The Influence of Certain Carbohydrates on Growth and Starch Formation in Detached Leaves

Mary Irene Brown

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The Influence of Certain Carbohydrates on Growth and Starch Formation in Detached Leaves

by

Mary Irene Brown

Presented in partial fulfillment of the requirement for the degree of Master of Arts.

State University of Montana

1929

(Signed) Chairman Examination Committee
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The growth of leaves and plants on nutrient solutions has been and will be of vital importance to the research worker in all fields of Botany because it furnishes an acceptable and fairly easy method of keeping the experimental material under conditions of controlled environment.

Leaves and plants grown in this manner have been the basis for work on Photosynthesis, Respiration, Nutrition, Assimilation, Growth and other studies involving practically all fields of Plant Physiology. Detached leaves on nutrient media have proven very satisfactory hosts for the study of parasitic fungi. And more recently, the study of metabolism and growth of Fungi, Algae, and other lower forms has dealt almost entirely with their reactions to various nutrient solutions.

Thus we see that the field is of wide-spread interest and a thorough and complete understanding of the factors concerning the growth of leaves on nutrient solutions is very important.

It has been the aim in this paper to present a study of growth and starch formation in leaves on various monosaccharide and disaccharide nutrient solutions; to evaluate these solutions as nutrient media; and to demonstrate the effects of varying two environmental factors, namely CO₂ and the color of light.

I am especially indebted to Dr. Charles W. Waters under
whose direction the work was carried on, and to Professor J. W. Severy, Chairman of the Department, for many helpful suggestions and criticisms.
DISCUSSION OF LITERATURE

Knudson (1916) states that "De Saussure found in 1804 that Bidens cannabin and Polygonum persicaria apparently absorbed sugar from an aqueous solution of the same. His work was of course not conducted under sterile conditions, and doubtless much of the sugar reported as being absorbed disappeared thru fermentation".

Parkin (1899) gives Boehm the credit for the first work on starch formation on nutrient solutions. He used a 20 percent solution of cane sugar and after eight or ten days found that species of Allium and Asphodelus were starch free while Galanthus nivalis, Arnithogalum umbellatum, Hyacinthus orientalis, Iris germanica and Veratrum nigrum contained starch. There was no marked difference in the production of starch between 10° and 20° C.

It was impossible to obtain the papers by A. Meyer in 1885 and 1886 but a very good account of his work appears in an article by Brown and Morris (1893). Meyer records the examination of a great many leaves for starch. As a general rule he states that Dicotyledons store starch plentifully in their leaves, and the Monocotyledons less so. He conducted an experiment to ascertain if leaves not normally containing starch would store starch under conditions most favorable to assimilation. Cut leaves were exposed to bright light in an atmosphere containing 3 percent CO₂ and, as a rule, the plants
whose leaves did not form starch under normal conditions did not do so when treated in the manner described, but two exceptions to this were found in Hemerocallis and Muscari.

In the following year Meyer published another paper. In this he formulates for the first time the proposition that starch only arises in the leaf cell if more of the nutritive starch-forming material is received than the leaf uses for its own nourishment and respiratory processes, and that the degree of concentration of the nutritive solution plays an important part in the result. Following in the main the experimental methods of Boehm, leaves which had been thoroughly depleted of starch in darkness were divided into pieces of about 4-6 cm. square and floated on the nutritive solution, or if the leaves were small, they were merely cut around the margin to open the tracheids, so that the solution might gain access to the parenchyma. Dextrose, levulose and galactose were converted into starch by the leaf parenchyma. The leaves of some plants produced starch from all three kinds of sugar, but this was not found to be the case with all. He found that almost all leaves that were capable of forming starch at all produced it abundantly from a 10 percent fructose solution, while only a few formed starch on dextrose. On the other hand, only a very few leaves could form starch from galactose. He believed that as a rule those forms of sugar which are naturally present in the plant are favorable to starch-production. As proof of this he states that "a good instance of this is
afforded by the *Compositae*, this group contains inulin as a reserve substance; the product of hydrolyses of inulin is levulose; consequently we find the *Compositae* especially well able to elaborate starch from levulose. On the other hand, from dextrose only small quantities of starch are elaborated by the leaf-cells of this order of plants and none at all from galactose. Then again, the *Sileneae* naturally contain galactose, and we find in consequence abundant starch formation in the leaves of these plants when floated on solutions of galactose.\[1]\n
Sapozhnikov is said by Palladin (1914) to have investigated this matter quantitatively. Leaves of *Astrapoea wallichii* previously rendered starch-free, formed in seven days from 4.6 to 5.3 g. of starch per square meter of leaf surface, when floating upon a 20 percent solution of cane sugar in darkness.\[2]\n
Palladin (1914) also states that "according to the experiments of Reinhardt and Sushkov the accumulation of starch in leaves floating upon cane-sugar solution depends upon a variety of conditions. This process occurs rapidly only at medium temperatures, while starch that was previously present disappears at higher or lower temperatures, in spite of the supply of sugar".\[3]\n
Spoehr (1926) concludes from the investigations of Winkler in 1898 that the minimal concentration of sucrose for starch formation is 0.2 percent, the optimal 10 percent and
that a 30 percent solution produces no starch at all. He states, however, that the concentration of solution which produces starch varies with different plants and that no general rule covering all plants can be made.

Acton (1899) studied the production of starch with nutrient solutions supplied to cut branches, to the roots of plants, and to the leaf surface. He found that starch was formed when glucose, glycerin, sucrose or inulin was supplied either direct to the roots of plants or to the leaf surface. He came to the conclusion that "green plants cannot normally obtain carbon for assimilation from any substances except carbohydrates or bodies closely related to them, not from aldehydes or their derivatives, and not from all carbohydrates even".

Parkin (1899) worked with the formation of starch in leaves of Monocots floated on solutions of cane-sugar, glucose, fructose, and maltose, varying in concentration from 5-20 percent. The leaves were cut into small fragments, \( \frac{1}{2} \) cm. in length, and floated on solutions in glass dishes. The experiments were performed at a temperature of from 12-15 degrees C., in the dark, under sterile conditions. Cane-sugar was found to be the best starch producer and the optimum concentration appeared to be somewhere near 10 percent. There was not much difference between glucose and fructose, but maltose was generally unfavorable. He found that leaf pieces had to float at least four days on the solutions before starch made its appearance.
After the work of Parkin, the subject of growth of leaves on nutrient solution seems to have been dropped for a period of fifteen years, and the growth of seedlings on nutrient solutions attracted the attention of Plant Physiologists. Most of these experiments dealt with the influence of minerals and are not of interest in this connection. However, the work of Lewis Knudson (1916) on the effect of various sugars is important in that his results show the varying effects of the same sugar on different species of plants.

Seedlings of corn, Canada field pea, timothy, radish, and cabbage were grown on a mineral nutrient solution plus various sugars. In the case of corn, glucose and fructose were most effective, saccharose was second, and maltose, third. The Canada field pea utilized saccharose, to greatest advantage with glucose, maltose and lactose following in the order named. Timothy could use only glucose and saccharose in the light but also lactose in the dark. Radish used glucose to the greatest extent with saccharose, maltose and lactose following. Maltose was most effective for growth of cabbage but a mixture of saccharose and maltose was still more valuable. Galactose was toxic to vetch, pea, corn and wheat, even at concentrations as low as .0125 percent. In a later paper Knudson (1917) proved that the toxicity of galactose could be prevented by the addition of minute traces of glucose and saccharose and that the toxic effect of galactose was due mainly to its injurious effects on roots.
Otto Gertz (1919) found that in the case of variegated leaves grown on glucose solutions, starch was formed in both the light and the dark areas.

A. W. Reinhard (1923) found that leaves of bean, white acacia and pea, produced starch in the absence of CO₂ if grown in a nutrient solution containing sugar. The formation of starch was favored by light - yellow light being more efficient than blue.

Spoehr and Mc Gee (1923) published in that same year their work on the respiration of detached leaves of sunflower and "Canada Wonder" bean grown on nutrient solutions containing different sugars and amino-acids. They found a relationship between the intensity of sugar content and amino-acid content of the leaves. They concluded that in general an increase in amino-acids increased the rate of respiration when the leaves contained an ample supply of carbohydrates.

Tolenaar (1925), a Dutch writer, has made the most important contribution in recent years to our knowledge of this subject. He particularly stresses the fact that consideration must be given to previous preparatory treatment of the experimental leaves as well as environmental factors during the experiment. For this reason he thinks that the results of Sachs, Schimper, Brown and Morris and other workers must be repeated and proven in order to be fully relied upon. In studying the effect of temperature, he finds that equal amounts of starch are formed in tobacco leaves in one day at
28 degrees C., in two days at 17 degrees C. and in three days at 12 degrees C. He finds that the minimum sucrose which will produce starch is one-hundredth percent at 28 degrees C. His interests, however, were chiefly with photosynthesis and he reached the conclusion that a monosaccharide sugar is the first detectable step in photosynthesis, that in most instances the process leads immediately to the formation of starch and that much of the starch is used directly in respiration rather than being transported.

Mains (1917) and Waters (1927) have not added much that is new to the field except that it is suitable for the study of pathological diseases. Waters tried glucose, fructose, starch, maltose, raffinose, asparagin, malt extract, peptone, and cane sugar. He found a 6 percent cane-sugar solution most satisfactory for all purposes of rust infection.

The literature dealing with the effects of increased amounts of CO₂ gas on starch formation, is very limited. The experiment by Meyer, previously described, is the only one dealing with detached leaves. Fischer (1920) performed several practical experiments in Germany in which waste gases of a steel mill containing about 5 percent CO₂ were piped to greenhouses and fields and the increased production noted. Tomatoes increased 175 percent over the control group and cucumbers 70 percent.

Borneman (1920) performed a similar experiment the same year with peas, oats, barley, potatoes, onions and kohlrabi.
The percentage increase in weight varied from 18.2 percent in kohlrabi to 210 percent in onions.

Fischer (1927) in a later experiment found increasing results in the yield of Tropaclum which is inclined to sterility. The supply of CO₂ in the air was increased from 0.3 percent to 1 percent with no injurious effects. He suggests that the use of increasing amounts of CO₂ may aid in studies on genetics.

Many similar experiments have been tried and for the most part indicate that increased amounts of CO₂ result in increased Photosynthesis but only in the presence of sunlight. A good review of the literature may be found in Spoehr's "Photosynthesis".

No work has been done on the effect of different wave lengths of light on starch formation in detached leaves but the differences in photosynthetic activity in light of different colors has been extensively studied and a good resume of the work done may be found in Palladin's "Plant Physiology" and in Spoehr's "Photosynthesis". Most experiments have shown that the red portion of the spectrum is most effective not only for the decomposition of CO₂ and H₂O but for starch formation.
METHODS AND APPARATUS

The Experimental Material

The leaves of all plants are not equally well suited to a study of this kind. Some leaves, e.g., geranium, do not retain their vigor on sugar solutions, but within a comparatively short time become brown and present a "water-soaked" appearance. Other leaves, which are particularly hardy and well-suited for this type of work, could not be used because of the difficulty of freeing them of chlorophyll for the purpose of making starch tests. It is also necessary that the leaves be broad enough to furnish a large number of pieces approximately equal in the number and size of veins, in thickness of the leaf tissue, and in other conditions such as healthfulness, sturdiness, etc., all of which might be influential in varying the results.

During the winter months, only greenhouse material was available, and many different types of hot-house plants were tried. Among these were leaves of rose, geranium, carnation, wandering Jew, chrysanthemum, sweet pea, snapdragon and tulip. Snapdragon and tulip proved very satisfactory. In the late spring and early summer, the wild flowers and cultivated plants around the campus could be used and the problem of securing material was not difficult.

Care was exercised in selecting leaves for each particular experiment in order to use those of the same age and
Consideration was also given to the conditions to which the plant had been exposed previous to cutting the leaves. The leaves for each experiment were all cut at the same time of day and allowed to remain in the dark for the same length of time, eg. until all were free from starch.

The pieces of leaves used in all the experiments were obtained by cutting the leaves under distilled water into circles 13 mm. in diameter, by means of a small copper cork borer. This proved very satisfactory as it could be easily sharpened and the circles cut were always very regular and of the same diameter through, and the leaf tissue was apparently not injured appreciably. These pieces were very convenient in measuring rate of growth and they presented an advantage in that all pieces exposed the same amount of absorbing surface to the solution.

The Solutions

Solutions were made from chemically pure sugars and distilled water and were kept in sterile flasks plugged with cotton stoppers until used. At first all solutions were sterilized but it was found that contamination took place almost as rapidly when the solutions were sterilized as when they were not. Waters (1927) also found this to be the case and explained it as being due to the inversion of the disaccharides into monosaccharides during sterilization, the latter being
more easily attacked by Fungi. He states, however, that according to Mudge (1917) practically no saccharose is inverted during sterilization and that if this be true he is at loss to explain his results.

The Apparatus

Petri-dishes were used for all the experiments. These and the flasks used for the solutions were all thoroughly washed in warm water, rinsed three times in tap water and three times in distilled water and sterilized in a hot air oven at 160 degrees C. for one hour.

The dark chamber in which the experiments were carried on was washed once or twice a week with a dilute solution of carbolic acid, in order to keep contamination at a minimum.

The Procedure

The de-starched leaves were cut into circles under distilled water and were transferred while wet to petri-dishes which had been partially filled with the sugar solutions. The leaf-portions were allowed to float freely on the top of the solution.

New solutions were put in freshly sterilized petri-dishes every three or four days and the leaf-portions were rinsed in distilled water and transferred to them. By this method the leaves were kept free from contamination for some time.

Most of the experiments dealing with starch formation were carried on in the dark in order that any photosynthetic
starch might be eliminated. The experiments on growth had to be carried on in the light, however, as it is impossible to keep leaves living in the dark for more than two or three weeks. Extreme chlorosis occurs after a short time which seems to cause a cessation of growth and finally death of the leaves; although starch is manufactured after the leave have become entirely white.

Starch tests were made according to Sachs' well-known method of decolorizing with alcohol and judging the amount of starch by degree of coloration with iodine. "The starch is deposited centrifugally from the vascular bundles outward, the more distant cells showing the starch last. When the cut surfaces are exposed the sugar evidently travels from cell to cell by diffusion but more rapidly thru the bundles." 1.

The results are denoted according to a system used by Miyaki (1917) in her article on the starch of Evergreen leaves. 0, 1, 2, 3, and 4 are used, designating amounts of starch as follows:

0 - no starch
1 - small amount around the margin
2 - heavy band around the margin
3 - some throughout the veins
4 - black throughout

In the experiments on growth, several pieces of leaves

were used and once a week the diameter of these was taken. The average diameter of the healthy pieces of leaves was used as the result of growth for the week. It was found, however, that in most cases the diameters of all pieces of leaves on a given solution varied little if the leaves were in good condition.
EXPERIMENTS AND RESULTS

Part I - Starch Formation

The Influence of Concentration of the Nutrient Solution on the Rate and Amount of Starch Formation

Snapdragon, dog tooth violet and tulip leaves were grown on .1, .5, 2, and 6 percent concentrations of glucose, saccharose and lactose in order to study the effect of concentration of the nutrient solution on starch formation. The experiment was carried on in the dark for a period of six days. Table 1 shows the results of three successive starch tests at two-day intervals.

A 6 percent solution of all sugars used in the experiment was most favorable. Decreasing percentages resulted not only in retarded starch formation but frequently in the case of .5 and .1 percent solutions, in no starch formation at all. There was little difference between the value of 2 percent and 6 percent solutions of saccharose and glucose. Lactose was apparently not utilized at all by the tulip and only to a very slight extent by the other two groups of leaves. This indicates that leaves of various species may show some selection in the sugars utilized in starch formation. The leaves of snapdragon, normally containing heavy starch, formed in nearly all cases more starch than the other two genera whose leaves do not normally store food in the form of starch.

In another experiment, leaf-portions of corn and bean
Table 1. - The effect of concentration of the nutrient solution on the rate and amount of starch formation in leaves of snapdragon, tulip and dogtooth violet.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Time of Starch Test</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 days</td>
<td>4 days</td>
<td>6 days</td>
<td>2 days</td>
<td>4 days</td>
<td>6 days</td>
<td>2 days</td>
</tr>
<tr>
<td>6% glucose</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2% glucose</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2-3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>.5% glucose</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>.1% glucose</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6% lactose</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2% lactose</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0-3</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>.5% lactose</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>.1% lactose</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6% saccharose</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2% saccharose</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2-3</td>
<td>3</td>
<td>2-3</td>
</tr>
<tr>
<td>.5% saccharose</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2-3</td>
</tr>
<tr>
<td>.1% saccharose</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>H₂O</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
were grown on solutions of saccharose increasing in concentration up to thirty-four percent. In all cases over 6 percent starch was formed throughout the veins and around the edge of the leaf circles. Increasing concentrations, however, failed to show a perceptible increase in the amount of starch. In no case was the leaf completely filled with starch in two days' time but in four days heavy starch was formed throughout the leaf on most concentrations.
The Influence of Species on Rate and Amount of Starch Formation by the Various Sugars

The preceding experiment indicated that leaves of various species may differ in the amount of starch formation on specific sugars. Leaves of eight genera were grown on 6 percent solutions of glucose, fructose, galactose, lactose, and saccharose. The results of starch tests after two days are indicated in table 2.

Table 2. The Influence of Species of Leaf on Amount of Starch Formation by Various Sugars

<table>
<thead>
<tr>
<th>Species</th>
<th>Solution</th>
<th>glucose</th>
<th>fructose</th>
<th>galactose</th>
<th>lactose</th>
<th>saccharose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquilegia canadensis</td>
<td></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Delphinium sp.</td>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Lychnis alba</td>
<td></td>
<td>2-3</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>2-3</td>
</tr>
<tr>
<td>Verbascum thapsus</td>
<td></td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Sorbus aucuparia</td>
<td></td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Parthenocissus vitacea</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Antirrhinum majus</td>
<td></td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Convallaria majalis</td>
<td></td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

These results show clearly a correlation between species and amount of starch formed on specific sugars. *Aquilegia* apparently utilized glucose most advantageously; *Verbascum*, fructose and saccharose; *Sorbus*, glucose and saccharose; and *Parthenocissus*, saccharose. Conversely, some sugars were
apparently of no value in starch formation for certain leaves. For example, *Aguilegia* made no use of fructose and galactose; *Verbascum*, of glucose, galactose, and lactose; *Sorbus* of galactose and lactose, and *Parthenocissus* of glucose, fructose, lactose, and galactose. Leaves of some plants, *e.g.* *Delphinium*, *Antirrhinum* and *Convallaria* could utilize all sugars but even here some selection is shown, *Convallaria* doing best on lactose and *Antirrhinum* on fructose.

Glucose and saccharose are apparently most beneficial for all species, galactose and fructose follow and lactose comes last.
The Influence of Various Rays of Light on Starch Formation in Detached Leaves

In this experiment, double walled bell-jars with colored liquids were used as light screens for isolating certain regions of the spectrum. Three jars were used; one contained a solution of Potassium dichromate, another a solution of ammoniacal copper oxide (a copper sulphate solution to which has been added an excess of ammonia water) and the last was simply a glass bell-jar. The first admits the rays of the less refrangible half of the spectrum (red, orange, yellow, and a part of the green), while the second transmits the remainder of the visible rays, mainly the blue and the violet. The glass bell-jar admits all but the ultra-violet rays. It was impossible to conduct an experiment using full sunlight due to the lack of a proper ventilating system to keep the air cool above the petri-dishes.

The leaves were floated on a 6 percent sugar solution in 100 cc. beakers and each day three sugars were tested under each of the bell jars. The bell jars were exposed to direct sunlight for a period of seven hours. The air in and around the bell-jars was kept cool by means of a garden sprinkler which was kept running over the jars throughout the experiment. The results for leaves of *Lychnis* are given in Table 3. The numbers 1, 2, and 3 are used to indicate the relative amounts of starch. Leaves of *Delphinium* were also used and gave similar results.
Table 3. Influence of various light rays on starch formation by various sugars.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Red light</th>
<th>Blue light</th>
<th>Whole spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Some in veins (2)</td>
<td>A little in veins (3)</td>
<td>Some in veins (1)</td>
</tr>
<tr>
<td>Fructose</td>
<td>Some throughout (2)</td>
<td>Little (3)</td>
<td>Black throughout (1)</td>
</tr>
<tr>
<td>Galactose</td>
<td>None</td>
<td>None</td>
<td>A little throughout</td>
</tr>
<tr>
<td>Saccharose</td>
<td>Black in veins (2)</td>
<td>Some in veins (3)</td>
<td>Black throughout (1)</td>
</tr>
<tr>
<td>Lactose</td>
<td>A little (3)</td>
<td>A little (3)</td>
<td>Some throughout (1)</td>
</tr>
<tr>
<td>H₂O</td>
<td>A little (2)</td>
<td>None (3)</td>
<td>A little throughout (1)</td>
</tr>
</tbody>
</table>

The whole spectrum proved more favorable for starch formation than either half, the red half being more valuable than the blue. Galactose was unfavorable for starch formation in all cases proving somewhat toxic in red light in that no starch was formed even by photosynthesis. Lactose was of little value except in the blue light where it resulted in the formation of a little starch although none was formed on distilled water. Saccharose was the most beneficial of the other three sugars except in full light, and here fructose showed a heavy black test throughout the pieces of leaves equal to that of saccharose. This might indicate that in the presence of sunlight, saccharose is broken down into glucose and fructose.
and that it is the fructose which is most valuable in starch formation.
The Effect of Increasing Amounts of Carbon Dioxide Gas on Starch Formation in Detached Leaves

The apparatus used is shown in plate I. A 3850 cc. bell jar was calibrated and used as a chamber within which the carbon dioxide content of the atmosphere was increased. Circles of leaves were grown in a low-stender dish within the bell jar on a 3 percent saccharose solution. A higher concentration of sugar was not used in order that the amount of starch formed in the controls would be small and any increase due to the effects of the gas would be more noticeable. Carbon dioxide was manufactured by the action of hydrochloric acid on marble. The gas was unwashed, but was tested each time before using by the lime water test.

The bottom of the bell jar was sealed to a glass plate, except in a narrow region which was kept up from the plate by the insertion of a small piece of glass tubing. This space permitted the entrance of the glass tube thru which the carbon dioxide was bubbled, and allowed for the entrance and expulsion of water.

Procedure: The stender dish containing the leaves was placed in the bell jar and water was drawn by displacement of air until its level was equal to one of the 100 cc. calibrations, the percentage of which to the whole volume, was equal to the percentage of carbon dioxide to be tested. The glass dish was allowed to float on the surface of the water
until its displacement by carbon dioxide.

A slight error in the water level resulted from the insertion of the glass rod between the bell jar and the glass bottom, but was adjusted by the insertion of an equal piece of glass tubing under the bottom on the opposite side of the bell jar.

After the complete displacement of the water by carbon dioxide, the glass tubing was removed and the remaining surface of the bell jar completely sealed to the bottom. This was facilitated by covering the bottom of the bell jar with petroleum jelly before putting it into the water. It was found that with practice the apparatus could be used successfully and with little error. However, the calibrations were not exact, and all that may be gained from the experiment is a knowledge of the effects of increasing amounts of carbon-dioxide gas on starch formation in leaves grown on 3 percent solutions of saccharose.

Leaves of *Lychnis* and *Convallaria* were used in the experiment. A group of leaf-circles grown in normal atmosphere on the same nutrient solution and under the same conditions of illumination, temperature, etc., was used as a check in judging the amount of starch formation in various percentages of carbon dioxide. The leaves were grown on the solutions for twenty-four hours. During this time, leaves in normal atmosphere produced a ring of starch around the edge and a small
amount throughout the veins.

Results

In the dark: **Lychnis** and **Convallaria** both showed increased production of starch with increased amounts of carbon dioxide up to approximately eight percent. Ten percent concentrations of carbon dioxide in excess of that normally present in the atmosphere did not decrease the amount of starch formation but apparently did not increase the amount shown by the check plants. Concentrations above ten percent resulted in decreasing amounts of starch formation until the amount of carbon dioxide in the bell jar was increased approximately twenty-five percent. Leaves grown in this atmosphere were entirely free from starch.

*In the light:* Carbon dioxide could be utilized to advantage in concentrations increasing to thirty percent above that normally found in the atmosphere. Further increase in the amounts of carbon dioxide showed a rapid decline in the amount of starch formed in the leaf portions. An additional increase of less than 10 percent resulted in the absence of starch within the leaves.
PART II - GROWTH

The Growth of Leaves on Sugar Solutions as Determined by Actual Measurements

The problem of obtaining actual measurements of the rate of growth proved to be very difficult. At first, an attempt was made to compare growth of whole leaves by measuring them before the experiment was started, and at weekly intervals throughout the experiment. This proved to be very inaccurate and difficult due to changes in shape of some leaves during growth and the improbability of obtaining measurements of width and length in the same area as was used at first. Later, an outline of the leaves was made on graph paper before the experiment was started, and at weekly intervals throughout the experiment, with a view to obtaining the area increase by means of a planimeter. This method proved satisfactory for leaves grown on solutions of weak concentration but it was found impossible to avoid serious contamination on 2 and 6 percent solutions. The accompanying graph shows the results of growth of bean leaves on 1 percent solutions of fructose, saccharose and glucose for two weeks.

The method, previously described, of judging growth by measuring increase in diameter of 13 mm. circles of leaves proved to be by far the most satisfactory method. But even with this method, it was difficult to secure a complete
Graph showing the growth of bean leaves floated on one percent sugar solutions.

Jan. 28.

Feb. 3.

Feb. 6.

Glucose

Fructose

Saccharose
series of measurements for growth on all sugars due to the very high mortality rate of pieces of leaves grown in this manner. The growth of snapdragon leaves on .1, .5, 2 and 6 percent solutions of glucose, lactose, and saccharose is shown in Table 4. The leaves were grown in diffused light.

Table 4. The growth of leaves on nutrient solutions as determined by increase in diameter of 13 mm. circles. The experiment was started May 10, 1929 with cut portions of snapdragon leaves.

<table>
<thead>
<tr>
<th>Solution</th>
<th>May 20</th>
<th>May 27</th>
<th>June 3</th>
<th>June 10</th>
<th>June 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% d-glucose</td>
<td>15</td>
<td>16.5</td>
<td>dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% d-glucose</td>
<td>14.5</td>
<td>15</td>
<td>dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.5% d-glucose</td>
<td>15</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>.1% d-glucose</td>
<td>15</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>6% lactose</td>
<td>14.5</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>dead</td>
</tr>
<tr>
<td>2% lactose</td>
<td>14.5</td>
<td>15</td>
<td>15</td>
<td>15.5</td>
<td>15.5</td>
</tr>
<tr>
<td>.5% lactose</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>.1% lactose</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>6% saccharose</td>
<td>14</td>
<td>15</td>
<td>dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% saccharose</td>
<td>14.5</td>
<td>15</td>
<td>16</td>
<td>18</td>
<td>dead</td>
</tr>
<tr>
<td>.5% saccharose</td>
<td>15</td>
<td>17</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.1% saccharose</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>H₂O</td>
<td>14</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
</tr>
</tbody>
</table>
The lower concentrations were of most value in growth. Saccharose was most beneficial while lactose and glucose appear to be of about equal value. The greatest amount of growth was found on a 2 percent saccharose solution. The most rapid growth was on a .5 percent saccharose solution. Strangely enough, the leaves remained in much better condition on all percentages of lactose than on the other sugars, although lactose was by far the poorest starch former of the three sugars and has never been found in plant tissue. Growth was much more rapid on all the sugars than on distilled water.
The Rate of Growth on Various Sugars Determined by Increase in Dry Weight

Fifty pieces of dandelion leaves, 13 mm. circles, were grown in the dark for six days on 6 percent solutions of fructose, glucose, galactose, saccharose and lactose. The original dry weight was determined by taking the average dry weight of three groups of leaves, each containing fifty, 13 mm. circles. The equality of the pieces of leaves cut in the manner described is shown by the fact that two of the groups of fifty pieces each were of exactly the same dry weight, while the third group varied only .034 g. The original dry-weight was .168 g. Table 5 shows the increase in dry weight and the percentage increase on the various sugars.

Table 5. Increase in dry weight of 50 pieces of dandelion leaves 13 mm. in diameter. July 2-8, 1929.

<table>
<thead>
<tr>
<th>Nutrient solution</th>
<th>Increase in dry weight in grams</th>
<th>Percentage increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>.247</td>
<td>147%</td>
</tr>
<tr>
<td>Fructose</td>
<td>.125</td>
<td>74%</td>
</tr>
<tr>
<td>Saccharose</td>
<td>.307</td>
<td>183%</td>
</tr>
<tr>
<td>Lactose</td>
<td>.101</td>
<td>60%</td>
</tr>
</tbody>
</table>

Saccharose and glucose showed an increase of over 50 percent more than any of the other sugars. Saccharose was
more beneficial than glucose, showing a 40 percent gain over the latter. There is little difference between fructose and lactose, the former showing an increase in dry weight of 74 percent and the latter of 60 percent.

The dandelion, however, is a plant normally forming starch. An experiment was carried out to see whether or not the percentage increase would be as great with leaves not normally forming starch.

Fifty pieces of tulip leaves were grown on a 6 percent saccharose solution for six days and another group was grown for an equal length of time and under the same conditions, on distilled water. The average original weight was .285 g. The weights and the percentage gain or loss are shown below.

Leaves grown on H₂O ............... dry weight .195 g....32% loss
Leaves grown on 6 % Saccharose... " " .511 g....79% gain

These results show the value of the sugar nutrient solutions for growth of leaves, both with leaves normally forming starch and with those whose leaves seldom containing starch. However, in the latter experiment the percent gain on 6 percent saccharose was much less than in the case of dandelion leaves. Another experiment was carried out using leaves of dogtooth violets, another plant not normally forming starch in its leaves. The leaves were grown on H₂O, glucose and saccharose for a period of six days. The solutions used this time were of 2 percent concentration. The original weight was .258 g.
The results are shown in table 6.

**Table 6.** The rate of growth of pieces of leaves of dogtooth violet, 13 mm. in diameter.

<table>
<thead>
<tr>
<th>Nutrient Solution</th>
<th>Amount of Gain or Loss in Dry Wt. in Grams</th>
<th>Percentage Gain or Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>0.073</td>
<td>28% Loss</td>
</tr>
<tr>
<td>2% Saccharose</td>
<td>0.192</td>
<td>74% Gain</td>
</tr>
<tr>
<td>2% Glucose</td>
<td>0.188</td>
<td>73% Gain</td>
</tr>
</tbody>
</table>

In this case with lower concentrations, the percentage gain was nearly as great as in the preceding experiment with 6 percent concentrations. This is not so surprising since the rate of growth of the leaf circles of dandelion was just as rapid on the lower concentrations. But here again, the percentage increase was not nearly so great as was the case with dandelion. This may be due to the fact that the dandelion is a plant normally forming starch and can thus assimilate food more rapidly from the nutrient solutions or it may be due to varying effects of specific sugars on the leaves in question.
The Influence of Equimolar Solutions of Glucose and Saccharose on Growth as Determined by Increase in Dry Weight

In all of the preceding experiments, saccharose seems to be more readily utilized than glucose but not to a very great extent. Since solutions of equal concentration are not all equi-molecular, the osmotic relations of the disaccharides and hexoses are different. A two percent glucose solution being equivalent to .111 gram molecular solution, while the 2 percent disaccharide is equivalent to a .058 gram molecular solution. Since the osmotic pressure is theoretically proportional to the concentration, the osmotic pressure of the glucose solution is twice as great as that of the disaccharide and, as compared with the disaccharide, glucose might be expected to act disadvantageously to the growth of the leaves because of less available carbon and if saccharose is inverted by the leaf, there is made available one molecule of glucose and one of fructose for every molecule of saccharose entering the leaf. Glucose, therefore, would have to penetrate twice as rapidly as saccharose to produce the same effect.

Fifty pieces of dandelion leaves (13 mm. circles) were grown on saccharose and glucose solutions of .02 gram molecular concentration. This was equivalent to approximately a 7 percent solution of saccharose and a 3.5 percent solution of glucose. The leaves were allowed to remain on the solutions six days.
Results. Leaves on the glucose solution showed an increase of only .052 g. in dry weight while leaves on the saccharose solution showed an increase of .155 g. Here again saccharose appears more beneficial than glucose and in much greater amounts than before. The osmotic pressures of the two solutions were equal, but saccharose still had the advantage of supplying more carbon to the leaf. However, since lactose in all the preceding experiments did not prove nearly as beneficial as saccharose, although it furnished the leaf with identical amounts of carbon, it is not probable that the carbon content of the sugars is responsible for their increased or decreased values. The differences in the values of the sugars must be due to certain specific properties of each, which are not as yet fully understood.
The Comparative Influences of Monosaccharide and Disaccharide Nutrient Solutions

The problem as to whether or not the disaccharides are utilized by the cells of the leaf directly or whether they are first broken down into two molecules of monosaccharides and are utilized as such, has not been satisfactorily answered. There was some indication in the results of the experiment on light that the latter might be the case. The fact that saccharose is more readily utilized than either glucose or fructose indicates, however, that it is not first inverted.

An experiment was carried out using 6 percent solutions of saccharose, and lactose; and 6 percent solutions containing equal molecular proportions of glucose and fructose, and glucose and galactose. Fifty pieces (13 mm. circles) of dandelion leaves were grown on each of the solutions in the dark for a period of 5 days. The original dry weight was .183 g. The results are shown in Table 7.

Table 7. Increase in dry weight of fifty pieces of Dandelion leaves on Monosaccharide and Disaccharide solutions.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Increase in dry wt. in grams</th>
<th>Percent increase in dry wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% Saccharose</td>
<td>.098</td>
<td>59%</td>
</tr>
<tr>
<td>3% Glucose and 3% Fructose</td>
<td>.082</td>
<td>49%</td>
</tr>
<tr>
<td>6% Lactose</td>
<td>.020</td>
<td>12%</td>
</tr>
<tr>
<td>3% Glucose and 3% Galactose</td>
<td>.067</td>
<td>40%</td>
</tr>
</tbody>
</table>
It will be noticed that the amount of increase in dry weight on 6 percent solutions of saccharose and lactose is much less than it was in previous experiments, (table 5). This is probably due to the fact that this experiment was carried on a month later and the growth processes of the leaves had begun to slow down considerably. However, the comparative results on the four solutions are of value.

Saccharose is of more value than a combination of glucose and fructose. This is not surprising since saccharose was more beneficial than either glucose or fructose alone. Lactose on the other hand, was not as beneficial as a combination of glucose and galactose. These results confirm the view that the disaccharides are utilized directly as such and are not first broken down into monosaccharide sugars.
Perhaps the most important factor brought to light in the present work is that the value of sugar both for starch formation and growth depends not on whether the sugar is a monosaccharide or a disaccharide but on the specific effects of the sugar on the plant studied.

A similar view was held by A. Meyer, 1885, but he attempted to make generalizations for such large groups as the Sileneae, Compositae, etc. The experiments of the writer have all led to the conclusion that no such generalization can be made. Lychnis, for example, formed as much starch on lactose and glucose as on galactose, although, according to Meyer, the Sileneae naturally contain a large amount of galactose and thus should form more starch on galactose than on solutions containing other sugars.

Meyer also stated that the Compositae are especially adapted to the manufacture of starch from fructose due to the storage of food in the form of inulin in most members of that group. It was found, however, in the case of species of Chrysanthemum and Senecio, that saccharose was a better starch former than fructose.

As a general rule, it seems probable that those sugars commonly present in the leaf may be most beneficial in starch formation. It is well known that there are variations both in the number of sugars found, and in the percentages of each,
in leaves of varying species. This factor may be correlated with the specific differences of leaves in the amount of starch formation on various sugars. The problem is one which would merit further study.

Saccharose and glucose are the two sugars most widely distributed in the plant kingdom and these proved most beneficial for both starch formation and growth. For some genera, however, it was found that fructose, galactose or lactose was most valuable. The latter fact is especially noteworthy since lactose has never been found in the plant kingdom. Knudson (1916) also found that the sugar was used in demonstrable quantities and was apparently assimilated. The fact that galactose was not toxic to leaves grown on a solution of the same, supports Knudson's view that the toxicity of galactose solutions to plants was due mainly to its injurious effects upon roots.

A second noteworthy point is that increasing concentrations of the nutrient solution resulted in increased starch formation but were unfavorable to growth.

The increased starch formation may be explained as due to the rapid accumulation of sugars within the cell, resulting both from the loss of water due to the concentration of the surrounding medium, and to the assimilation of sugars from the nutrient solution. The loss of water would not be injurious until the concentration of the medium was sufficient to cause plasmolysis and thus would not interfere with the formation
of starch.

Growth, on the other hand, is conditioned by turgor, and any rise in concentration of the surrounding medium above that of the cell, would not only decrease turgor pressure within the cell, but would increase the osmotic pressure from without. Both conditions are extremely unfavorable to growth.

The growth of leaves on solutions of high concentration resulted in noticeable thick circles with little increase in diameter. On solutions of weak concentration the leaf circles showed no perceptible difference in thickness, but increased considerably in diameter.

Similar changes in growth were found by Livingston (1903) who proved conclusively that such changes in the growth of *Stigeoclonium* resulted from changes in the concentration of the medium and were entirely dependent upon its osmotic pressure. The value of lower concentrations for growth is thought to be due both to the increase in turgidity within the cells and to the lower osmotic pressure of the nutrient solution.

It is apparent from the experiment on light that those rays most influential in the photosynthetic process are also influential in the transformation of sugars absorbed from the nutrient solution into starch. Since the greater value of the red rays of the spectrum in photosynthesis is explained as being due to their greater amount of energy, it seems probable that this may account for their beneficial effect in
starch production. The blue and violet rays are of little influence because they represent smaller amounts of energy.

An increased production of starch was noted when leaves were grown in the light on a 3 percent saccharose solution in an atmosphere enriched in carbon dioxide. There is no indication, however, that the increased amount of starch is due to an interrelation between the amounts of carbon dioxide in the atmosphere and the conversion of the sugars in the nutrient solution. The starch may be simply that resulting from increased photosynthesis. However, starch produced in leaves grown in the dark could only be formed thru a transformation of the sugars in the nutrient solution. Since leaves grown in the dark could use increasing amounts of carbon dioxide up to eight percent to advantage, it is evident that the process of conversion of sugars into starch by the protoplasm, or enzymes secreted by it, is aided by an increase in the carbon dioxide content of the atmosphere.

The actual amount of increase in dry weight found on the various sugars is of little value since the leaves were subjected to such highly artificial conditions and the results are dependent on such factors as age and development of leaves, amount of carbon dioxide in the atmosphere, etc. Since many pieces of leaves, usually 20, were grown within a single petri-dish, the increased gains in dry weight were due not alone to the absorption and assimilation of sugar, but in part to their having a greater supply of carbon dioxide produced thru
the increased respiration of the leaves. The comparative results, however, are important, and these confirm the view that saccharose is most beneficial to the plant with glucose, fructose and lactose following in the order named.

The beneficial effects of saccharose may be explained as being due to its advantage over monosaccharides in that it furnishes the leaf with twice the amount of carbon with only one-half the osmotic pressure. With equi-molecular solutions, saccharose is still at an advantage because of the increased percentage of carbon over the monosaccharide sugars, although the osmotic pressures of the two solutions are the same. The superior qualities of saccharose for growth are also definitely shown by the results of actual measurements. The greatest amount of growth was found on a 2 percent saccharose solution. Leaves on this solution showed an increase in diameter of 2 mm. more than leaf-circles on any of the other solutions.

We may definitely conclude, then, from the results of this paper, that a 5 percent saccharose solution is most valuable for starch formation and growth; that glucose, fructose, galactose and lactose may also be utilized, their values decreasing in the order named; that the amount of starch formation may be increased by increasing amounts of carbon dioxide in the atmosphere and that those rays most influential in the photosynthetic process are most useful in this process of starch formation.
SUMMARY

1. Cut circles of leaves, 15 mm. in diameter, were used throughout all the experiments and were allowed to float directly on the nutrient solution.

2. Cut pieces of leaves of snapdragon, tulip and dogtooth violet were grown on solutions of glucose, saccharose and lactose increasing in concentration from 1 percent to 6 percent for a period of 6 days. A 6 percent solution proved most valuable in starch formation.

3. Leaves of corn and bean were grown on saccharose solutions increasing in concentration up to 34 percent with no injurious effects. Increasing concentrations above six percent failed to increase the amount of starch formation.

4. Saccharose was most advantageous for most genera both for starch formation and growth with glucose, fructose, galactose, and lactose following in the order named.

5. Specific sugars may produce heavy amounts of starch in some species of leaves and no starch at all in others. No general rule as to the value of specific sugars for starch formation in large groups of plants was apparent from the genera studied.

6. The full spectrum was found more influential in starch formation in Lychnis and Convallaria than either the red or the blue portions of the spectrum. The red half of the spectrum was more influential than the blue.
6. Leaves of *Lychnis* and *Convallaria* grown in the dark on a 3 percent saccharose solution utilized increasing concentrations of CO₂ to advantage up to 8 percent. A 10 percent solution was neutral, while further increase in concentration resulted in decreased starch formation.

Increasing concentrations of CO₂ up to 30 percent were of value in starch formation in the light but there was no evidence that this was not due to increased photosynthesis.

7. Solutions of low concentration were most favorable for growth of snapdragon leaves on glucose, saccharose and lactose. Increased concentrations were injurious presumably due to the increased osmotic pressure of the solution and the decrease of turgor pressure within the cell.

8. Saccharose produced the greatest increase in dry weight in dandelion leaves with glucose, lactose, and fructose following in the order named.

9. Leaves of tulip and dogtooth violet, plants not normally forming starch, showed an increase in dry weight on 6 percent solutions of saccharose and glucose.

10. Leaves of dandelion grown on equi-molecular solutions of saccharose and glucose showed a greater increase in dry weight on the former.

11. Leaves of dandelion grown on solutions of 6 percent saccharose, 6 percent lactose, 3 percent glucose plus 3 percent fructose, and 3 percent glucose plus 3 percent galactose failed to show any indication that the disaccharides are first
broken down into monosaccharides before being utilized. The differences in the amounts of increase in dry weight were thought to be due mainly to differences in the specific values of the sugars.
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