(4.3 milliosmoles per liter) in which *Microcystis* was grown was used as the negative control.

Table 1 illustrates diarrheagenic dose-response obtained by *Microcystis* nondialyzable toxin. Two milliliters of the nondialyzable fraction of *Microcystis* toxin produced 0.33 ml of fluid per centimeter of small intestine of guinea pigs (Table 1), and 2.78 Lb units of cholera toxin produced 0.38 ml of fluid per centimeter.

One-tenth of the preparation of cholera toxin used in Table 1 produced fluid accumulation in loops (Table 2). Also, when 3 ml of the dialyzable portion of the *Microcystis* whole cell lysate is injected in the loops, no fluid accumulation occurs. However, this dialyzable fraction kills rats when injected intraperitoneally in 0.5-ml volumes.

The foregoing data offer new insight into the possible causes of diarrhea and diarrheal epidemics where no common source etiology is known and no person to person transmission can be established (*10*). It is expected that further research will determine the environmental conditions under which toxins are best produced by various species of *Microcystis* and possibly species belonging to other genera of blue-green algae. The use of surface water for drinking purposes, where waterbloom is of common occurrence, may be a possible health hazard.

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Carbon Fixation and Isotope Discrimination by a Crassulacean Plant: Dependence on the Photoperiod

Abstract. *Variations of more than 1 percent are observed in the carbon-13 to carbon-12 ratio of extracts of leaves of the succulent* Kalanchoe blossfeldiana *when the photoperiod is changed from long to short days. This indicates that the mechanism of carbon fixation switches from the Calvin (C_3) pathway to the Hatch-Slack (C_4) pathway of primary enzymic operation. The variations observed in the isotope compositions are tentatively explained by a model.*

The carbon isotope composition of a plant is a good indicator of the metabolic pathway by which it assimilates carbon. In particular, isotope analyses have proved convenient for differentiating plants (*1, 2*) which photosynthesize via the enzyme ribulose-1,5-diphosphate carboxylase (RuDPC) (the Calvin, or C_3, pathway) from plants which photosynthesize via the enzyme phosphoenolpyruvate carboxylase (PEPC) [the Hatch-Slack, or C_4, pathway (*3*)]. These results suggested that the isotope method could be used to study the pathway of CO_2 fixation in succulent plants with crassulacean acid metabolism (CAM) (*4*). In these plants both carboxylases are present, but the main reaction for CO_2 fixation is via PEPC operating mainly in the dark. As it is known that photoperiodism controls the intensity of CAM operation (*5, 6*), the isotope method is useful for studying the relative degree of operation of both carboxylases according to the photoperiodic environmental conditions.

The carbon fixed by a terrestrial plant has lower ^{13}C and ^{14}C concentrations than the atmospheric CO_2. This depletion is due to isotope discrimination or fractionation. Although the effect is larger (about double) for ^{14}C, the assay on the stable isotope ^{13}C is much easier and more accurate. Moreover, information on the natural isotope composition of different metabolites along a carbon pathway has proved to be a powerful means of shedding light on some biosynthetic processes (*7*). For C_4 plants, the discrimination causes the relative ^{13}C content in the plant to be slightly lower (about 5 per mil) than in the CO_2 of the surrounding atmosphere, while C_3 plants show a depletion of about 2 percent [see histograms in (*2, 8, 9*)]. Two distinct groups can be observed with modes at about −12 per mil, on the $\delta^{13}C_{PDB}$ scale (*10*), for the C_4 plants and −28 per mil for the C_3 plants. [The composition of the free atmosphere on the same scale is about −6.7 per mil (*11*).] The first analyses of CAM plants indicated isotope compositions either similar to those of C_4 plants or intermediate between those of C_3 and and C_4 plants (for example, −18 per mil) (*12*). The distribution of δ values for these plants has a mode at about −17 per mil with a larger scatter to

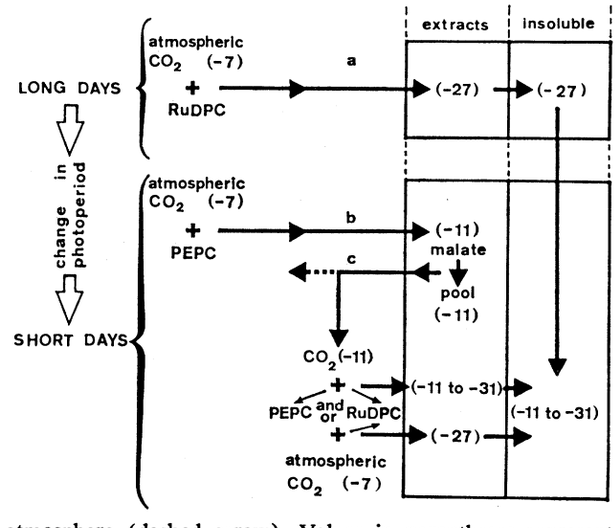

Fig. 1. Tentative model for carbon isotope discrimination by plants with crassulacean acid metabolism. (a) Photosynthesis by a C_3-type pathway via the enzyme RuDPC. (b) Fixation of CO_2 in the dark by a C_4-type pathway and eventual C_4 photosynthesis via the enzyme PEPC. (c) Decarboxylating reaction by malic enzyme; the CO_2 produced is partially refixed by the plant (via RuDPC or PEPC or both) and partially released to the atmosphere (dashed arrow). Values in parentheses represent $\delta^{13}C_{PDB}$ ($\pm$ 2 per mil). The values we observed (Table 1) are in the range predicted by the model. According to this model the isotope composition of CAM plants may vary in a range of values, reflecting different environmental conditions.

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both higher and lower values, covering most of the range of values known for terrestrial plants grown in nature (*8*). Lerman (*8*) suggested (i) that the range may be due to fixation via both C_3 and C_4 pathways and (ii) that consequently the isotope ratio might be affected by environmental conditions such as thermoperiod, photoperiod, and water conditions because the degree of CAM varies with them (*13*). The influence of photoperiod had been shown by Queiroz (*5, 6*) to have particularly clear-cut effects in the young leaves of *Kalanchoe blossfeldiana* 'Tom Thumb': Under long days (or short days followed by long nights interrupted by a flash of red light, which establishes the involvement of phytochrome) (*14*), these leaves display very low CAM, as evaluated either in vivo by CO_2 exchange and malate content or in vitro by enzyme activity; the change from long days to short days induces strong, typical CAM. One week after plants originally grown in long days are transferred to short days, fixation of CO_2 via PEPC is induced (*15*). Then the activity of this enzyme increases progressively and accumulation of malic acid takes place, until a maximum enzyme activity and acid content is attained after 50 to 60 short days. After this, both acid content and enzyme activity decrease rapidly (*15*). We compared *K. blossfeldiana* plants after about 45 short days to long-day plants (*14*). Isotope composition was determined for young and old leaves, on total tissue and on two raw fractions. One fraction, the "extract," was soluble in water; the other was insoluble (*16*). The analyses were made according to techniques described elsewhere (*17*).

The results in Table 1 show that:

1) The isotope compositions are characteristic for each photoperiod, being rather homogeneous for the long-day leaves and having a wide spread for the different fractions of the short-day leaves. The differences between long-day and short-day leaves are rather small for the insoluble fractions (3 to 5 per mil), large for the total tissue (7 to 10 per mil), and very large for the extracts (13 per mil). This indicates a striking difference in carbon metabolism between the two groups of plants and between tissue fractions. As the extract represents the recent metabolism of the plant (the insoluble fraction integrates the products of CO_2 fixation during the whole growth period), the analysis of extracts is the only way to ascertain the first step of carbon fixation. Whereas the analyzed extract of short-day plants is only about 2 per mil depleted in ^{13}C compared to the atmosphere of the growth rooms (Table 1), the extract of the long-day plants is about 15 per mil depleted. This indicates that in the short-day plants the carbon in the soluble fraction was initially fixed via PEPC (*1, 2, 8, 9, 18*), and in the long-day plants it was initially fixed predominantly via RuDPC (*18, 19*).

2) In each group a difference in isotope composition between leaves of different age is observed. The variations of δ in the total tissue are caused by variations of δ in the extracts and the insoluble matter. Extracts were also prepared from leaves of different ages, and higher (less negative) δ values were observed for the older leaves; this correlates with the increase of CAM, which is age-dependent (*20*). For the insoluble fraction, the variations with age may mean that both enzymes operate in different proportions in the primary carboxylation leading to the cell wall material of leaves of different age, or they may be due to variation in the size of the malate pool (Fig. 1), as demonstrated for *Bryophyllum daigremontianum* (*20*). In our case, variations of δ value with age might also be due to the old leaves in the short-day plants conserving a large part of the cell wall material that was fixed during the initial period of long days (*14*).

The large difference in δ between long-day and short-day plants cannot be related to the different length of time available for the processes of decarboxylation and respiration; discrimination by respiration is a much smaller effect (*7, 19*) than we observed, and decarboxylation of the malate pool does not produce a detectable change in its δ value (*20*).

In summary, our results suggest a switching from RuDPC-dominant activity to PEPC-dominant activity when the photoperiod is changed from long to short days; this is in agreement with the interpretation of variations of enzyme activity reported by Queiroz (*6*).

The tentative model shown in Fig. 1 is an attempt to integrate the effect of photoperiod in the leaf of a plant grown under long days and transferred to short days. Under long days, CO_2 from the atmosphere ($\delta = -7$ per mil) would be photosynthetically fixed through RuDPC and would produce soluble and cell wall matter with similar isotope composition (−27 per mil) (*18, 19*). After the change to short days, atmospheric CO_2 would be fixed in the dark by PEPC to produce soluble matter, mainly malic acid (−11 per mil) (*18*). In the following day, part of this malic acid would be decarboxylated by malic enzyme (*4, 6, 13*) to produce CO_2 (−11 per mil). At least part of this CO_2 will be photosynthetically refixed by RuDPC or PEPC or both without leaving the tissues; we assume that the isotope composition of the photosynthates lies within the range −11 to −31 per mil: the first figure would occur if all the released CO_2 were refixed, because a complete (or quantitative) reaction does not discriminate, and the second figure would occur if only a relatively small part of the −11 per mil CO_2 were refixed (*20*). A further complication arises because, within a certain range of temperature and water conditions, atmospheric CO_2 may enter the leaf through the stomata and be fixed either by PEPC, still active during daytime (*21, 22*), or RuDPC. The latter reaction would

Table 1. Carbon isotope composition of leaves of *Kalanchoe blossfeldiana* 'Tom Thumb' under different photoperiods (*14*), normalized to an atmosphere with $\delta = -7$ per mil. The isotope discriminations are, approximately, the difference between the tabulated values and −7 per mil. The normalization was effected by adding 3 per mil to the measured values, because the mean $\delta^{13}C_{PDB}$ of the CO_2 in the atmosphere of the growth rooms was about −10 per mil, that is, about 3 per mil lower than in the free atmosphere (*11*). The mean value was estimated from analyses of C_3 plants of different ages. The atmospheric $\delta^{13}C$ fluctuates around the mean value; this was observed by making spot analyses of a greenhouse atmosphere (*20*). However, the atmospheric fluctuations were smaller than the observed differences of isotope composition; for example, between the extracts and insoluble fractions, and between the extracts of plants grown in different photoperiods. Moreover, the similar trend observed for the age effect in both experiments [this table and (*20*)] indicates that our results have not been much affected by the fluctuation of the isotope composition of the growth-room atmosphere. In some recent reports (*23*) the δ value of the atmosphere in which the plants were grown has not been considered, thus making it difficult to intercompare results.

Age of leaves	$\delta^{13}C_{PDB}$ (per mil) in		
	Insoluble fraction	Total tissue	Extract
	Long days		
Young	−24.4	−23.2	−22.1
Old	−26.7	−21.3	
	Short days		
Young	−21.3	−13.0	
Old	−21.8	−14.6	−9.1

yield photosynthates with an isotope composition of −27 per mil. In this case, the composition of the total photosynthetic products would be the resultant of the combination of the fraction at −11 to −31 per mil and the fraction at −27 per mil. The isotope composition of the total extract would be the combination of the compositions of the total photosynthate and the accumulated malic acid (−11 per mil). When the number of short days increases, the composition of the insoluble fraction will be progressively modified by the contribution of the newly formed carbon chains.

It has been reported (*23*) that environmental changes could shift the isotope composition of a CAM plant from values typical of a C_4 plant to values typical of a C_3 plant. Our results establish that such a shift can be obtained by photoperiodic control. However, we emphasize the importance of measuring different fractions instead of total leaf tissue for evaluating the metabolic pathways in a CAM plant. Analyses of extracts provide information about the relative contribution of each of the carboxylating enzymes to the primary CO_2 fixation, while the difference between the δ values of the insoluble fractions and extracts gives information concerning the size of the malate pool. Analyses of total tissue might produce, for example, values intermediate between those typical of plants of the C_3 and C_4 type (for example, −14 to −22 per mil), suggesting large contribution of both enzymes, while in reality such leaves fix all CO_2 via PEPC [see Table 1 and (*20*)].

Thus, a biophysical method facilitates the understanding of biochemical data in the study of metabolic responses. Environmental parameters such as temperature (*24*) and thermoperiod (*21*) produce similar biochemical changes, and will also leave a characteristic δ imprint in CAM plants (*8, 25*). This suggests that the isotope method might be applied in ecological studies of recent and fossil CAM plants, in particular, to reconstruct the past climate by isotope analyses of fossil remains of CAM plants (*25, 26*).

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Redirection of Filial Attachments in Rhesus Monkeys: Dogs as Mother Surrogates

Abstract. *Rhesus infants raised from birth with their mothers, age-mates, or cloth surrogates for periods varying from 1 to 10 months were separated from these objects and placed with dogs. Contrary to previous suggestions that were consistent with the notions of a critical period for attachment formation and irreversibility of filial bonds, the monkeys formed strong and specific attachments to their canine surrogates.*

The newborn of many species of birds and mammals form an attachment to a parent or appropriate substitute. The strength of attachment is usually inferred from behaviors that maintain proximity to the parent figure and from reactions to separation. Such bonds are often described as though they were once-in-a-lifetime events—restricted to a narrow period early in life, specific to a particular object, exclusive, and enduring (*1*). Actually, these aspects of attachment behavior have been systematically examined mainly in birds but almost never in mammals, including the nonhuman primates.

We investigated the specificity, exclusiveness, and reversibility of filial bonds in an initial experiment with eight immature laboratory-born rhesus