Reprinted from
22 March 1974, Volume 183, pages 1206-1207

SCIENCE

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*).

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.

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 li-

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

gated 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 (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.

K. M. S. Aziz

Cholera Research Laboratory,
Institute of Public Health,
Dacca-12, Bangladesh

References and Notes

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19 September 1973