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Persistence of Enteroviruses in Lake Water

JOHN E. HERRMANN[1], KENNETH D. KOSTENBADER, JR., AND DEAN O. CLIVER
Food Research Institute and Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706

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Two enteroviruses were inactivated more rapidly in a lake than in sterile lake water; then their coat proteins were degraded and, perhaps, used by microorganisms.

Viruses must be stable in water in order to be transmitted by the water route. Enteric viruses are often more persistent in water under laboratory conditions than under more natural conditions (1). We reported previously that coxsackievirus type A9 (CA9) is inactivated faster if proteolytic bacteria are present with it in laboratory suspensions of lake or well water (3). The coat of this virus is degraded by proteolytic enzymes of both microbial and animal origin (3, 6). Poliovirus type 1 (PO1) is protease resistant but is susceptible to some species of proteolytic bacteria, notably *Pseudomonas aeruginosa* (3). We wanted to learn whether these viruses would be affected in a lake as they are in the laboratory.

The study site was Lake Wingra in Madison, Wis. The CA9 (strain Bozek) and PO1 (strain CHAT) viruses were propagated (2), labeled with [^{14}C]leucine (4), purified (6), and assayed for radioactivity (6), specificity of labeling (5), and infectivity in plaque-forming units (PFU; [2]) per milliliter by methods we have used previously. More than 95% of the label was virus-associated. We mixed 10 ml of lake water with 0.1 ml of virus suspension ($>10^7$ PFU) in a dialysis bag and suspended it 1 m below the lake surface from a raft approximately 200 m from the shore in late May. At the same time, we sterilized 10 ml of lake water by filtration at 220-nm porosity (Millipore) and added the same quantity of virus for laboratory incubation. A 1-ml sample, taken after 2 h on day 0 and repeated at 3-day intervals through day 21, served for infectivity assay (0.1 ml), radioactivity assay (0.1 ml), and a crude filtrability determination (0.8 ml). We diluted the 0.8 ml of sample with 7.2 ml of 5% (vol/vol) fetal calf serum in phosphate-buffered saline (pH 7.2), passed this through a filter (25 mm by 220 nm porosity), and measured the radioactivity on the top surface of the filter in a planchet counter. The samples for radioactivity assay from day 9 were lost.

Inactivation of both viruses was more rapid in the lake than in the sterile lake water suspensions in the laboratory (Fig. 1). Temperatures ranged from 21 to 23 C in the laboratory and from 19 to 25 C where the samples were in the lake, so temperature was probably not a significant factor. CA9 was clearly the less stable of the two viruses under approximately natural conditions.

Loss of infectivity was more rapid than loss of the ^{14}C label from the dialysis bags (Fig. 2). A 99% loss of infectivity took 4 to 14 days in the lake; that proportion of radioactivity had not been lost by day 21. This does not mean that all of the label was still virus-associated. A good

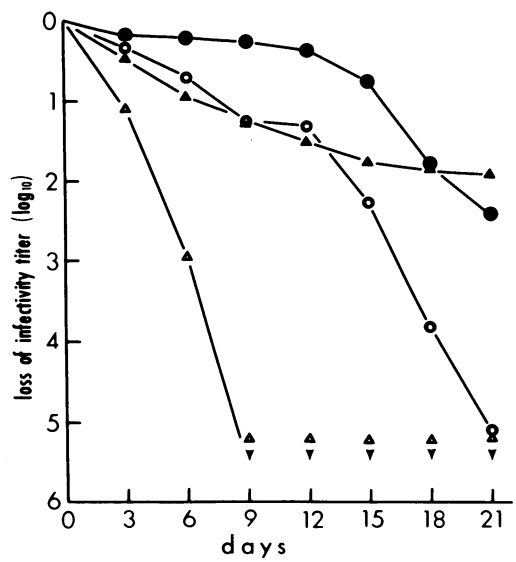

FIG. 1. *Inactivation of poliovirus type 1 (circles, ●, ○) and coxsackievirus A9 (triangles, ▲, △) in Lake Wingra (open symbols) and in sterile lake water in the laboratory (filled symbols). The arrowheads denote a value greater than that indicated by the position of the triangle.*

[1] Present address: Department of Microbiology, Harvard School of Public Health, Boston, Mass. 02115.

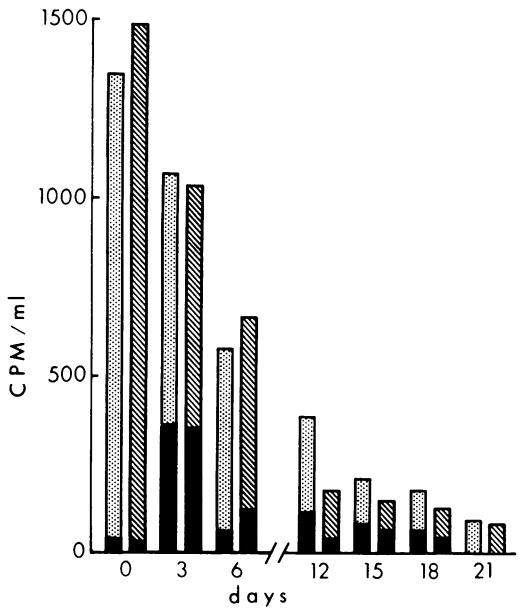

FIG. 2. *Levels of ^{14}C radioactivity measured in dialysis tubes inoculated with poliovirus type 1 (dot-shaded bars) and coxsackievirus A9 (diagonally-shaded bars); the heavily shaded part of each bar represents the portion retained by a 220-nm filter.*

deal of radioactivity in samples from days 3 through 18 was retained on the 220-nm filters; this was at background level by day 21. Dissolution of the virus and dissipation of the radioactivity apparently proceeded at nearly the same rates with both viruses, despite their different rates of inactivation.

The viruses were inactivated more rapidly in the lake than in sterile suspensions of lake water. This and our previous findings suggest that retention of the label on the 220-nm filters resulted from microbial utilization of [^{14}C]leucine from the virus coat protein. Enteroviruses appear to be biodegradable under natural conditions, but we have still to determine whether degradation occurs as or after the virus loses infectivity.

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