrupy, and remained so, refusing to crystallize, even when exposed over strong sulphuric acid. It was freely soluble in cold water, and its solution reduced Fehling's fluid when boiled with it. As I was unable to get it into crystalline form, I could not determine its specific rotatory power. It had a feebler reducing power than dextrose or laevulose, and this power was considerably increased by boiling it for a few minutes with about two per cent. of HCl.

Having extracted this sugar from the concentrated dialysates by treatment with absolute alcohol, there remained a residue about equal in bulk to the sugar taken up. A great deal of this dissolved freely in cold water, which is not the case with unaltered inulin. The rest remained insoluble till heat was applied. This consisted of inulin that had dialysed through the parchment during the later stages of the digestion. An experiment was conducted on this power of dialysis, some inulin-solution, without any ferment, being dialysed in a fresh well-tested parchment tube for several days, when the dialysate, on being concentrated, deposited a residue which the microscope showed to consist chiefly of the well-known sphaero-crystals of inulin.

The two constituents of the residue, after separation of the sugar, were separated from one another by treatment with alcohol. Careful experiments showed that inulin was insoluble in alcohol of sixty-five per cent. strength. On making the solution of the residue up to this strength of spirit, there was a precipitate which gradually separated out and settled to the bottom of the fluid. On filtering and adding further alcohol, no change took place till about eighty-two per

cent. of spirit was present. Then opalescence set in again, and gradually a very finely-granular precipitate separated out, one much more finely-granular than the first one. This consisted of the intermediate body, which had been found to be soluble in cold water. In 100 parts of the residue there were about 62·5 parts of inulin which had passed the dialyser unchanged, and 37·5 parts of the second body.

On concentration of the watery solution of this residue it deposited sphaero-crystals of inulin and a quantity of other crystalline matter. These crystals appeared generally as plates, sometimes pentagonal, sometimes rhomboidal or oblong, with here and there needle-like prisms forming part of a rosette. They could under the polarising microscope be readily distinguished from those of inulin, the latter not being so strongly doubly-refractive, and having the form of circles showing the cross so characteristic of the sphaero-crystal. The others were probably due to the intermediate body.

Some samples of inulin contain a certain portion of this body, which can be separated from the inulin by fractional precipitation with alcohol, as already described. In one sample I tested there was 12–14 per cent. of it. It has a greater power of dialysis than inulin has, but to separate it by this method is not easy. In the case of some of the last-mentioned sample of inulin, when the dialysate was concentrated after the process had gone on for five days, this intermediate body formed sixty-six per cent. of the total precipitate which could be thrown down by alcohol.

This product then differs from inulin in the following particulars:—

1. It is more soluble in cold water.
2. It has a greater power of dialysis.
3. It has a different crystalline form.
4. It is soluble in alcohol of sixty-five per cent. strength, not being precipitated by less than eighty-two per cent.

Returning to the experiments quoted on p. 229 it is clear that this body occurred in the dialysates in consequence of its

formation during the digestion and not from having been present in the inulin used, for the dialysates of the first three days were rejected. If any had been mixed with the inulin taken for experiment this would have escaped during that time, as its dialysing power is so great compared with that of inulin. Its occurrence as a consequence of the action of the ferment recalls the occurrence of dextrin during the action of the ordinary amylolytic animal ferments. The body too somewhat resembles dextrin, being soluble in stronger percentages of alcohol than the original carbohydrate, but not soluble in a greater percentage than eighty-two, at which point dextrin also is precipitated. Unlike dextrin, it gives no reaction with iodine, but this is not remarkable, as inulin differs from starch in the same respect.

The slowness with which the ferment-extract was found to work is probably due to there being an extremely small quantity present at any particular time. The progress of germination in the tuber of the artichoke is extremely slow and gradual. As the plant continues to develop, the tuber becomes more and more exhausted, but it contains inulin for months, until in fact the new tubers are being formed on the underground stems that have been developed from the parent tuber. The interior is the first to be exhausted, the outside often continuing hard and succulent till it is only a thin shell, while the inside is spongy and dry. The ferment is only to be looked for at any moment in the cells which are parting with their carbohydrate contents.

It cannot be found at all until the young stems begin to emerge from the tuber, and then its presence is maintained till the store of inulin is all exhausted. The quantity that can be extracted from the minced tubers is small, for a single digestion with glycerine takes it nearly all up, hardly any being found in a subsequent extraction. An examination of the plant while the germination is going on enables the course of events to be followed fairly well. In the resting tuber before germination begins, the inulin is found to occupy nearly all the cells in its interior, which consist almost entirely of paren-

chymatous tissue, the fibro-vascular tissue being extremely reduced. The microscopical tests for inulin are very imperfect, depending on precipitation in particular forms by alcohol or glycerine and chiefly on the occurrence of the well-known sphaero-crystals. In working at the micro-chemical reactions of inulin I was however fortunate in finding a test which always indicated it when present and enabled me to see exactly where in the sections the cells contained it. This was a solution of orcin in alcohol. On warming with strong HCl a section soaked in this reagent, the cells containing the inulin were stained a deep orange-red. The commercial preparations of inulin which I had gave the reaction in a very marked manner, and on treating, in the way described, sections in which the sphaero-crystals had been deposited, these dissolved leaving an orange-red area which they had occupied.

I found too that solutions of inulin boiled with strong HCl, to which a little orcin in alcohol had been added, took on this deep orange-red tint. Phloroglucin was as efficacious as orcin, the colour being rather more brown. On the tube cooling the clear orange-red colour was replaced by a brown precipitate. In tracing the progress of the inulin I used the orcin-reaction.

As the young stem grew, the inulin could be seen to follow its increase in length, occupying the centre of the shoot, and leaving the circumference free. It did not reach so far upwards as the growing-point but stopped abruptly just behind the actively growing zone, so far as I could make out. It was accompanied in its progress by sugar, which extended rather further forwards, but which also could not be detected with certainty in the growing-point. This agrees with observations which have been made in the cases of growing-points supplied with sugar at the expense of starch. From the power of dialysing which inulin has been shown to possess, this travelling of it towards the growing-point does not seem remarkable. Its occurrence just behind the growing cells may be due to an actual transit of the stored inulin before

being converted into sugar, it being thus brought near to the point where it is changed. On the other hand, it may with greater probability be suggested to have another origin altogether, and to be caused by the supply of sugar being too rapid for the needs of the growing cells. The surplus sugar might in such a case be reconverted into inulin temporarily, till wanted. Such reconversion is a matter of constant occurrence in the case of supplies of starch.

Should the first-mentioned view of its presence be the correct one, and the inulin itself be able to travel from the tuber to the growing-point, it suggests the question of the necessity of any ferment-transformation. But the easy transport of the carbohydrate-material is only one of the requisite conditions of the nutrition of the growing cells. We can hardly suppose, at any rate in the case of inulin, that it is transformed into sugar merely to be more easily moved about the plant. There still remains the question of the condition in which the carbohydrate must be to serve as nourishment to the cells of the growing-point. This must apparently be sugar, and hence we have always sugar supplied to the growing tissue.

I have said above that the presence of the ferment is only to be expected where the carbohydrate material is being rendered available for use. It follows from this that it should not appear in the tubers till the onset of germination, and that consequently in a resting tuber none should be discoverable. In an experiment on this point some developing tubers were taken from the plant on which they were being formed, and their stalks carefully cut off close up to the tubers, so as to leave nothing but the latter. These were then mashed up carefully and covered with glycerine. After two days' exposure to this liquid, the extract was filtered off. It was found to contain a good deal of sugar. Two dialysers were prepared, A and B. In A were put 10 cc. of this glycerine-extract and 30 cc. of inulin-solution. B was made up similarly, but the extract was well boiled before adding it to the inulin. Outside the dialysers 200 cc. of water were placed. As sugar dialyses rapidly through parchment-paper, the dialysates were changed

frequently, and were tested carefully at intervals to see whether any difference of reducing power between the two dialysates could be detected as time went on. After three days both the dialysates were quite free from sugar, and during this period there was never any difference of reducing power to be observed. Had any ferment been present in the extract, the dialysate of A should have given evidence of its activity by an increased reducing power, but of this, as I have said, there was no indication. The sugar originally present in the extract was equally present in both dialysers, and it gradually dialysed out at the same rate in both. When this quantity had disappeared, there remained nothing in A that would reduce Fehling's fluid, as there would had ferment been present. Both dialysers at the end of the experiment contained nearly as much inulin as at first, only a little having dialysed out during the experiment. Hence no ferment is present in the developing tubers.

There remains for consideration the condition in which the antecedent of the ferment exists in the tubers till the onset of germination. From analogy with other ferments, both animal and vegetable, it appears probable that it is present in the form of a zymogen. In many cases this can be proved to be the antecedent form of ferment, as e.g. in the gastric and pancreatic glands in the animal body. An extract of these glands, taken while they are quite fresh, is found to possess no digestive powers, but to become active when warmed with a weak acid. A gland that has been kept warm for some hours before the extract is made is found to contain large quantities of the ferment. My first experiments on this point were made with very small tubers, and were not conclusive. Later in the year I was able to use tubers that had attained their full size, and with these I was more successful.

Some full-grown artichokes were procured; half of them were at once extracted with glycerine, as in the other cases described. The remainder were sliced each into about four pieces, and were put into a beaker over a bath at 35° C, and kept there for twenty-four hours, after which they were minced,

and an extract made, as in the other case. The first of these two extracts proved to be quite inert, while the second showed a power of converting inulin into sugar. The experiments were carried out as before in well-tested dialysers, the dialysates being examined at intervals for sugar.

I was unable to obtain any evidence of the formation of ferment in the extract of the unwarmed tubers by the action of acid alone. I tried hydrochloric, acetic, citric, and malic acids, but none had any effect. This might be due to the destruction of the ferment by the acid as fast as it was formed, for it is most sensitive in this direction, as already pointed out. When the extract was treated with a solution of acid-albumin in ·2 per cent. H Cl it did give rise to a certain amount of ferment, though less than was obtained by warming the tubers for twenty-four hours before the extract was made.

The result of the investigation into the germination of the artichoke tuber may be briefly summarised as follows :—

1. The stored inulin in the tuber is made available for the use of the plant by ferment-action.

2. This ferment is not diastase, but a special body working on inulin.

3. Its action is to produce from the inulin a sugar and an intermediate or collateral product.

4. The latter differs from inulin in its solubility in water and alcohol, its crystalline form, and its power of dialysis.

5. The ferment does not exist as such prior to the commencement of germination, but is present in the resting tuber in the form of a zymogen, from which it can be developed by the action of warmth, or, under certain conditions, by that of acid.

6. Its activity is only manifested in a neutral or very faintly acid medium, and it is destroyed by prolonged contact with acids or alkalis.