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Diarrhea Toxin Obtained from a Waterbloom-Producing Species, Microcystis aeruginosa Kützing

Abstract. *A diarrhea-producing toxin from a blue-green alga,* Microcystis aeruginosa *Kützing, was obtained from standing laboratory cultures. The nondialyzable fraction of the lysate from whole cells produced fluid accumulation in the ligated small intestinal loops in guinea pigs.*

Algal species have been suspected of producing toxin since 1878 (*1*), and three different types of toxin were obtained from several genera of the Phylum Cyanophyta commonly known as the blue-green algae (*2*). Pathological changes in the liver, heart, lungs, and kidney due to the effects of the extracts from *Microcystis aeruginosa* Kützing, a member of the phylum Cyanophyta, were also reported (*3*). Although no diarrheagenic toxin has been isolated previously from any species of the phylum Cyanophyta (*4*), I have pointed out the importance of further studies on toxic phytoplankton as a cause of gastroenteritis when no known etiological agent could be identified (*5*).

A diarrhea toxin obtained from *Microcystis aeruginosa* laboratory cultures has now been isolated and assayed. Since *Microcystis* is widely distributed in many parts of the world in freshwater sources used by man, domestic animals, and wildlife, the findings reported may have a bearing on their health.

Initial studies with centrifuged waterbloom consisting predominantly of *Microcystis aeruginosa* obtained from a city pond in Dacca, Bangladesh, demonstrated a lethal factor, which was assayed by intraperitoneal injection in rats; but these crude preparations were inconsistent in causing fluid accumulation in the mammalian small intestine. Subsequently a *Microcystis* clone was established from a single cell cultured from a suspension of disrupted colonies separated by the extinction dilution method. The disruption of the colonies was effected by a cell disruption bomb (*6*) at 1000 pounds per square inch. *Microcystis* cells were cultured in one liter of a synthetic, dialyzable aqueous media in 4-liter flasks at 27° ± 1°C for 30 days in low light. *Microcystis* cells were concentrated by the cell disruption bomb, at 500 pounds per square inch for 20 minutes, followed by slow equilibration to atmospheric pressure. Virtually clear fluid was taken from the top, and usually 10^8 *Microcystis* cells per milliliter remained at the bottom of the bomb. The concentrated cell suspension was then frozen and thawed five times and broken by shaking in a flask with glass beads. This suspension was then centrifuged, and the supernatant was sterilized by passing it through a series of Millipore filters. The sterile filtrate was then concentrated 43-fold by vacuum dialysis and reconstituted with distilled water to a concentration tenfold that of the original. This preparation was sterilized again by Millipore filtration and the osmolarity was 11.7 milliosmoles per liter.

This preparation was assayed in ligated intestinal loops of guinea pigs essentially by the described technique (*7*). Modifications included the use of five loops per animal and harvesting at 6 hours. The dialyzable component (24.3 milliosmoles per liter) was assayed in the same way. A preparation of cholera toxin (*8*) containing 278 Lb units or 133 Ll units per milliliter (*9*) was used as the positive control, and the medium

Table 1. Fluid accumulation in the ligated small intestine loops of guinea pigs as a result of the action of the nondialyzable component of *Microcystis* whole cell lysate.

Injection into loop (ml)	Averages of three small intestine loops in three guinea pigs		
	Length of loop (cm)	Fluid accumulation per loop (ml)	Fluid per centimeter (ml)
	Microcystis *toxin*		
2	11.9	3.9	0.33
1	10.2	2.5	0.24
0.5	8.8	1.2	0.14
	*Cholera toxin**		
1	11.2	4.2	0.38
	Growth media for Microcystis		
2	8.0	0	0

* Gel borate buffer containing 0.01 ml (2.78 Lb units) of cholera toxin.

Table 2. Fluid accumulation in the ligated small intestine loops of guinea pigs as a result of the action of the dialyzable component of *Microcystis* whole cell lysate. Cholera toxin was used as a control.

Injection into loop (ml)	Averages of two small intestine loops in two guinea pigs		
	Length of loop (cm)	Fluid accumulation per loop (ml)	Fluid per centimeter (ml)
	Cholera toxin in 2 ml of buffer		
0.01	11.2	4.6	0.42
0.005	8.1	2.6	0.36
0.001	9.5	3.0	0.31
	Dialyzed Microcystis *cell lysate*		
3	14.5	0.1	0
	Growth media for Microcystis		
2	6.4	0	0

(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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19 September 1973

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

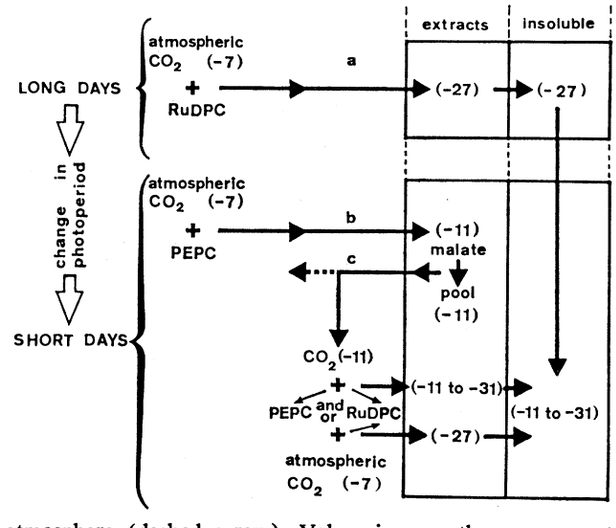

Fig. 1. Tentative model for carbon isotope discrimination by plants with crassulacean acid metabolism. (a) Photosynthesis by a C_3-type pathway via the enzyme RuDPC. (b) Fixation of CO_2 in the dark by a C_4-type pathway and eventual C_4 photosynthesis via the enzyme PEPC. (c) Decarboxylating reaction 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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