THE BEHAVIOR OF THE ORGANIC ACIDS AND STARCH OF BRYOPHYLLUM LEAVES DURING CULTURE IN CONTINUOUS LIGHT

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When leaves of Bryophyllum calycinum are picked at daybreak and cultured in water in darkness, certain changes in composition occur that are not observed in such leaves when they are exposed to the normal diurnal alternation of light and darkness. In effect the leaves are exposed to a night period of abnormal length and the stresses that are thus set up give rise to reactions the ultimate physiological expression of which seems to be the early initiation of a reproductive phase. The formation of roots at the indentations of the margins of the leaves is stimulated and becomes detectable at the end of about 75 hours. Continued culture in darkness promotes the rapid growth of these roots and the ultimate formation of numerous plantlets.

The outstanding chemical changes that have been observed (1) are a rapid and continuous drop in the quantity of malic acid, a prompt but moderate decrease in isocitric acid which takes place during the first 24 hours but does not continue thereafter, and an increase in citric acid which is maintained for about 60 hours. The net result is the loss of about one-third of the ether-soluble organic acids initially present and this is accompanied by an increase in pH. Provided that the starch content is low at the beginning of the experiment, it is possible to demonstrate the formation of a small quantity of this substance (1, 2).

A somewhat similar sequence of events occurs in leaves picked in the afternoon at the time of high starch content and low acidity if the culture period in darkness is prolonged beyond the length of a normal night (2). Although the changes in composition at first follow those seen in leaves exposed to the normal diurnal alternation of light and darkness (3, 4), the changes that subsequently occur resemble those just mentioned.

The outcome of these experiments aroused interest in the behavior of Bryophyllum leaves exposed to an abnormally prolonged period of illumination. This is a situation that could not occur in a state of nature save in arctic regions, and is one which would presumably subject the leaves to stresses of a unique kind. The responses elicited may be expected to throw some light on the capacity of the organic acid components to undergo
chemical change and thus lead to a better understanding of the main functions of the enzyme systems involved. It was a matter of particular concern to discover whether or not isocitric acid would share in the expected transformations of the organic acids. This substance remains unchanged in quantity during normal diurnal variations of illumination.

Leaves collected at daybreak were chosen for the experiment in order to establish with the material studied that the usual enrichment in starch and loss of organic acids would take place during a period that corresponds to that of a normal day. A test of the adequacy of the illuminating equipment to bring about these changes was thereby also afforded.

**EXPERIMENTAL**

The plants were derived from the clone that has been studied in this laboratory for a number of years and were grown in sand with the use of a complete culture solution (5). Collection was begun at 5.00 a.m., May 23, 1951 (sunrise 5.27 a.m.), and ten samples of 60 leaflets each were taken by the statistical method (6) from the top three pairs of fully developed five-leaflet leaves on twenty plants. The fresh leaf control sample was placed in the drying oven at 6.00 a.m., this being taken as the zero time of the experiment. The remaining samples were placed in V-shaped culture troughs close under a bank of fluorescent lights in a room with controlled temperature and humidity, this operation being completed by 6.50. The intensity of the light at the surface of the leaves was approximately 750 foot-candles. Sufficient water to immerse the petioles was added to each trough as soon as the leaves were arranged, the petioles being held securely in place by means of a glass rod. The temperature of the room was adjusted to 20°, and the relative humidity to 50 per cent; however, the temperature directly under the lights was a little higher and varied with the operation of the cooling equipment between the limits of 21° and 26°. Individual samples were removed at the times indicated in Table I and were prepared for analysis by being dried at 80°. The tissue was then broken up, equilibrated at 24° and 50 per cent relative humidity, and ground in a Wiley mill. The samples were stored in closed bottles in the air-conditioned room and were analyzed by methods outlined in an earlier publication (7); isocitric acid was determined as described by Hargreaves, Abrahams, and Vickery (8).

The initial and final weights of the samples and some of the analytical data are shown in Table I. The coefficient of variation of the fresh weight (1.8 per cent) furnishes a measure of the accuracy with which the samples duplicated each other in initial weight, and the similarly low coefficients of variation of the nitrogen content and ash, as calculated in terms of gm. per kilo of initial fresh weight, indicate that the composition was satisfactorily constant. The protein nitrogen also remained constant at 2.02 ±
0.024 gm. per kilo, the coefficient of variation being 1.2 per cent. The higher coefficient of variation (2.4 per cent) of the equilibrated dry weight arises in part from the fact that the samples increased in organic solids during the experimental period, as is shown by the figures in the 8th column.

The data in the 9th column show that synthesis of starch followed the expected course in the early hours of the experiment and that the illuminat-

### Table I

Analytical Data on Samples of Excised B. calycinum Leaflets Collected by Statistical Method and Subjected to Culture in Water in Continuous Light

<table>
<thead>
<tr>
<th>Culture period</th>
<th>Per sample</th>
<th>Per kg. initial fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh weight at start</td>
<td>Fresh weight at end</td>
</tr>
<tr>
<td>hrs.</td>
<td>gm.</td>
<td>gm.</td>
</tr>
<tr>
<td>0</td>
<td>170.6</td>
<td>170.6</td>
</tr>
<tr>
<td>5</td>
<td>174.8</td>
<td>177.2</td>
</tr>
<tr>
<td>10</td>
<td>169.2</td>
<td>172.8</td>
</tr>
<tr>
<td>15</td>
<td>174.2</td>
<td>175.0</td>
</tr>
<tr>
<td>20</td>
<td>175.6</td>
<td>172.2</td>
</tr>
<tr>
<td>26</td>
<td>170.0</td>
<td>166.7</td>
</tr>
<tr>
<td>32</td>
<td>171.6</td>
<td>165.5</td>
</tr>
<tr>
<td>38</td>
<td>171.1</td>
<td>161.6</td>
</tr>
<tr>
<td>50</td>
<td>166.0</td>
<td>156.1</td>
</tr>
<tr>
<td>64</td>
<td>167.9</td>
<td>150.8</td>
</tr>
<tr>
<td>Mean</td>
<td>171.1</td>
<td>125.3</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>±3.08</td>
<td>±3.0</td>
</tr>
<tr>
<td>Coefficient of variation, %</td>
<td>1.8</td>
<td>2.4</td>
</tr>
</tbody>
</table>

The starch content of these samples was initially somewhat high. Sets of samples of Bryophyllum leaves collected at daybreak on different occasions may vary between fairly wide limits in starch content, possibly because of variation in such factors as the weather conditions during the days preceding collection or the temperature of the greenhouse during the night. Observations in the range from 2.2 to 13 gm. per kilo of initial fresh weight have been made. The upper limit attained after illumination under normal conditions also varies, figures in the range between 20 and 32 gm. per kilo having been obtained in samples collected in the afternoons of sunny days.

The general behavior of the carbohydrates of the leaves collected for
the present experiment is shown in Fig. 1. Photosynthesis obviously took place at a moderately rapid rate during the first 20 hours, as is indicated by the increase in organic solids plotted as gm. per kilo of initial fresh weight at the top of the figure. However, the broken line, which shows the fresh weight of each sample at the expiration of the experimental period as a percentage of the initial fresh weight of that same sample, furnishes a clear indication of the physiological stress which was initiated when the period of illumination began to exceed that of a normal day. Excised leaves of Bryophyllum exposed to the normal alternations of light and darkness do not change notably in fresh weight for several days but retain their turgidity and general appearance of health. In the present case, however, the leaves increased slightly in fresh weight during the first 10 hours, presumably mainly through the uptake of a little water, and then began to lose in fresh weight, so that after 20 hours a few leaves showed evidence of flaccidity. This effect became increasingly severe and, at the end of the experimental period, almost all of the leaves were notably flaccid, although there was no evidence of chlorophyll degeneration and the protein nitrogen was not affected. Unfortunately, a sample was not ex-

![Fig. 1. Organic solids, starch, and soluble carbohydrates of leaves of B. calycinum cultured in water in continuous light for 64 hours.](http://www.jbc.org/)
posed to darkness at the end of the period of illumination in order to discover whether irreversible damage had been done to the enzymatic systems in the cells.

The curve for the starch content (Fig. 1) shows that, although there was an initial period of 5 hours when synthesis of starch appeared to have been somewhat sluggish as compared with rates (approximately 1 gm. per hour per kilo of fresh weight (4)) that have been observed in leaves exposed to the more intense illumination of a greenhouse, the rate of synthesis during the second 5 hour period was high, being maintained at 2 gm. per hour per kilo. The maximum of nearly 30 gm. of starch per kilo was reached in 20 hours and compares favorably with the starch content observed in samples of *Bryophyllum* leaves collected from greenhouse plants in the afternoon of sunny days.

After 15 hours of illumination, the starch content of the leaves remained essentially constant. The fluctuations in the seven samples taken from the 15 hour point to the end of the experiment at 64 hours amounted to only ±3 per cent of the mean amount of starch present, a variation that is commensurate with, although probably at the upper limit of, the experimental error of the determination. It seems evident therefore that, under continuous illumination, starch accumulates in *Bryophyllum* leaves and is subsequently maintained at a limit that is presumably determined by physiological conditions within the cells of the particular sample under study. It is possible that the minor variations in starch content that were observed may have been in part related to the small variations in the actual temperature of the leaves during the experiment. It was impossible with the available equipment to keep the leaves at a fixed temperature.

The curves in the lower part of Fig. 1 show the behavior of the simple carbohydrate components. The material recorded as "total soluble" carbohydrate represents the reducing power, calculated as glucose, of an extract of the tissue after treatment with invertase. It includes true glucose, sucrose (which remained constant), and the unfermentable carbohydrate, an analytical component that represents the reducing power, calculated as glucose, of a solution that has been treated with yeast. The total soluble carbohydrate is plotted on a scale five times larger than the starch and follows an interesting bimodal curve. It accumulated during the first 10 hours, owing presumably to the formation of glucose, then diminished as synthesis of starch continued, and finally increased again during the interval from 24 to 50 hours when both glucose and starch remained essentially constant. During this period, unfermentable carbohydrate was formed in an amount that corresponds to the increase in total soluble carbohydrate. It seems reasonable from the correspondence in magnitudes to assume that this increase in unfermentable carbohydrate is also correlated with the increase in organic solids which occurred at the same time.
The behavior of the organic acids is shown in Fig. 2. The change in pH follows a course during the first 15 hours of the experiment which is precisely that to be anticipated from the well known effect of diurnal variation of light on the acidity of the leaves of this species. It is to be noted, however, that the increase in pH began sluggishly, exactly as was observed for the beginning of the increase in starch, but then rose rapidly as synthesis of starch proceeded with maximal intensity. After the expiration of 15 hours, the pH followed a somewhat irregular course, rising and falling through intervals of about 0.3 unit; that is, by amounts that would seem to be greater than can be attributed to experimental error.

The explanation of this behavior is apparent from the curve for the total organic acids. After an initial sluggish drop, the total organic acids decreased rapidly, so that after 20 hours they had attained a level that was about 60 per cent of the amount originally present. This level was maintained until the end of the experiment although with fluctuations that amounted to ±6 per cent of the mean quantity present.

Fig. 2. Organic acids of leaves of *B. calycinum* cultured in water in continuous light for 64 hours.
The determinations of total ether-soluble organic acids in the present experiment were carried out by a newly improved technique. Examination of the differences between independently obtained duplicate determinations on each sample showed that these differences had a standard deviation in terms of a single determination of 3.2 m.eq.; in other words, the precision of a single determination at the mean level of organic acids present after the initial drop was approximately 1 per cent. Accordingly, in spite of the fact that a small sampling error (Table I) is included, there seems good reason to attribute significance to the observed variations in total acidity. Comparison of the curve for pH with that for the total organic acids shows that the variations in these two quantities are faithfully reflected in each other. The correlation coefficient between the data

**Table II**

Correlation Coefficients between Changes in Quantity of Certain Pairs of Components of Bryophyllum Leaves Cultured in Continuous Light

<table>
<thead>
<tr>
<th>Component Pair</th>
<th>Correlation Coefficient</th>
<th>r*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch vs. pH</td>
<td>+0.965</td>
<td></td>
</tr>
<tr>
<td>Total organic acids vs. pH</td>
<td>-0.969</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; starch</td>
<td>-0.981</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; malic acid</td>
<td>+0.996</td>
<td></td>
</tr>
<tr>
<td>Malic acid vs. starch</td>
<td>-0.989</td>
<td></td>
</tr>
<tr>
<td>Citric &quot; &quot; &quot;</td>
<td>-0.661</td>
<td></td>
</tr>
</tbody>
</table>

* Correlation coefficient, \( r = 0.872 \) for probability \( P = 0.001 \) with 8 degrees of freedom; \( r = 0.632 \) for \( P = 0.05 \) with 8 degrees of freedom.

for pH and those for total organic acids was accordingly calculated (Table II) and found to be -0.969; the probability that this is the result of chance is considerably less than 1 in 1000 (\( r = 0.872 \) for \( P = 0.001 \) with 8 degrees of freedom). Since determinations of pH are entirely independent of determinations of total acidity by titration, the argument that the small fluctuations in acidity observed are real is thus a strong one.

In further support of this belief, the correlation coefficient between the total organic acidity and starch was also computed. It was found to be -0.981 (Table II), and the inference is clear that there is a sensitive relationship between the organic acids and the starch under the conditions of the experiment. However, no statement can be made in explanation of the minor fluctuations of both starch and organic acids observed after 20 hours save to point out that there were uncontrolled variations of a few degrees of temperature of the leaves.

The results of the detailed examination of the organic acids are plotted
in the lower part of Fig. 2. Aside from a small temporary decrease in undetermined organic acids early in the experiments, malic acid was the only component which underwent conspicuous change. Table II shows correlation coefficients computed from the data for several of the pairs of components. It is obvious that most of the variation in pH and in total organic acids arose from changes in the quantity of malic acid and that these changes closely reflect the variation in the starch. The correlation coefficients between these sets of data are all significant well beyond the 0.001 point.

The behavior of the citric acid, however, provides an exception, for the correlation with the behavior of the starch was significant only at the 5 per cent point. In previous experiments on the diurnal variation of acidity in Bryophyllum under greenhouse conditions, a correlation between the variation of citric acid and that of starch significant at the 0.001 point has been observed (4). It would appear, however, that so close a relationship is not invariably seen.

Special importance should be attached to the observation that isocitric acid underwent no material change. A single point at 15 hours showed a slight elevation of doubtful significance. However, the subsequent data decrease in a manner suggestive of an over-all loss of about 7 per cent of the isocitric acid initially present; nevertheless, this is evidence of at most a minor utilization of isocitric acid during the period in which the leaves were subjected to stress.

DISCUSSION

That the diurnal variation of acidity in B. calycinum leaves is accompanied by a complementary variation in starch content was first made clear by the investigations of Wolf (9) and has been amply confirmed by subsequent workers (3, 4, 10). Direct qualitative evidence that radioactive carbon dioxide absorbed in darkness by this species is assimilated into the organic acids has been obtained by Thurlow and Bonner (11) and also by Varner and Burrell (12). The latter workers have further shown that, if leaves which have taken up radioactive carbon in darkness are then illuminated in the absence of the isotope, extensive labeling of the starch can promptly be detected, although they observed no significant labeling of starch in leaves maintained in darkness. The evidence thus seems conclusive for the transformation of malic acid into starch when Bryophyllum leaves are subjected to illumination.

The present data afford an opportunity to test whether or not this transformation is quantitative. If so, it should be possible to show that all of the carbon of the malic acid that disappeared in the early hours of the experiment is accounted for by the carbon of the starch that was
formed. The calculation is given in Table III. The molar ratio of the carbon of malic acid which disappeared to that of the starch formed was 0.77. However, it is to be noted that there was an increase in organic solids which amounted to 4.1 gm. during the first 15 hours. If it is assumed that this quantity represents starch that was photosynthesized, and if 4.1 gm. are accordingly deducted from the total increase in starch, the remainder should be that part of the starch which arose from the transformation of malic acid. When this correction is made, the ratio of the molar quantities of carbon is 1.04. The ratio reaches the theoretical value for quantitative transformation (1.00) if it is assumed that about 0.5 gm. of the 4.1 gm. of photosynthesized material represents products other than starch, a not unreasonable assumption. In any case, the close approach

TABLE III

<table>
<thead>
<tr>
<th>Substance</th>
<th>Δ substance</th>
<th>Δ carbon</th>
<th>Δ carbon</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in malic acid</td>
<td>14.99</td>
<td>5.365</td>
<td>447.1</td>
<td>0.77</td>
</tr>
<tr>
<td>Increase in starch</td>
<td>15.75</td>
<td>6.994</td>
<td>582.8</td>
<td>1.04*</td>
</tr>
<tr>
<td>&quot; &quot; &quot; corrected for photosynthesis of 4.1 gm. organic solids</td>
<td>11.65</td>
<td>5.174</td>
<td>431.2</td>
<td>1.04*</td>
</tr>
</tbody>
</table>

* Similar calculations at the 10 hour point give a ratio of 1.02, and at the 20 hour point 1.06.

of the ratio to the theoretical value when the data for starch are corrected for the presumed photosynthesis affords strong support for the hypothesis that malic acid is quantitatively transformed into starch when Bryophyllum leaves are exposed to light. In view of the absence of information regarding the detailed functions of the enzyme systems active in the leaves of this species, speculation on the chemical mechanisms involved is at present premature.

The important point in connection with the present experiment is, however, the behavior of starch and of malic acid in the period after about 15 hours of illumination. The data suggest that there is a definite limit to the quantity of starch that can be formed. When collected at sunrise, the leaves contained 264 m.eq. of malic acid per kilo of fresh weight. After 15 hours of illumination, only 15 per cent of this quantity remained, the mean value being 45.9 m.eq. Fluctuations of the order of ±30 per cent of this mean value occurred during the rest of the experimental period. Notwithstanding the fact that these fluctuations are not well correlated
with those of the starch in the same interval,\(^1\) the behavior is consonant with the view that a reversible reaction or series of reactions had been driven to an extreme position which was maintained only by the continuous influx of light energy. Under such circumstances, the system would be expected to be particularly sensitive to alterations in other conditions such as minor changes in temperature.

Exactly what determines the upper limit to the quantity of starch that can be formed, aside from the practically complete exhaustion of the supply of malic acid, is by no means clear. The photosynthetic mechanism might be expected to provide for a more or less continuous although slow formation. On the contrary, after 24 hours of illumination, there seems to have been a change in the nature of the photosynthetic product which accumulated. Notwithstanding that the starch content underwent small fluctuations, the main event appears to have been the formation of unfermentable carbohydrate, although a limit to the quantity of this component synthesized seems to have been approached at 50 hours; subsequently there was a fall in total soluble carbohydrate and in unfermentable sugar. Whether or not the enzyme systems in the leaves had at this time been driven to what may be regarded as a "breaking point," i.e. whether irreversible damage had been initiated, will require the study of leaves cultured in light for an even more prolonged period.

The increase in unfermentable carbohydrate that began after 25 hours of culture in light led to an inquiry into the identity of the sugar which then began to accumulate. The recent work of Benson et al. (13) on the nature of the carbohydrates formed in the early phases of photosynthesis drew attention to the possibility that the substance might be sedoheptulose or ribulose, and the well known presence of the former sugar in succulent plants suggested an examination of these later samples for heptose. Through the courtesy of Dr. E. Racker of the Department of Biochemistry of Yale University, a new method to determine heptoses, developed by Dr. Z. Dische of Columbia University, was made available, and Dr. Dische kindly consented to its use in advance of publication. The data obtained are shown in Table IV. The coefficient of correlation between the data for heptose and for unfermentable sugar (3rd and 4th columns) is 0.903, and, accordingly, since \( r = 0.882 \) for \( P = 0.02 \) with 4 degrees of freedom, the chances are less than one in fifty that the similarity between the slopes of the respective curves (Fig. 1) that represent these data is fortuitous.

\(^1\) The correlation coefficient between the data for malic acid and for starch in the period from 15 to 64 hours is \(-0.51\), which is not significant even at the 10 per cent level (\( r = 0.67 \) for \( P = 0.1 \) with 5 degrees of freedom). However, the variations in apparent starch content were close to the limits of accuracy of the analytical method, and thus a close correlation could scarcely be expected.
The differences between the data at each point for these two components are shown in the last column. There is no indication of a sequential change, and the figures are constant with a mean value of 0.96 ± 0.15. This suggests that, within the limits of accuracy of the respective analytical methods for heptose and for unfermentable sugar and with consideration of the other experimental errors involved, the fraction designated as unfermentable sugar consists of at least two components, one of which remains essentially constant and the other, which is a heptose, increased during the late period of the culture in light. That the newly synthesized heptose is sedoheptulose rather than some other 7 carbon sugar is at present an assumption, although this seems likely. The precise identification of the sugar must await examination by more specific methods.

The failure of citric acid to change in conformity with the change in malic acid in the present experiment is of interest; it diminished slowly and in a somewhat irregular manner by about 30 per cent of the quantity initially present. In marked contrast, when *Bryophyllum* leaves are maintained in darkness, citric acid increases continuously. Under normal conditions of diurnal alternation of light, citric acid undergoes changes that follow those of the malic acid (3, 4) and there is much evidence in the literature to indicate that this behavior is usually observed (14, 15). Nevertheless, in an early experiment with plants from the same clone as that used in the present study (10), a case was encountered in which the diurnal variation of citric acid was unusually small. It may be pointed out that the intensity of the light to which the present samples of leaves were exposed was...

**TABLE IV**

*Effect of Culture of B. calycinum Leaves in Continuous Light upon Content of Heptose*

<table>
<thead>
<tr>
<th>Time of culture (hrs.)</th>
<th>Heptose (per cent)</th>
<th>Unfermentable sugar (gm. per kg.)</th>
<th>Difference (gm. per kg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.31</td>
<td>0.37</td>
<td>1.24</td>
</tr>
<tr>
<td>26</td>
<td>0.53</td>
<td>0.67</td>
<td>1.55</td>
</tr>
<tr>
<td>32</td>
<td>0.68</td>
<td>0.86</td>
<td>2.01</td>
</tr>
<tr>
<td>38</td>
<td>0.96</td>
<td>1.22</td>
<td>2.18</td>
</tr>
<tr>
<td>50</td>
<td>1.43</td>
<td>1.85</td>
<td>2.98</td>
</tr>
<tr>
<td>64</td>
<td>1.50</td>
<td>1.93</td>
<td>2.70</td>
</tr>
</tbody>
</table>

*Method of Z. Dische (unpublished).*

† Grateful acknowledgment is made to Dr. G. de la Haba of the Department of Biochemistry, Yale University, for the quantitative information obtained with solutions of pure sedoheptulose from which these data were calculated.
posed was low in comparison with daylight illumination in the greenhouse and may not have been sufficient to elicit a pronounced response from the mechanisms which affect the quantity of citric acid. Alternatively, it is not impossible that the continuous loss of citric acid may have been an expression of the physiological stress to which the leaves were subjected. However this may be, the suggestion is implicit in the present observation that citric acid is not necessarily in the main pathway of the enzymatic reactions that bring about the synthesis of starch from malic acid in this species. Further experimentation will be required to decide the matter.

Previous studies of the behavior of isocitric acid during normal diurnal variation of illumination have shown that this substance takes no detectable part in the changes of the organic acids. Accordingly, the observation that there was no significant change in the content of isocitric acid during the first 24 hours of the present experiment is not surprising. It is of interest that there was no subsequent change, save for a small continuous loss after the leaves had passed into a stage at which evidences of physiological stress were becoming increasingly apparent. Isocitric acid is promptly called upon when *Bryophyllum* leaves are exposed to the stresses occasioned by exposure to an abnormal period of darkness. However, it does not seem to be involved to any important extent in the reactions brought about by prolonged exposure to light. The evidence that isocitric acid is not in the main pathway of the reactions concerned with the interconversion of malic acid and starch is thus becoming more and more impressive.

Grateful acknowledgment is made to Dr. Israel Zelitch for the determinations of heptose, to Marjorie D. Abrahams, Katherine A. Clark, and Laurence S. Nolan for technical assistance, and to the Department of Genetics of this Station for the use of the illuminating equipment.

**SUMMARY**

Leaves of *Bryophyllum calycinum* picked at sunrise at the time of high acid and low starch content were exposed to continuous illumination for 64 hours. After about 20 hours, the leaves began to lose water and become flaccid; about 10 per cent of the fresh weight had been lost at the end of the experiment.

An increase in organic solids was observed which continued, although at a diminishing rate, for about 50 hours. Starch accumulated rapidly for 15 to 20 hours and was then maintained, with minor fluctuations in amount, at a high level. Glucose accumulated in small amount for 10 hours and then diminished to a level that was subsequently maintained. Unfermentable carbohydrate remained constant for 26 hours but then slowly increased, owing to the formation of a heptose sugar. The pH
increased rapidly for 15 hours and the total organic acids diminished for the same period, the change being almost entirely due to the decrease in malic acid. During the rest of the experimental period, the total organic acids, malic acid, pH, and starch underwent only minor fluctuations in the amounts present. Citric acid diminished to a small extent throughout the period studied but isocitric acid remained essentially unchanged.

The behavior of the starch and malic acid is closely correlated and, if correction is made for the production by photosynthesis of a quantity of starch equal to the increase in organic solids, the molar relationship between the carbon of the starch and that of the malic acid is such as to indicate quantitative interconversion during the first 20 hours of the experiment.

The formation of a heptose late in the experimental period at a time when accumulation of starch had ceased is in accordance with observations in the literature that sedoheptulose is a product of photosynthesis. However, no explanation can at present be advanced to account for the change in the nature of the photosynthetic product which accumulated as the exposure to light was prolonged.

Additional evidence has been secured that isocitric acid is not directly involved in the transformation of malic acid to starch in this species under the influence of light.

BIBLIOGRAPHY

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J. Biol. Chem. 1953, 205:369-381.

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