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THE EFFECT OF ABNORMALLY PROLONGED ALTERNATING PERIODS OF LIGHT AND DARKNESS UPON THE COMPOSITION OF BRYOPHYLLUM CALYCINUM LEAVES[1]

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In a state of nature, succulent plants such as *Bryophyllum calycinum* respond to the diurnal alternations of light and darkness with a closely correlated rhythmic change in the organic acid and starch content of the leaves. However, when the leaves are exposed to continuous darkness, or, alternatively, to continuous light (9), and the changes in composition which occur are compared with those observed under conditions of normal diurnal alternation of light and darkness, certain differences in the behavior of a number of the components begin to become evident as soon as the period of darkness, or of illumination, exceeds the length of a normal night or of a normal day.

Bennet-Clark (1) some twenty years ago showed that the rapid rise of organic acids that occurs when leaves are first placed in the dark is succeeded by a slow fall which begins after about 12 hours. He also reviewed the early literature on the marked changes in the respiratory quotient which take place at this critical period, an aspect of the problem which has been the theme of much recent research especially by Thomas and his associates (6). Wolf (14) has also examined the changes in the respiratory quotient, as well as in the acidity and the carbohydrate composition, in experiments in which leaves of succulent species have been cultured in darkness for several days. All of these workers have found that the respiratory quotient is close to zero at the start of the dark period but rises to unity in about 12 hours.

In order to obtain additional information on the alterations which occur in the normal course of the chemical reactions on prolonged culture either in light or darkness, samples of *Bryophyllum calycinum* leaves have been exposed to continuous artificial illumination for 24 hours and then to 24 hours of darkness. No intervening period of gradual reduction of the intensity of the light in simulation of twilight was employed since the interest was concentrated upon the response, whether immediate or delayed, to sudden darkness following upon an abnormally long period of illumination.

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EXPERIMENTAL PROCEDURE

The ten samples of leaflets used were collected by the statistical method (13), starting at 5:55 A.M. (sunrise 5:53 A.M.) on October 7, 1952, from 20 plants that had been transplanted to sand in February. The plants had been grown with use of a culture solution through the summer, all branches and suckers being removed, and were about 4 ft high. Each sample contained 60 leaflets 3 of which had come from each of the 20 plants and each of the 30 leaflet positions (i.e., 5 leaflets from each of the 6 leaves) on an individual plant was represented twice in each sample. Because of occasional variations from plant to plant, such as loss or malformation of an individual leaflet, a few substitutions of other but similar leaflets were necessary as the sampling process was carried out. The technique for handling the samples and references to the analytical methods employed have been given in a previous paper (12). Zero hour was taken as 7:00 A.M. when the control sample was placed in the drying oven.

The culture troughs were set up in an insulated room with controlled temperature under a bank of fluorescent lights which gave an intensity of from 700 to 800 fc at the surface of the leaves. During the light period, the temperature remained essentially constant at 20 to 22° C but, when the lights were turned off after 24 hours, the cooling equipment brought the temperature down to 16° C after 8 hours. The compressor was then turned off and the temperature slowly rose to 20° C. The temperature record is shown in table I.

Samples were removed at the times shown in table I, these being chosen so as to give maximal information on the anticipated changes in rate of the chemi-

TABLE I

FUNDAMENTAL DATA ON SAMPLES OF BRYOPHYLLUM CALYCINUM LEAVES COLLECTED BY THE STATISTICAL METHOD AND SUBJECTED TO CULTURE IN WATER FOR 24 HRS IN LIGHT FOLLOWED BY 24 HRS IN DARKNESS

SAMPLE NO.	CULTURE PERIOD	CONDITIONS OF CULTURE *	PER SAMPLE			PER KG INITIAL FW				
			FW AT START	FW AT END	EQUILIBRATED DRY WT	EQUILIBRATED DRY WT	TOTAL N	PROTEIN N	ASH	ALKALINITY OF ASH
	hr	*°C*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*meq*
1	0	.....	413.28	413.3	50.11	121.3	2.21	1.76	10.4	213.0
2	8	L:20	420.32	413.7	49.83	118.6	2.22	1.73	10.3	211.1
3	12	L:22	420.57	406.5	50.34	119.7	2.25	1.72	10.5	209.5
4	18	L:22	415.45	385.2	50.15	120.7	2.16	1.70	10.6	215.6
5	24	L:22	424.71	385.6	51.85	122.1	2.28	1.72	10.5	217.6
6	28	D:18	414.19	374.7	50.54	122.0	2.24	1.70	10.5	215.1
7	32	D:16	408.83	378.8	50.72	124.1	2.23	1.73	10.8	214.8
8	36	D:17	408.10	377.1	51.32	125.8	2.24	1.72	10.7	215.2
9	41	D:20	421.02	386.2	52.71	125.2	2.29	1.70	10.8	219.6
10	48	D:20	414.03	378.6	50.56	122.1	2.19	1.72	10.4	216.0
Mean			416.05		50.81		2.23	1.72	10.55	214.8
S.D.			5.43		0.89		± 0.04	± 0.02	± 0.16	± 2.9
C.V. (%)			1.3		1.8		1.8	1.2	1.5	1.4

* The symbol L signifies in light; D, in darkness.

cal reactions under study. Table I also shows the initial and final weights of the samples together with evidence on the constancy of their composition. In spite of the slight liberties that were necessarily taken with the rigid application of the statistical method of collection, the initial fresh weights of the samples were exceptionally constant, the coefficient of variation being only 1.3 %. The composition with respect to total nitrogen, protein nitrogen, ash, and alkalinity of ash was also constant within satisfactory limits.

EXPERIMENTAL RESULTS

Inasmuch as the data for organic solids contain the analytical errors in the determinations of both moisture and ash, as well as the sampling error, the curve in figure 1 for this component expressed as gm per kilo of initial fresh weight is somewhat irregular. It shows, however, that there was a slow but apparently continuous uptake of organic material throughout the period of illumination and that this uptake continued for about 12 hours after the lights were turned off. That the organic solids of Bryophyllum leaves may increase for many hours under continuous illumination has been shown in a previous paper (9), and that an increase can also be detected during the first 12 to 16 hours when leaves are exposed to darkness is also known (10). Subsequent loss of organic material after a more protracted period of darkness has been repeatedly observed and is to be expected after the process of acid formation has been completed and losses of organic matter from respiration begin to dominate the situation. The present set of samples accordingly behaved, with respect to the increase or loss of organic solids, in a manner which conformed with previous observations.

The curve for the fresh weight at each point of observation, expressed as a percentage of the initial weight of the same sample, shows an effect of prolonged exposure to light that became apparent as soon as 12 hours had elapsed. Excised leaves exposed to normal diurnal alternations of illumination under similar experimental conditions as a rule lose from 2 to 3 % of their fresh weight during the light period but recover nearly all of this loss at night (5). They at no time within 3 days become notably flaccid. After 12 to 16 hours under continuous light, however, Bryophyllum leaves begin to lose weight and flaccidity soon becomes obvious. This phenomenon was also evident in the present instance. The leaves after 12 hours in light were noted to be slightly flaccid and the leaves in all subsequent samples were recorded as being limp although there was evidence of recovery of some of the fresh weight after the lights had been turned off for 8 hours. It seems clear that significant and apparently only partially reversible damage may be done to the water-holding capacity of the leaves when the illumination exceeds in length a normal daylight period. However, this behavior is not invariably encountered, for, in an unpublished experiment carried out in July 1953, the leaves lost only 3 % during 34 hours of illumination and recovered completely during an ensuing 24-hour dark period. Nevertheless, a second exposure to light brought about excessive loss of fresh weight after 16 hours, and it would therefore appear that this somewhat younger and possibly more vigorous lot of leaves ultimately also suffered damage.

The behavior of the starch (fig 1) followed the anticipated course. Starch increased rapidly for 12 hours and reached a maximum in 18 hours but subsequently remained constant until the lights were turned off. Starch then fell rapidly for 12 hours, but the reactions in which starch was being utilized abruptly slowed down at that point and there was little further loss.

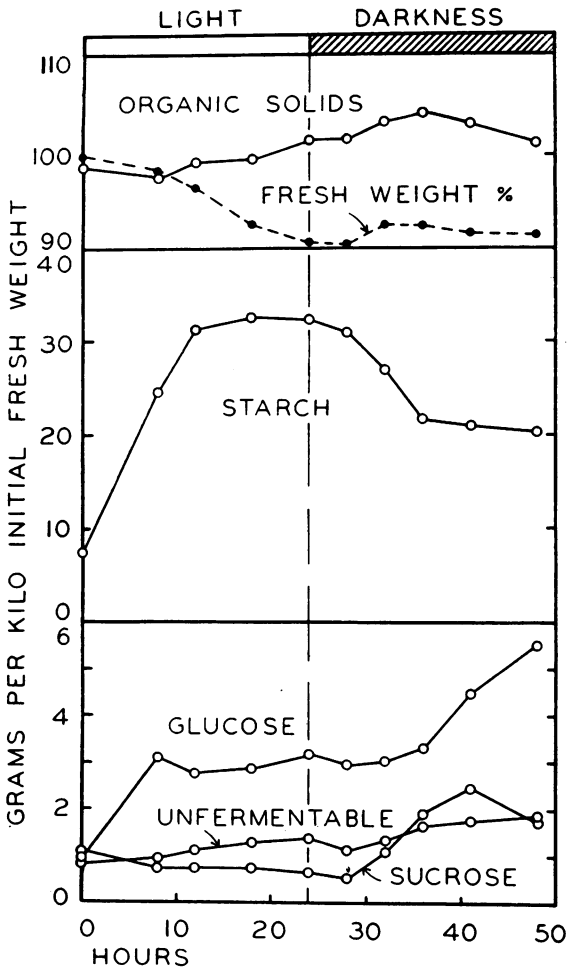

FIG. 1. Changes in organic solids, starch, and soluble reducing carbohydrates in excised leaves of *Bryophyllum calycinum* cultured in water at room temperature for 24 hours in light and 24 hours in darkness.

The sudden change in the rate of utilization of starch after 12 to 16 hours of darkness has been repeatedly encountered (e.g., (3) and unpublished experiments), but different lots of leaves may reach this stage at quite different levels of starch content, the present lot being unusually high in starch when the change occurred. However, the starch metabolism in these leaves was not notably sluggish, as is shown by the large increase during the first 12 hours of exposure to light. It seems possible that excision of the leaves may place a limitation upon the subsequent range of the response of the starch content to changes in light conditions. Indications of such an effect are to be seen in earlier experiments on diurnal variation of starch for 3 and for 2 successive days (5, 10). Alternatively, the failure of the starch to decrease to a low level may have been a delayed result of the prolonged exposure to light.

The behavior of the simple carbohydrates is plotted at the bottom of figure 1 on a scale of ordinates five times larger than that used in plotting the starch. In conformity with previous observations, glucose responded to illumination of the leaves with an immediate increase. However, the increase in 8 hours amounted to only about 2 gm per kilo while that of starch in the same interval was 17 gm. Whether or not the increase in glucose was a result of photosynthesis is not certain. Glucose subsequently remained fairly constant until the critical point after 12 hours of darkness was reached when it again increased.

The so-called unfermentable carbohydrate underwent little change throughout the experiment. There was a slow and nearly continuous rise but it is clear that the moderate drop in temperature in the first few hours of the dark period did not result in an increase in any way comparable to the change in this component that has been observed to occur at 6° C (12).

Sucrose remained constant throughout the light period, as was anticipated, but increased notably

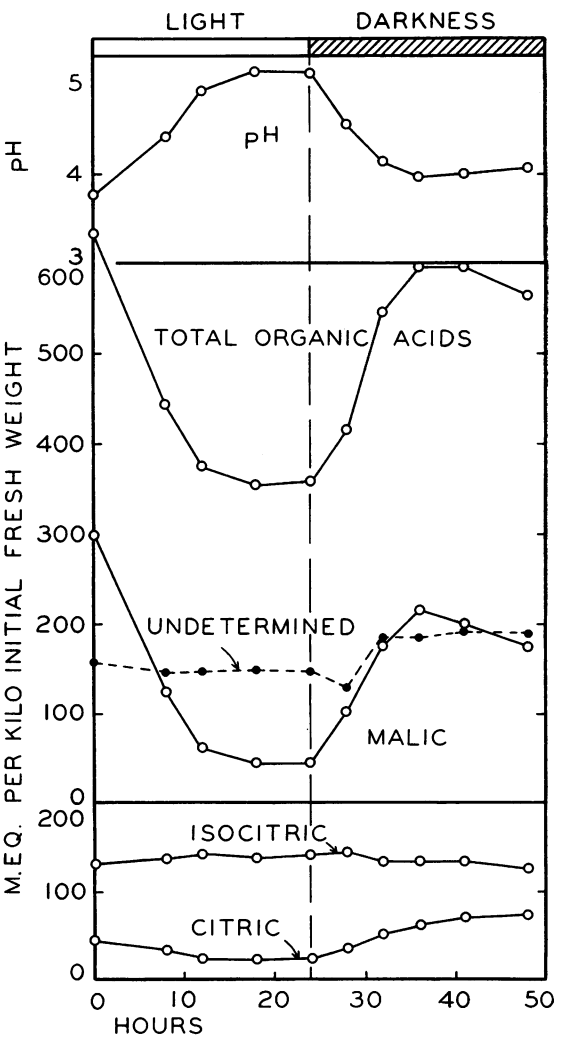

FIG. 2. Changes in organic acids in excised leaves of *Bryophyllum calycinum* cultured in water at room temperature for 24 hours in light and 24 hours in darkness.

shortly after the lights were turned off. It diminished again in the last 7 hours of the experiment. The significance of this behavior is not clear, but the timing of the change was definitely out of phase with the alterations in both starch and glucose.

The behavior of the organic acids is shown in figure 2. The pH increased rapidly for the first 12 hours of illumination and reached a maximum at 18 hours where it remained constant. An immediate drop in pH occurred after the lights were turned off and continued for 12 hours. However, in the last 12 hours of darkness, the pH rose slightly. The explanation of these effects is clearly evident in the curve for total organic acids. Acids were utilized rapidly for the first 12 hours and then slowly for the next 6 hours. There was no further change until after the lights were turned off when a rapid synthesis of organic acids began. This continued for 12 hours but then abruptly ceased and there was a small net loss of acids in the last 7 hours of the dark period. The correlation between the organic acidity and the pH is complete, the coefficient of correlation being -0.973 ($r = 0.872$ for $P = 0.001$ with 8 degrees of freedom).

As is evident from the curves in the lower part of the figure, by far the greater part of the fluctuation in total organic acidity is due to the change in the quantity of malic acid. This dropped rapidly for 12 hours, slowly for another 6 hours, and then remained constant. When the leaves were darkened, malic acid increased rapidly for 12 hours to a maximum and then decreased slowly for the rest of the experimental period. The symmetry of the curve for malic acid with the curve for starch in figure 1 is obvious and the coefficient of correlation between the data for these two components was -0.935 ($r = 0.872$ for $P = 0.001$ with 8 degrees of freedom). An intimate relationship between the reactions which affect the concentrations of these two substances must therefore exist.

Isocitric acid remained essentially constant throughout. The curve suggests a slight increase during the light period and a small decrease towards the end of the dark period, but the data as a whole have a coefficient of variation of 4.1 % which is little if at all in excess of the analytical error. It accordingly seems unlikely that isocitric acid responded significantly to the experimental conditions.

Citric acid behaved as would have been anticipated from previous studies. It decreased during the first 12 hours of the light period to a level which then remained constant, and increased continuously throughout the dark period. Although the changes were not large in terms of actual amount, they were substantial in terms of relative quantities. About one half of the citric acid present at the start had disappeared after 12 hours of illumination; after the lights were turned off, citric acid increased to about three times its minimal level.

The group of undetermined acids remained constant throughout the light period but at first dropped and then increased sharply after the dark period began. Too much significance cannot at present be attached to the apparent changes in these unknown acids since the data are obtained by difference. However, the increase that occurred after 8 hours of darkness was substantial and the higher level was maintained to the end of the experiment. The explanation must await investigation of the detailed composition of this fraction.

Discussion

Consideration of the data plotted in figures 1 and 2, in comparison with the results of studies of the composition of Bryophyllum leaves subjected to normal diurnal alternation of light and darkness (10), or to prolonged illumination (9) or prolonged darkness (11), indicates that the extension of the light or dark period beyond what may be regarded as "normal" has certain well defined effects. The most obvious one with respect to abnormal length of exposure to light is the loss of water-holding capacity of the tissues since this soon becomes apparent owing to the onset of flaccidity. The effect upon the starch and organic acids emphasizes the adaptation of the metabolism of the leaves of this species to the usual length of a daylight period. The normal behavior can be expressed by the statement that malic acid rapidly decreases and starch rapidly increases, these changes being accompanied by a small decrease in citric acid. At the expiration of about 12 hours, however, the rate of change rather suddenly diminishes and the composition of the leaves soon becomes stabilized. Further exposure to light brings about no significant additional alteration in either starch or malic acid content although citric acid may continue to decrease at a slow rate. In effect, the fluctuations in starch and malic acid are rather precisely adjusted in rhythm to the normal diurnal variation in illumination.

There seems to be an upper limit beyond which the starch content of any given lot of Bryophyllum leaves does not increase, although this maximum may differ between fairly wide limits in different lots. Figures close to 30 gm per kilo of initial fresh weight are commonly obtained, but an occasional set of samples may contain as little as 20 gm or as much as 38 gm after exposure to light. There is also a lower limit at which the malic acid content of a given lot of leaves becomes more or less constant, even if illumination is prolonged, and this usually lies between 20 to 40 meq per kilo. Exposure of the leaves to light thus brings them in a period of about 12 hours to a state at which the rates of the chemical reactions in which starch and malic acid are concerned appear to have attained a fairly constant relationship, although there is some evidence that the composition may then vary slightly in response to minor changes in conditions such, for example, as temperature.

Even after the leaves have been exposed to light for an abnormally long period, a sequence of chemical events begins when the lights are turned off which seems to be identical with that observed after nightfall in leaves exposed to the normal diurnal variation of illumination. Starch decreases, malic acid is synthesized with great rapidity, and citric acid increases

slowly. These reactions continue for about 12 hours. At this point, however, a relatively sudden change in the general course of the metabolism supervenes. The rate of decrease of starch becomes slow or stops entirely and, in a few cases observed in this laboratory, a small increase of starch occurred. Malic acid reaches a maximum and then begins slowly to decrease, and if the leaves are kept in darkness for a long time, this substance may even diminish to the level observed at the end of a sunny day.

In a recent paper (12), the behavior of malic acid in leaves cultured in darkness has been attributed to the competition between reactions that bring about its synthesis and reactions that make use of malic acid or of one of its precursors for the production of other metabolic products. These latter reactions appear to be especially sensitive to the temperature at which the experiment is carried out and are greatly depressed in rate if the temperature of the leaves is of the order of 10° C or less. Accordingly, at low temperature, accumulation of malic acid can continue for a considerably longer time than is observed at temperatures of 20° C or upwards. The timing of the sudden apparent alteration of the metabolism of malic acid shown in figure 2 is thus contingent upon the temperature at which the culture experiment is carried out. Nevertheless, even at a temperature as low as 9° C (4), the reactions which make use of malic acid or one of its precursors ultimately predominate; the point at which inflection of the curve occurs is merely postponed.

The molar relationships between the cumulative changes in malic acid and starch at each point of observation are shown in figure 3. After 8 hours of exposure to light, 88.0 millimoles of malic acid had disappeared and 106 millimoles ($C_6H_{10}O_5$ taken as 1 mole) of starch had been formed. Accordingly, the molar ratio of the change in malic acid to that of starch at this point was 0.83; that is to say, for every mole of starch formed during the first 8 hours, 0.83 moles of malic acid disappeared. The ratios of the changes in the two subsequent intervals of 4 and 6 hours between measurements were, respectively, 0.76 and 0.95. However, the actual change in this 6-hour period was small and there was practically no change in either starch or malic acid in the final 6-hour period of exposure to light. As a result, the ratios of the cumulative changes at each point remained essentially constant.

Data from an earlier experiment on culture in continuous light (9) are plotted as a broken line in figure 3. Disregarding the high value for the ratio after the first 5 hours, during which the total change in the quantities of starch and malic acid was small and the reaction was unusually slow, there is again evidence of a considerable degree of constancy. The cumulative ratio ranges from 1.2 to 1.0 and the ratios in the two intervals between 5 and 10 hours and between 10 and 15 hours when a large amount of starch was being formed were respectively 1.04 and 0.99. It would thus appear that throughout the period beginning a few hours after these two lots of Bryophyllum leaves were first exposed to light and during which the major formation of starch and the major loss of malic acid were taking place, there was an approximately constant molar relationship between the quantities of these two substances which underwent chemical change. This relationship was such that, for every mole of starch formed, close to 1 mole of malic acid was used up.

If the transformation of malic acid into starch were quantitative and no other mechanism for the formation of starch were operative, 1.5 moles of malic acid would be needed to supply the carbon for 1 mole of starch. However, if carbon from some other source were available for the formation of starch, for example by photosynthesis, the theoretical ratio would be depressed. The observation that the ratio is not far

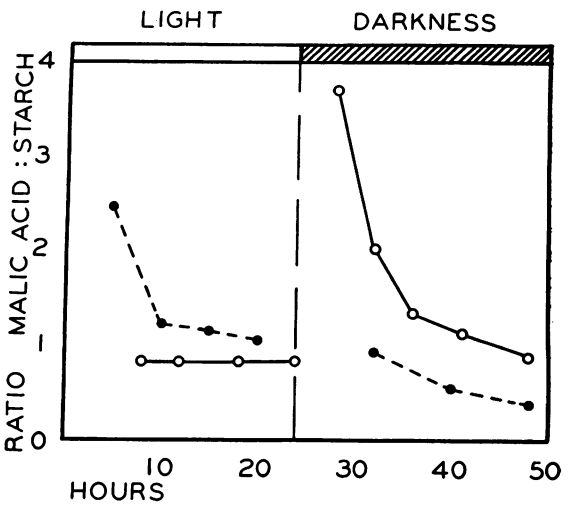

FIG. 3. Variation in the ratio at each point of observation of the cumulative change in malic acid to the cumulative change in starch in excised leaves of *Bryophyllum calycinum* cultured in water for 24 hours in light and 24 hours in darkness. Analogous data from two previously published experiments (9, 12) are plotted as broken lines for comparison.

from 1.0 during the period when starch is being produced in maximal quantity suggests that the average net effect consists in the utilization of a 4-carbon unit derived from malic acid together with a 2-carbon unit, or possibly of two 1-carbon units, for the formation of the 6-carbon unit which is finally converted into starch. The constancy of the cumulative ratio over the greater part of the period in which the leaves were exposed to light suggests that an over-all reaction of this general nature was the major one that occurred.

Nevertheless, so simple a quantitative relationship as this is not invariably observed. In an experiment carried out in 1949 (10), the increase in starch after 10 hours of illumination was 56.6 millimoles and the decrease in malic acid was 92.7 millimoles, the ratio being 1.6. It can only be assumed, in this case, that a part of the malic acid which disappeared may have been utilized in reactions other than the formation of starch. Notwithstanding this, however, the negative

correlation between the data for starch and malic acid was as highly significant as it was in the present case.

The ratios between the quantities of malic acid formed and starch used during the period of darkness are plotted at the right in figure 3. In the first 4-hour period, 28.8 millimoles of malic acid were synthesized and the loss of starch amounted to 7.8 millimoles, the molar ratio of malic acid to starch in this interval being therefore 3.7. After 8 hours of darkness, the cumulative ratio between the quantities involved had dropped to 2.0, and it subsequently fell to 0.9 at the end of 24 hours. Plotted as a broken line are data from an experiment (12) in which the behavior of the starch and malic acid was observed in leaves collected in the afternoon and cultured in darkness at 24° C. This curve is roughly parallel to that for the present experiment although placed lower on the diagram.

Obviously the situation during the early hours after Bryophyllum leaves are deprived of light is extremely complex. Carbon from some source other than starch must have been extensively drawn upon for the synthesis of malic acid in the first 4 hours and it will be recalled that this falls within the period when, according to the literature, the respiratory quotient is not far from zero and when it may even assume negative values if the air surrounding the leaves is enriched in carbon dioxide (2, 6). Under the latter circumstances, acid formation is enhanced, and Thomas and Ranson have shown that the formation of acid is distinctly greater in ordinary air containing 0.05 % of carbon dioxide than it is in air deprived of carbon dioxide. Furthermore, both Thurlow and Bonner (7) and Varner and Burrell (8) have demonstrated that malic acid quickly becomes labeled with isotope if Bryophyllum leaves are exposed in darkness to an atmosphere containing radioactive carbon dioxide. Accordingly, there is little doubt that a part of the carbon of the malic acid formed in the early phase of the exposure of the leaves to darkness was derived from externally supplied carbon dioxide. The increase in organic solids (fig 1) which occurred during the first 12 hours of darkness in the present experiment provides additional evidence in favor of this view.

In the interval between 4 and 8 hours of exposure to darkness (28th to 32nd hour), the molar ratio of the malic acid formed to the starch used dropped to 1.5 and in the period from 8 to 12 hours to 0.64. Accordingly, it would appear that the share in the synthesis of malic acid taken by substances other than starch diminished materially throughout these two intervals. After 12 hours had elapsed, malic acid reached a maximum and no significant further drain upon the supply of starch was evident. Reactions quite different in their effect upon the composition of the tissues then assumed dominance, the most easily recognized being reactions presumably involved in the general respiratory process that led to the decrease of malic acid. At this critical point, therefore, the leaves passed into a phase that became increasingly abnormal as the period of culture was prolonged.

To recapitulate the evidence in favor of such a view, the respiratory quotient rises from zero or a small positive quantity to approximately unity (1, 6, 14), utilization of starch and formation of malic acid cease, and malic acid begins to decrease. At least in the present case, glucose also began to increase. These considerations suggest that, in experiments designed for the study of the details of the reactions that occur in darkened succulent leaves, attention should be given to the preparation of the samples for the contemplated tests. The literature contains many cases in which leaves have been retained in darkness for prolonged periods in order to make sure that the starch content had been brought to its lowest possible point and that the acid content had reached a maximum. The present data suggest that such efforts may be self-defeating if the object of the tests is to study the "normal" behavior. The experience of this laboratory indicates that the adaptation of the metabolism of the Bryophyllum plant to the length of a night is so close that exposure of the leaves to darkness for much more than 12 hours at ordinary temperature brings about a situation in which the major chemical reactions that can then be demonstrated are no longer those characteristic of the behavior of leaves of plants exposed to normal conditions.

Emphasis should perhaps be placed on one further point. Although all of the evidence indicates that reactions occur in Bryophyllum leaves in which malic acid is converted into starch in light and starch is converted into malic acid in darkness, this apparently reversible interconversion is the result of a complex sequence of reactions which follow pathways that may well differ in detail in the two directions. Furthermore, there are accompanying reactions, in particular photosynthesis in light and carbon fixation in darkness, to say nothing of the correlated metabolism of citric acid, which have their effect upon the main result. The situation is thus far from simple from the chemical point of view and detailed explanation in terms of definite enzymatically controlled reactions is still a matter for future research.

Summary

Leaves of *Bryophyllum calycinum* picked at sunrise were cultured in water in light for 24 hours followed by culture in darkness for 24 hours at room temperature. The behavior of the starch, organic acids, and simple carbohydrates during the first 12 hours of each of these two periods conformed in general with that to be expected from previous studies of the changes in composition of this tissue when the leaves are exposed to the normal diurnal variation of illumination. However, in the second 12 hours of each period, the rates of the reactions which affect the starch and malic acid content became very slow and malic acid began to decrease towards the end of the period of darkness. Organic solids increased throughout the light period and for the first 12 hours of the dark period but then began to decrease. Glucose increased during the first 8 hours of the light period to a level that was maintained until the last 12 hours of the dark period when it again increased.

The conclusion is drawn that the time required at

room temperature for the major changes in starch and organic acids to occur is rather closely adapted to the period of the normal diurnal variation in illumination. The range through which the concentration of these components may move in response to the diurnal alteration of light and darkness is moderately constant for any given lot of leaves and at ordinary temperature is not increased by prolonged exposure either to light or to darkness.

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CHLORINE—A MICRONUTRIENT ELEMENT FOR HIGHER PLANTS[1]

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This article presents evidence of the essential nature of chlorine for the growth of higher plants and the proposal for its classification with the micronutrient elements. The experiments serve to support many past observations suggesting beneficial effects derived from fertilizers containing chlorine. Of particular interest are the controlled culture solution experiments of Eaton (2) and Raleigh (6) which showed highly significant increases in yields of tomatoes and cotton, and of beets, respectively, when supplied with additional chlorine. It also supports earlier work of Lipman in 1937 (4) who, after directing his attention specifically to chlorine as a growth factor for buckwheat concluded that "if chlorine is not essential, it is certainly highly beneficial."

Evidence of chlorine as a plant micronutrient offered in this paper seems conclusive beyond doubt, since inherent chlorine contaminations in culture solutions were controlled well enough to produce the nutritional disease in severe form, showing leaf symptoms of wilt, chlorosis, necrosis, and an unusual bronze discoloration which in combination are characteristic of no other known nutritional or pathological[3] disease of tomatoes. It has also been possible to maintain control at different levels of chlorine so that other significant data have been made available including yield versus chlorine supply, and the chlorine contents of roots, stems, and leaves at the various levels of chlorine deficiency. Further observations have been made

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