THE INTERRELATION BETWEEN CO_2 METABOLISM AND PHOTOPERIODISM IN KALANCHOË [1]

F. G. GREGORY,[2] IRWIN SPEAR,[3] AND K. V. THIMANN
BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS

Many studies have been made of the flowering response of plants exposed to alternating conditions of light and darkness. The present state of knowledge on this subject is reviewed by Lang (12). These studies have given rise to a number of schemes which attempt to explain photoperiodism by means of specific reactions which are supposed to occur in the dark and light phases of photoperiodic cycles, and by the formation in the leaf of flower-promoting and/or inhibiting substances under the influence of light and dark reactions. Diurnal endogenous rhythms of alternating "photophil" and "skotophil" phases have also been invoked to account for the varying behavior of long and short day plants (4), but no direct evidence of diurnal changes in metabolism has so far been presented to substantiate this theory.

In contrast with this wealth of speculation little has been done to investigate changes in the metabolism of plants, resulting from alterations in the photoperiodic regimes to which the plants are submitted. In spite of the well-established fact that a supply of CO_2 during the light phase is essential for the flowering response of short day plants (soybean: Parker and Borthwick, 13; Kalanchoë: Harder and von Witsch, 11) the change in assimilation rate during induction has not been directly investigated, nor has the need for CO_2 during the dark phase of the cycle.

Some interesting experiments have been carried out on isolated leaves and shoots taken from plants exposed to different day-lengths (Bode, 2; Schmitz, 15). These data, which will be taken up in more detail later, apparently show that, in Kalanchoë plants, the length of day affects both the photosynthetic rate in the light and the pattern of CO_2 production in the dark.

The suggestive nature of these findings, and particularly the general absence of basic information in this field, indicated the need for a more thorough investigation of assimilatory and respiratory changes in relation to photoperiodism. Such an investigation, it was felt, must of necessity be carried out with whole plants, at least at first, so that correlations between metabolic behavior and flowering could be made where possible.

It is well known that the group of plants known as succulents have diurnal fluctuations in their organic acid level and that they have the ability to fix large amounts of carbon dioxide in the dark (1, 3, 5, 18, 19, 20). With these facts in mind, it was decided to study the CO_2 metabolism of a succulent plant as a function of photoperiod.

The plant selected for study was *Kalanchoë Blossfeldiana*, a short-day plant, whose compactness, ease of handling, and slow growth rate make it well suited to the present purpose. It had already been used for investigations both of flowering (Harder, 9) and of leaf metabolism (2, 15). The only disadvantage of Kalanchoë is the large number of short days needed for floral induction. Under the conditions of temperature and light used, a minimum of 13 or 14 short days are required for any flower primordia to be subsequently formed, while a maximum response is only obtained with 20 to 25 short days. This disadvantage has so far not proved serious.

MATERIALS AND METHODS

Kalanchoë Blossfeldiana var. Tom Thumb was used for most of the experiments described, but in the preliminary experiments at Imperial College, London, another dwarf variety, Ernst Theide, was employed. The plants at Harvard were raised from cuttings taken from plants which had been maintained on long days. They were then grown in air-conditioned light-rooms where the temperature was maintained at 19°C and the relative humidity at 75 %. The length of day was maintained at 16 hours. A light intensity of 1500 fc at the level of the plants was obtained by using incandescent and fluorescent lamps, balanced in such a way as to produce a spectral distribution of light intensity similar to that of sunlight.

For most of these experiments, the plants were enclosed in gas-tight chambers as shown in figure 1. The seal is effected by affixing a slit piece of 7/16″ (O.D.) gum rubber tubing around the lower portion of the stem by filling in the space with latex. The tubing is slightly larger than the hole in the brass base plate of the chamber. The base plate is assembled and when bolted together forms a gas-tight seal around the rubber tubing enclosing the stem. The Lucite cylinder slips over the plant, and is pressed against a rubber gasket in the base plate by another brass plate at the upper end. Air is circulated by means of a small inlet nozzle in the base plate and an outlet in the top of the Lucite cylinder. The orifice of the inlet is only 0.5 mm in diameter, and this causes the air to enter in a jet, thereby facilitating mixing. When tested, the chamber did not leak even when subjected to 14 to 20 lbs pressure per square inch, which is many times that used. This type of chamber has proven to be very effective, especially since long-term experiments with the same plant are possible.

The use of a gas-tight chamber, though essential

[1] Received May 20, 1953.

[2] Imperial College of Science and Technology, London S.W. 7, England.

[3] The contents of this paper constitute a part of a thesis submitted by I. Spear to the Graduate School of Harvard University in partial fulfillment of the requirements for the Ph.D. degree, May 1953. Present address: Department of Botany, University of Texas, Austin, Texas.

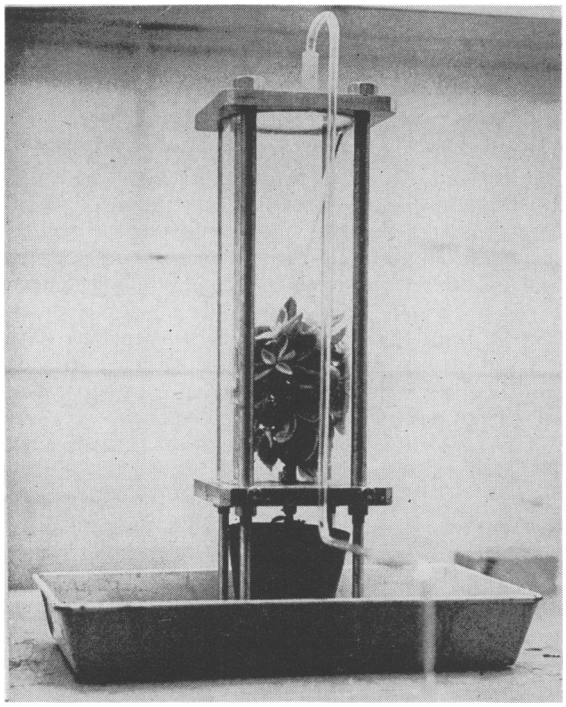

FIG. 1. Gas-tight plant chamber used to study CO_2-metabolism of *Kalanchoë Blossfeldiana*. Description in text.

for metabolic experiments, has the disadvantage that the temperature within the chamber varies with the lighting. Although the room was maintained at 19°C the temperature within the chamber reached 29°C (by thermometer) under the lights. Although the leaves were doubtless cooled somewhat by transpiration in the rapid current of air, still their temperature was certainly not constant.

Most of the CO_2 measurements were made by means of an Infra Red Gas Analyzer (supplied by Messrs. Sir Howard Grubb, Parsons and Company), which gave instantaneous direct readings of the CO_2 content of the air entering and leaving the chambers. Because the CO_2 content of the incoming air was not completely constant, the data are presented as a percentage of the CO_2 content of the incoming air. The absolute values for CO_2 content varied between 0.04 and 0.05 %. For the preliminary experiments NaOH absorption towers were used in addition. For this purpose and for the regulation of flow rates in all of the experiments, a modification of the apparatus described by Porter, Pal and Martin (14) was used. A rate of flow of 27.5 ± 0.5 liters per hour was maintained through the chamber during the 24 or more hours of a run. A detailed description of the modified apparatus, and of the construction of the chambers, is given by Spear (17).

RESULTS

PRELIMINARY EXPERIMENTS: The first experiments were performed at Imperial College, London, in the greenhouse, with the plants therefore subject to varying conditions of temperature and light. Cuttings with their bases in diluted Hoagland's solution were used. The total CO_2 exchange during a period of several hours in the light or in darkness was determined by absorption in towers containing NaOH. The results of one series are summarized in table I. Although the data show some variability, particularly in the light phase, certain general conclusions are evident. In all but one case, there is a net uptake of CO_2 in the dark, indicating that the rate of fixation exceeds that of respiration. For the plants on short day it can be seen that, as the number of short days of pretreatment increases, the uptake of CO_2 in the dark increases, reaching very considerable values. Correspondingly, the net uptake of CO_2 in the light (photosynthesis) decreases. The plants on long day show no such trend, but only minor variations. It is evident, therefore, that the total CO_2 metabolism is strongly influenced by photoperiodic treatment.

Other preliminary experiments made it clear that closer control of external conditions was essential, and that whole plants rather than cuttings would be desirable. All of the succeeding experiments were therefore performed in the air-conditioned light rooms at Harvard, using whole plants. The plants were set at a fixed distance from the lights, with the entire shoot enclosed in the chambers shown in figure 1.

THE DIURNAL VARIATION OF CO_2 METABOLISM: Plants which had always been maintained on non-flower-inducing 16-hour photoperiods ("long days") were placed in the chambers and their CO_2 exchange was followed for one or two 24-hour cycles with long days. They were then transferred to 8-hour photoperiods ("short days"), and were at intervals replaced in the chambers for 24 hours or more, in order to study their gas exchange under short day conditions after having received increasing numbers of short days.

Figures 2 and 3 present the data from a single plant so treated. For ease of presentation the dark and light phases are presented separately, figure 2 giving the CO_2 metabolism in the dark phase. The broken lines show the CO_2 uptake during the 8-hour dark periods following exposure to 16 hours light. Two successive "nights" are shown for the plant, which had not previously received any short days. When the lights go off at midnight (24 hrs.) photosynthesis ceases but there remains a small, though

TABLE I

UPTAKE OF CARBON DIOXIDE BY KALANCHOË CUTTINGS ON LONG AND SHORT DAYS

NUMBER OF SHORT DAYS	CO_2 UPTAKE IN MG CO_2/HR × DM^2			
	EXPERIMENTAL PLANTS ON SHORT DAY		CONTROL PLANTS ON LONG DAY	
	Dark	*Light*	*Dark*	*Light*
13	0.05	1.27	0.03	0.55
22	0.44	0.92	...	0.80
28	0.47	0.87	−0.03	0.70

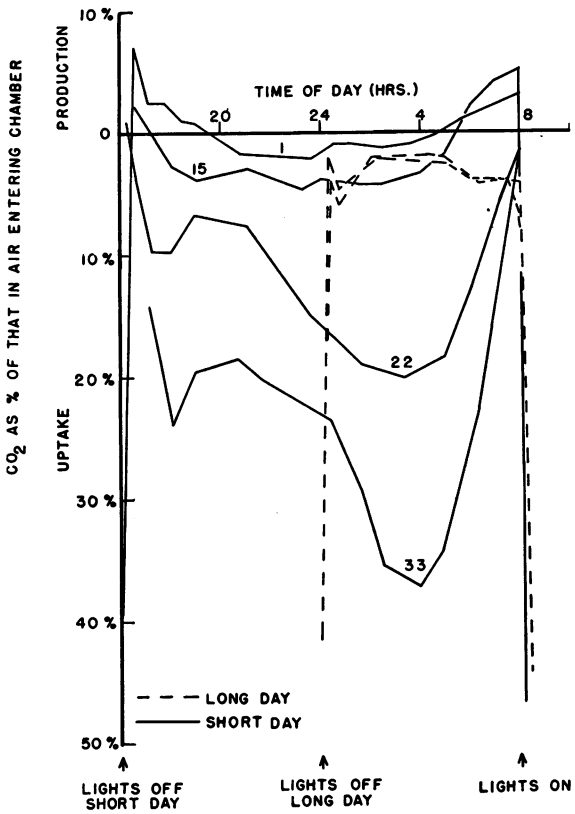

FIG. 2. Carbon dioxide metabolism of Kalanchoë in dark phase under long-day conditions (broken lines) and during the 1st, 15th, 22nd, and 33rd long nights (solid lines).

significant, net dark uptake of CO_2. When the lights come on at 8:00 A.M. photosynthesis resumes and there is a precipitous increase in the rate of uptake of CO_2. This behavior is as would be expected for a succulent plant.

The solid lines show the corresponding behavior in the 16-hour dark phases following 8-hour photoperiods. In the first of these long nights (topmost curve of fig 2) the net CO_2 exchange is small, but in the 15th, 22nd, and 33rd long nights there is a steady increase in the rate of dark fixation of CO_2. The fixation passes through a characteristic cycle, increasing to reach a maximum not later than 12 hours after the onset of darkness, and then decreasing to reach values close to zero before the lights come on at 8:00 A.M.

Figure 3 shows the course of CO_2 metabolism during the light phase. Again the broken lines represent the CO_2 uptake under long day conditions, before the plant has been exposed to short days. In the two experiments recorded, photosynthesis begins rapidly and after a slight recession shows a steady tendency to increase throughout the light period, falling very abruptly as soon as the light is turned off. The solid lines show that the course of CO_2 exchange after exposure to short day cycles is very different. Already after one short day there is a temporary fall in the rate of uptake, followed by a steady rise throughout the rest of the light period. In the other curves these changes become progressively more marked. After 14 cycles of short days the preliminary fall in photosynthesis brings the uptake almost to zero in 2–4 hours; after 21 short days the uptake after 2 hours gives place to a loss of CO_2, while after 32 cycles loss of CO_2 begins soon after the light is turned on, again passing through a maximum. The maximum point is followed in all cases by a rapid increase in CO_2 uptake, and by the end of the light period all the curves approach the same maximum value, which is not very different from that of the plants kept in long days. In other words, plants which have received 33 short days show a net loss of CO_2 during the light phase, and a large net uptake of CO_2 in the dark.

These experiments have been repeated four times with similar results.

INTERRUPTION OF DARK PERIOD: In "short day plants," that is, plants which initiate flowers only after one or more long nights, the effectiveness of a long dark period may be completely annulled by a short period of illumination (8), particularly when given near the mid-point of the dark period (10). It was therefore important to determine whether a brief interruption of the dark period affected the metabolic changes described above.

In the first experiment of this sort, a plant was studied under 16-hour long days as before, and then transferred to 8-hour short days. After six such short days it was replaced in the chamber and found to have a CO_2 metabolism characteristic of plants ex-

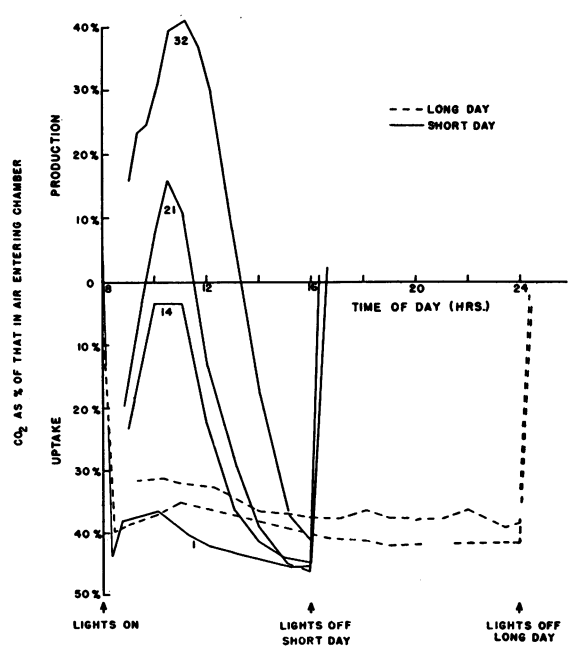

FIG. 3. Carbon dioxide metabolism of Kalanchoë in light phase under long-day conditions (broken lines) and after 1, 14, 21, and 32 long nights (solid lines). Same plants as in figure 2.

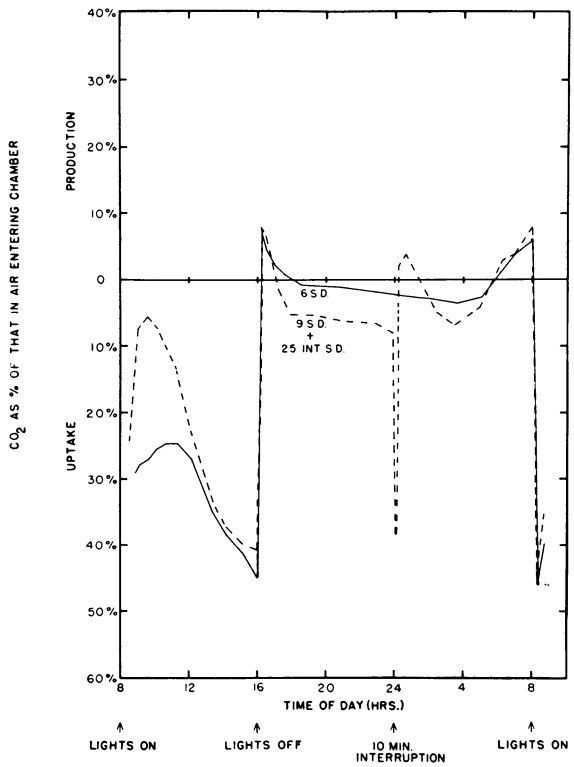

FIG. 4. The diurnal variation in CO_2 metabolism of Kalanchoë after 6 short-day cycles (solid line), and after 9 short-day cycles with 25 additional interrupted short days (broken line). The 10-minute interruption with 1500 fc was at midnight (24 hrs.), the mid-point of the dark period.

posed to a few short-day cycles (i.e., between those shown for 1 and 14 days in figures 2 and 3). The data are shown in figure 4 (solid line). After nine short days the 16-hour dark period was interrupted at its mid-point by 10 minutes of full light (1500 fc). This treatment was continued daily for an additional 25 days, and the CO_2 metabolism of the plant was re-examined at intervals. It was found that the interruption markedly decreased the further development of the characteristic short day pattern, so that there was relatively little increase in the dark uptake of CO_2, and no net production of CO_2 in the light (fig 4, broken line, cf. fig 2 and 3). It appears that interrupting the long dark period, after 9 unbroken short days, is not completely effective in arresting the metabolic changes, but does so to a considerable extent. However, this plant when returned to long days later produced two single flower primordia, even though under the conditions of these experiments no other plants have ever formed flowers with a treatment of less than 13 uninterrupted short days.

In subsequent experiments no pretreatment with short days was given, but plants grown in long days were exposed for prolonged periods to cycles of 8 hours light and 16 hours darkness, with and without interruption of the dark period. The data from one of these experiments is presented in figures 5 and 6. Here again, both plants were first studied under long days, of which two such cycles are averaged and presented in figure 5A. The plants were then transferred to 8-hour photoperiods and their carbon dioxide metabolism was re-examined during the 1st, 7th, 14th, 21st, and 35th cycle (figs 5B through 6D respectively). The broken lines in figures 5 and 6 refer to the plant that received interrupted long nights, while the solid lines refer to the uninterrupted control. The interruption comprised 10 minutes of full light intensity. Although the plants were selected for uniformity their leaf areas were not identical, which accounts for the small discrepancy in the replicates in figure 5A.

It is evident that the plant grown in uninterrupted long nights developed a progressively increasing dark CO_2 uptake and an associated transient production of CO_2 in light, quite similar to those shown in figures 2 and 3. In the companion plant, however, which received briefly interrupted dark periods, this progressive fixation and production of CO_2 is almost completely absent. It is to be noted that the total duration of its light and dark periods does not differ appreciably from that of the controls. It follows that the metabolic changes resulting from short days are true photoperiodic phenomena and are not the result of differences in total light received.

EFFECT OF PROLONGED DARK AND PROLONGED LIGHT PERIODS: It is clear from figures 2, 6C and 6D that the CO_2 fixation in darkness decreases rapidly after the first 12 hours and has usually ceased before the end of the dark period. A study was therefore made of the CO_2 relations in prolonged darkness. Figure 7 shows the effect of such a prolonged dark period on a plant which had previously received 34 short day cycles. During the first 12 hours of dark the typical increase in dark fixation of CO_2 occurs, followed by a rapid decline, so that after 16 hours (i.e., at 8:00 A.M.), dark uptake gives place to continuous production of CO_2. Presumably this is the normal rate of respiration for this plant. The slow decrease in the rate of CO_2 production during the remainder of the 48 hours of darkness may be explained by a decreasing supply of substrate. It is interesting and significant that the 15- to 16-hour period which seems to be required for the dark fixation to run its course corresponds exactly to the optimal length of the dark period, as determined by Harder (9), on the basis of numbers of flowers formed. This observation may provide a physiological meaning for such an optimum. It will be noted also that there is no sign of an endogenous rhythm in the dark CO_2 fixation, nor in the normal respiration rate.

When the lights were turned on at the end of this 48-hour dark period the burst of CO_2 production was similar to that noted after a 16-hour dark period. This means that the substrate responsible for this burst of CO_2 has not been decomposed in prolonged darkness.

The effect of prolonging the light period to 24

hours on plants pretreated with 34 short-day cycles is shown in figure 8. Following upon the usual immediate burst of CO_2 production, which is complete in 4 hours or so, photosynthesis rapidly increases and then remains constant. Again there is no sign of an endogenous rhythm.

Discussion

1. The Fixation of CO_2 in Dark: In plants pretreated with short-day cycles, CO_2 uptake ceases immediately on darkening and then begins again at an increasing rate, reaching a maximum velocity in about 12 hours. The plants kept in long days show only a very slow uptake of CO_2 in darkness. The most probable interpretation of this phenomenon is that an enzyme system which catalyzes the dark uptake of CO_2 is produced during the long dark period. The properties which this enzyme must have are discussed further below.

In so far as they are comparable these data agree with those of Schmitz (15), who found that when leafy shoots from plants receiving long nights of 12 or 18 hours' duration were placed in the dark in CO_2-free air, they evolved very little CO_2 (at 20°C) for the first part of the dark period, but that after 12 hours of darkness CO_2 was evolved. Schmitz's data can all be interpreted in terms of a retention of CO_2 following the light phase. When the duration of the light phase was decreased the amount of CO_2 retained was also decreased. Even under these conditions the CO_2 evolution generally showed a minimum at about 12 hours.

The increase and subsequent decrease in the rate of dark CO_2 fixation in plants on short days (or long nights) may be due to two factors: (a) stomatal movements, (b) the production and removal of a CO_2 acceptor by dark reactions.

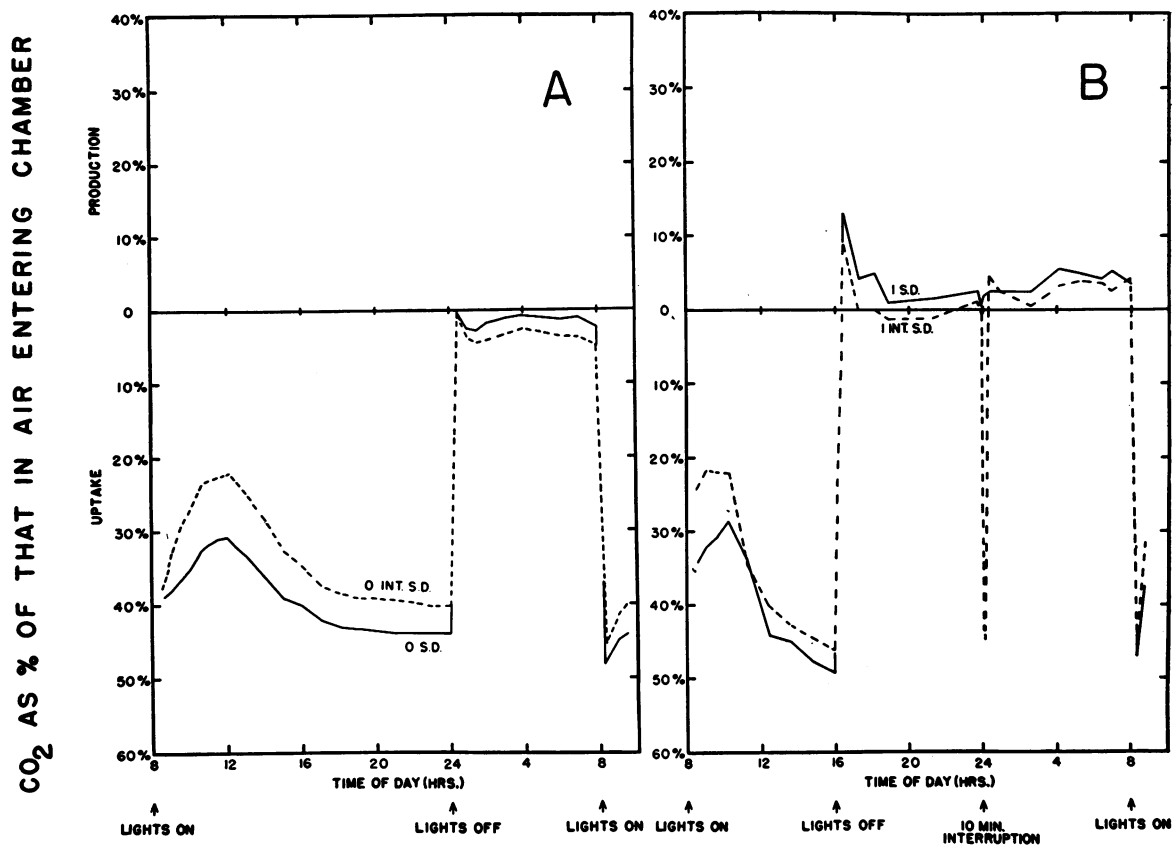

Fig. 5. The diurnal variation in CO_2 metabolism of Kalanchoë under long-day conditions (A) and after one long night (B) with (dashed line) and without (solid line) 10-minute interruption of the long dark period by 1500 fc. In A the dashed line represents the metabolism of the plant which will receive the interrupted nights.

(a) Schwabe (16) has shown in the Chrysanthemum that stomatal behavior in the dark period depends upon the previous photoperiodic regime. Plants given short days show a rapid closure at first, followed by opening in darkness, so that by the end of a 16-hour dark period the aperture is not much less than the maximum. Plants given long days show immediate stomatal closure and no appreciable opening in the dark. Schwabe has qualitatively observed similar behavior in Kalanchoë. If this behavior were to apply to the plants in our experiments it would mean that in the plants given long days external CO_2 would be largely excluded, and it might help to explain the

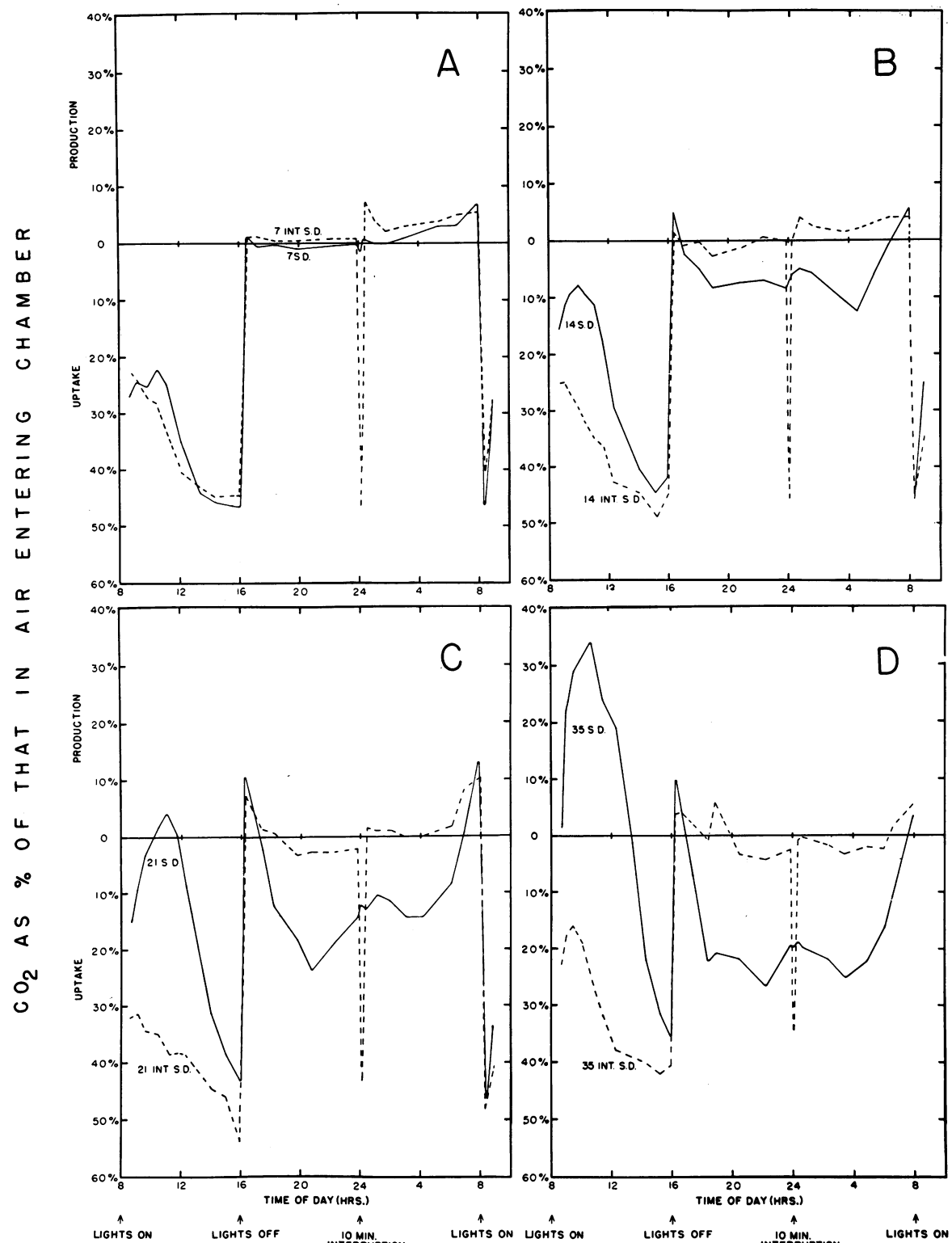

FIG. 6. The diurnal variation in CO_2 metabolism of Kalanchoë after 7 (A), 14 (B), 21 (C), and 35 (D) long nights with (broken lines) and without (solid lines) 10-minute interruptions of the long dark period by 1500 fc. The interrupting light was at midnight (24 hrs.).

absence of dark fixation. However, in plants given short days, the significant production of CO_2 observed at the beginning of the dark period, and the fact that dark fixation of CO_2 rapidly declines after reaching its maximum (just when the stomata are opening in Schwabe's experiments), do not support the idea that stomatal movement is the main factor controlling the CO_2 exchange. It is to be noted also that Schmitz's experiments with plants on very short days show vigorous CO_2 evolution during the first hours of the dark period.

(b) The acceptor for CO_2 is at present unknown. The increasing rate of fixation in the dark might be due to the necessity of a dark reaction for the formation of an acceptor. Since dark fixation is dependent on the preceding light period (fig 6) the precursor from which the acceptor is formed might be carbohydrate or a related photosynthetic product. In plants on long days, the concentration of this material may well become higher, but these plants do not possess, according to the above theory, sufficient of the enzyme system for appreciable CO_2 fixation. In Schmitz's experiments the plants given higher light intensity gave the greater CO_2 fixation in the dark. In the absence of any evidence this explanation seems perfectly plausible.

The cessation of dark fixation could then have two possible explanations. First, it could be due to exhaustion of the acumulated CO_2 acceptor. Secondly, however, it could be due to translocation of the acceptor out of the leaves. To complete the translocation could well require 12 hours. This alternative is supported by the experiment of Bode (2) which showed that increased metabolism occurred in a leaf kept on long day, but so situated that products were translocated to it from a leaf below given short-day treatment. No decision can be made between these two possibilities at present.

2. The CO_2 Burst in Light: The burst of CO_2 production, which occurs for several hours after the beginning of the light period, is presumably due to the destruction of a thermo- or photo-labile substance or substances. Since carbon dioxide is given off, the reaction would appear to be the decarboxylation of an organic acid. This substance is not metabolized away during a long dark period of 48 hours (fig 7). Whether its decarboxylation is due primarily to light or to high temperature cannot be decided from the present data, but must await additional experiments.

There appears to be a direct relationship between the fixation of CO_2 in the dark and its production in the light. In plants kept on long days, neither process occurs appreciably. As one of the processes increases, following successive exposures to short days, so does the other. Taking 43 % uptake as the average level of the CO_2 metabolism during photosynthe-

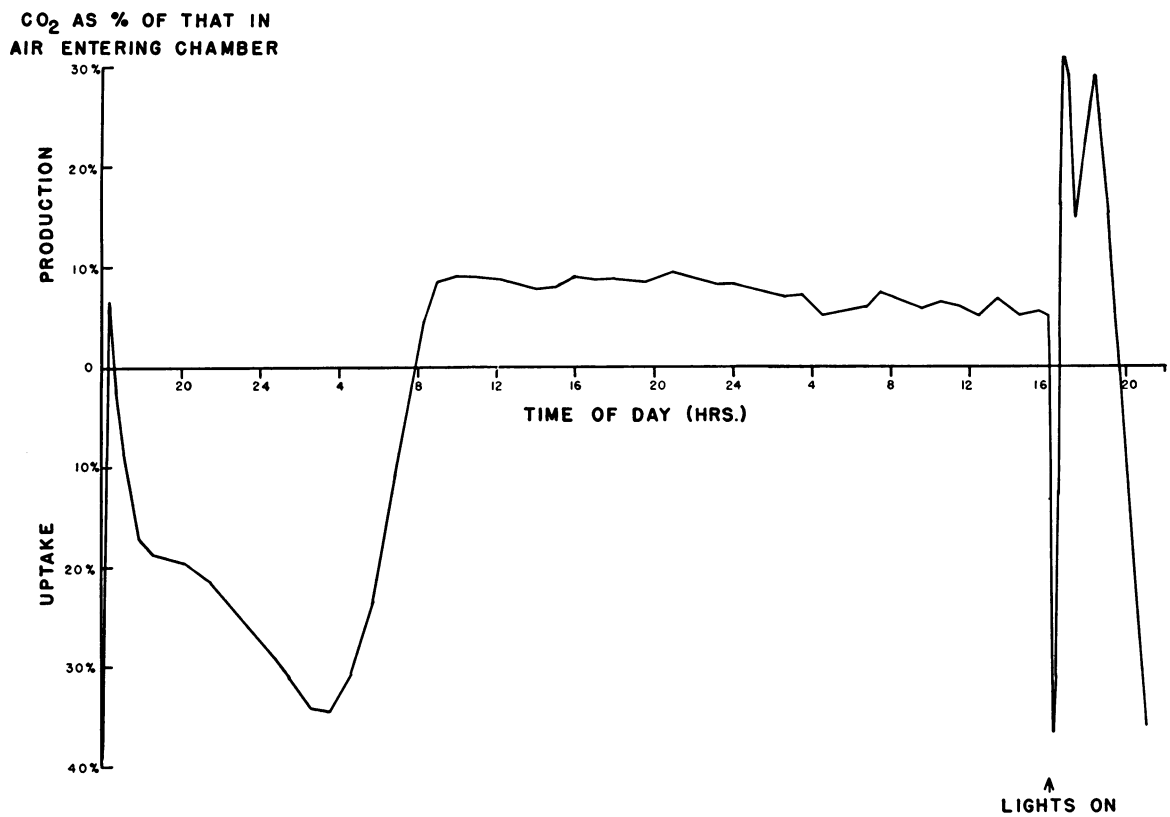

Fig. 7. The CO_2 metabolism of Kalanchoë during a prolonged dark period of 48 hours and 4 additional hours in the light.

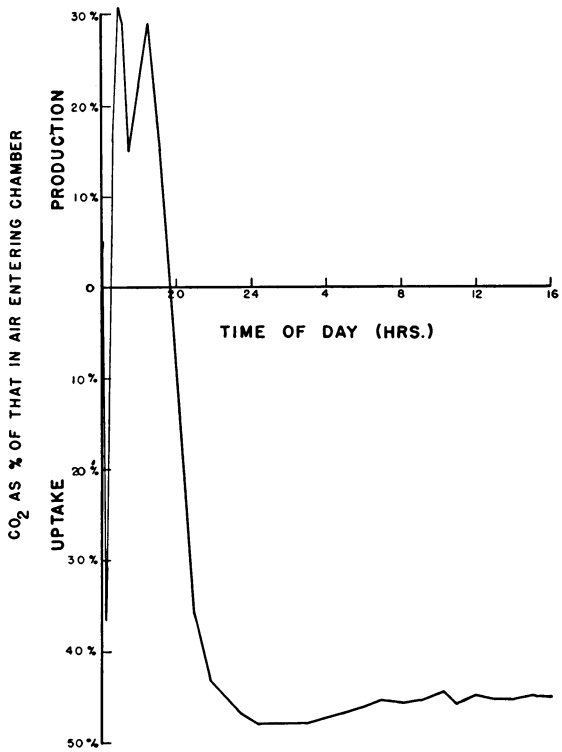

FIG. 8. The CO_2 metabolism of Kalanchoë during a prolonged light period of 24 hours.

sis, the extent of CO_2 production in light can be estimated as the area under the curve (see for instance the 32-short-day curve (fig 3). Similarly if we take 0 % as the average CO_2 production in dark of long-day controls, the dark uptake can be similarly assessed as the area above the curve (33-day curve of fig 2). These areas are almost identical. It is probable, therefore, that the dark fixation directly forms the material decarboxylated in light. It is suggested that this CO_2 burst may be similar in nature to the much smaller burst encountered in Chlorella (6).

3. THE EFFECT OF REPEATED SHORT-DAY CYCLES: The outstanding fact about the fixation of CO_2 in darkness and the associated outburst of CO_2 in light is that the processes are intensified as the number of short days given as pretreatment is increased.

The summation of the effects of repeated cycles of short days could be accounted for by the following hypotheses:

(a) The necessary enzyme system, or coenzyme, for the dark fixation is gradually developed after repeated short day cycles; that is, it requires a long dark period for its formation.

(b) The substrate for the dark fixation, i.e., the CO_2 acceptor, is formed by rapid dark reactions from a precursor which is formed in the light. Hence plants on long-day cycles would contain the precursor but not the enzyme.

The experiments of Bode (2) and of Schmitz (15) discussed above are of further interest in this connection. Bode found that detached leaves from Kalanchoë plants kept in short days had a photosynthetic rate 50 % higher (on basis of leaf area) than those from plants in long days. He also found that the respiration rate was higher, but since these measurements relate to oxygen uptake in the absence of CO_2 their bearing on the present experiment is doubtful. The increased photosynthetic rate, however, should be applicable to our experiments and would mean that the enzyme postulated in (a) above could be concerned also in light fixation of CO_2. The true photosynthetic rate cannot be deduced from the present CO_2 measurements, since it is masked by the liberation of CO_2 in the light.

An important criticism of Bode's experiments must be made. The metabolism of the detached leaves was not studied throughout the cycle but apparently only for relatively brief periods. In view of the large fluctuations in metabolism which we have noted, even within a single light or dark phase, the assumptions of this simplified technique do not seem justified. The same criticism, of course, applies to any method based on integration of the metabolism over long periods. It is for this reason that the data of table I have not been given any detailed interpretation.

Schmitz carried out some experiments with whole plants on 48-hour cycles. Among other things he found that if the light period exceeded 19 hours the plants did not flower. In the interpretation of our results presented above there is no reason why excessively long light periods should in themselves cause loss of the characteristic short-day metabolism. Hence, if indeed the CO_2 metabolism is related to flowering, the CO_2 fixation enzyme, or the precursor, must be gradually destroyed in light. Only if the light phase is below a critical maximum length can some CO_2 acceptor survive, and only thus can the effects of repeated long nights show summation.

4. THE INTERRUPTION OF THE DARK PERIOD: The fact that the interruption of the dark period by brief exposure to light leads to failure of flower induction was shown first by Hamner and Bonner (8) and for Kalanchoë by Harder and Bode (10); it is evidently a general phenomenon in short day plants.

The data in figures 5 and 6 show that, with a brief light exposure of 10 minutes in the middle of the dark phase, plants pretreated with short days fail to show CO_2 fixation in the dark and correspondingly fail to evolve CO_2 in the light. They thus behave, with minor differences, like plants kept continually in long days. The 10 minutes of light interruption is insufficient to release more than a very small fraction of the CO_2 normally appearing in the light phase, and hence this action of light must be exerted, not on the CO_2-containing compound itself, but on the enzyme or substance responsible for producing it. This material, whose concentration builds up in long nights, is destroyed by brief exposure to light during the dark period, but reaches a stable, light-resistant state before the end of the 16 hours of darkness. Its behavior is thus identical with that of the postulated flower-inducing substance (for instance, Gregory, 7).

5. The Relation of the CO_2 Metabolism to Flower Induction: The metabolism of CO_2 investigated in these experiments so closely resembles the effects on flowering induced by similar treatments that some causal relationship seems probable. This is particularly striking in regard to interruption midway in the dark period, which completely prevents flower induction. This was ascribed by Gregory (7) to the formation during the dark period of the precursor of a "flowering hormone." This precursor reached a maximum concentration by the end of the critical dark period, but, if it remained in the leaf, it was destroyed by exposure to light. Thus the interruptions by light had progressively greater effect as the precursor accumulated. However, towards the midpoint of the dark period the precursor was supposed to be translocated from the leaf, so that later interruptions had less and less effect. Before this suggestion can be accepted as holding for the CO_2 acceptor it will be necessary to investigate the effects of interruptions at various times during the dark period. There are many other problems, including the nature of the labile substance and its destruction by light or by heat or both. These are being actively studied.

Summary

The diurnal carbon dioxide metabolism of *Kalanchoë Blossfeldiana* was studied under long days, short days, and short days with interrupted nights. Sodium hydroxide absorption towers and an infra red gas analyzer were used for determining carbon dioxide. A gas-tight chamber is described which encloses only the shoot portion of rooted plants, and enables their study in complete gaseous isolation from the roots. With this apparatus the dark uptake of CO_2 by succulent plants reported by other workers is confirmed for Kalanchoë. It is shown, however, that exposure to repeated short-day cycles increases both the amount and the rate of the net dark fixation of carbon dioxide. The fixation passes through a maximum and may be replaced by a brief period of CO_2 production before the end of the dark period. If the plants are kept in darkness after 16 hours, a steady loss of CO_2 by respiration takes place and continues at a nearly constant level.

Exposure to repeated short days also causes a burst of CO_2 on illumination; the amount of CO_2 evolved parallels the amount of CO_2 absorbed during the preceding dark period. This CO_2 burst also occurs, though only to a very small extent, in plants kept in long days. The two phenomena, i.e., the dark fixation and the CO_2 production in light, are ascribed to the destruction by light or high temperature of a substance, probably an organic acid, produced by CO_2 fixation in the previous dark period. This photo- or thermo-labile substance is not metabolized away in 48 hours of darkness, but appears to require illumination, or high temperature, or both, for its destruction.

Interrupting a long dark period at its mid-point with 10 minutes of light completely inhibits the dark fixation of CO_2 and also the associated CO_2 production on illumination. Various mechanisms to account for the results obtained are critically discussed.

Acknowledgment is made of a Parker Fellowship held by one of us (I. S.) during part of this work and of support from the Maria Moors Cabot Foundation for Botanical Research of Harvard University and the National Science Foundation.

Literature Cited

1. Bennet-Clark, T. A. The role of the organic acids in plant metabolism. Part I. New Phytol. 32: 37–71. 1933.
2. Bode, O. Über Zusammenhänge zwischen CO_2-Assimilation und Photoperiodismus bei *Kalanchoe Blossfieldiana*. Planta 33: 278–289. 1942.
3. Bonner, W. D., Jr. and Bonner, J. The role of carbon dioxide in acid formation by succulent plants. Amer. Jour. Bot. 35: 113–117. 1948.
4. Bünning, E. Endonome Tagesrhythmik und Photoperiodismus bei Kurztagspflanzen. Biol. Zentr. 64: 161–183. 1944.
5. De Saussure, T. Recherches chimiques sur la végetation. Paris. 1804.
6. Emerson, R. and Lewis, C. M. Carbon dioxide exchange and the measurement of the quantum yield of photosynthesis. Amer. Jour. Bot. 28: 789–804. 1941.
7. Gregory, F. G. The control of flowering in plants. Symp. Soc. Exptl. Biol. II: 75–103. Cambridge, University Press. 1948.
8. Hamner, K. C. and Bonner, J. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. 100: 388–431. 1938.
9. Harder, R. Vegetative and reproductive development of *Kalanchoë Blossfeldiana* as influenced by photoperiodism. Symp. Soc. Exptl. Biol. II: 117–138. 1948.
10. Harder, R. and Bode, O. Über die Wirkung von Zwischenbelichtungen während der Dunkelperiode auf das Blühen, die Verlaubung und die Blattsukkulenz bei der Kurztagspflanze *Kalanchoë Blossfeldiana*. Planta 33: 469–504. 1943.
11. Harder, R. and von Witsch, H. Über die Bedeutung der Kohlensäure und der photoperiodische Belichtung für die Blütenbildung bei *Kalanchoë Blossfeldiana*. Naturwiss. 29: 770–771. 1941.
12. Lang, A. Physiology of flowering. Ann. Rev. Plant Physiol. 3: 265–306. 1952.
13. Parker, M. W. and Borthwick, H. A. Floral initiation in Biloxi soybeans as influenced by photosynthetic activity during the induction period. Bot. Gaz. 102: 256–268. 1940.
14. Porter, H. K., Pal, N., and Martin, R. V. Physiological studies in plant nutrition. XV. Assimilation of carbon by the ear of barley and its relation to the accumulation of dry matter in the grain. Ann. Bot. (London) 14: 55–68. 1950.
15. Schmitz, J. Über Beziehungen zwischen Blütenbildung in verschiedenen Licht-Dunkelkombinationen und Atmungsrhythmik bei wechselnden photoperiodischen Bedingungen (Untersuchungen an *Kalanchoë Blossfeldiana*). Planta 39: 271–308. 1951.
16. Schwabe, W. W. Effects of photoperiodic treatment on stomatal movement. Nature 169: 1053–1055. 1952.
17. Spear, I. The interrelation between carbon dioxide

metabolism and photoperiodism. Thesis, Harvard University, May 1953.
18. THOMAS, M. Physiological studies on acid metabolism in green plants. I. CO_2 fixation and CO_2 liberation in crassulacean acid metabolism. New Phytol. 48: 390–420. 1949.
19. THOMAS, M. and BEEVERS, H. Physiological studies on acid metabolism in green plants. II. Evidence of CO_2 fixation in Bryophyllum and the study of diurnal variation of acidity in this genus. New Phytol. 48: 421–447. 1949.
20. WOLF, J. Beiträge zur Kenntnis des säurestoffwechsels sukkulenter Crassulaceen. Planta 37: 510–534. 1949.

THE ABSORPTION OF INORGANIC IONS BY CHLORELLA PYRENOIDOSA [1,2]

H. J. KNAUSS AND J. W. PORTER
THE BIOLOGY SECTION, RADIOLOGICAL SCIENCES DEPARTMENT,
GENERAL ELECTRIC COMPANY, RICHLAND, WASHINGTON

In the course of experiments on the effect of radioactive elements on the growth of *Chlorella pyrenoidosa*, Porter and Knauss (9), determinations were made of the content of a radioactive element in algal cells when the ratio of that element to its stable isotope was kept constant, but the total concentration of the two isotopes in the solution was varied. Calculations were then made, assuming no isotope effect, of the total contents of the two isotopes in the algal cells. These determinations were made for the elements P, S, Ca, Fe, Mn, Zn, Cu, and Sr.

A limited amount of information is available on the effect of the nutrient concentration of an element on the uptake of that element by algae. Scott (11) has reported that the ratio of uptake of calcium and magnesium by Chlorella depends upon the ratio of these two elements in the nutrient solution. Scott (12) has also studied the behavior of phosphate-deficient cells during a restoration period when adequate phosphate was supplied to the nutrient medium. He found the phosphate uptake to be proportional to the nutrient concentration only at levels that were limiting for growth.

More information on the effect of nutrient concentration of an element on its uptake has been obtained through studies with higher plants and diatoms. Beckenbach et al (1), in making a statistical analysis of the relationship between the ionic content of the culture solution and the element content in corn plant tissues, found the most important single factor affecting the absorption and accumulation of an element in plant tissues to be its absolute concentration in the nutrient solution. Similar results were reported by Tidmore (14) on corn, sorghum, and tomatoes, and by Beeson et al (2) on tomato plants. Recently, Rediske and Selders (10) reported the uptake of strontium by plants to be proportional to the concentration of this element in the nutrient solution. Goldberg et al (5), working with diatoms, found the phosphate uptake to be proportional to the concentration in the medium over the range 0.5 to 3.5 μg/l. Similar findings were reported by Ketchum (7).

[1] Received May 22, 1953.
[2] This paper is based on work performed under Contract No. W-31-109-Eng-52 for the Atomic Energy Commission.

EXPERIMENTAL METHODS

The strain of Chlorella used was American Type Culture No. 7516. Inocula to use in experiments were obtained by the same procedure reported previously (9).

Seven and one half ml of nutrient solution (KNO_3, 3.0 gm/l; $MgSO_4 \cdot 7\,H_2O$, 4.8 gm/l; $Ca(NO_3)_2$, 1.0 gm/l; KH_2PO_4, 3.0 gm/l; $MnSO_4 \cdot H_2O$, 2.0 mg/l; H_3BO_3, 2.0 mg/l; $CuSO_4 \cdot 5\,H_2O$, 0.2 mg/l, and $ZnSO_4 \cdot 7\,H_2O$, 0.2 mg/l), less the element being investigated, were added to 20×150 mm culture tubes. Varying amounts of the element being studied and the appropriate amounts of water were added to each tube. After sterilization each tube received 0.2 ml of a sterile iron solution (1 gm $FeSO_4 \cdot 7\,H_2O$/500 ml), 1.0 ml of inoculum (an optical density of 0.60 at 660 mμ/18 mm tube, approximately 17×10^6 cells/ml) and amounts of a solution containing a radioactive isotope of the element under study to yield a final specific activity of one mc/gm of that element. In experiments on the absorption of copper the specific activity was 3 to 8 mc/gm of element. Radioisotopes used were P^{32}, S^{35}, Ca^{45}, Fe^{59}, Mn^{54}, Cu^{64}, Zn^{65}, and Sr^{90}. The final volume of solution was 18.2 ml, and the final pH was 4.2 to 4.6. Duplicate tubes were set up for each concentration of the element under study. Control tubes, lacking in radioactivity, were also inoculated in each experiment.

The cotton plugs of the tubes were replaced with sterile 0.5 mm capillary tubing wrapped in cotton. The cells were then aerated through these tubes at a rate of approximately 100 ml per minute with a mixture of 8 to 9 % CO_2 in air. The gas mixture was saturated with moisture by passing through water. Light intensity (white fluorescent) incident to the tubes during the growth period was 300 to 500 fc.

After the growth period, usually 72 hours, the suspensions of cells were made to a common volume such that light absorption readings could be made. These were then made on the cell suspensions in 18 mm tubes at 660 mμ with a Beckman Model DU Spectrophotometer. From these readings the dry weight for each sample was determined by reference to a previously determined relationship between dry weight and optical density (fig 1).

The procedure of a typical experiment in which