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Effects of Ingesting Mercury-Containing Bacteria on Mercury Tolerance and Growth Rates of Ciliates

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Abstract. The ciliate *Uronema nigricans* was found to acquire tolerance to mercury after being fed mercury-laden bacteria followed by exposure of washed suspensions of these ciliates to various concentrations of mercury in solution. Significant differences in percent mortality were observed for ciliates fed mercury-laden bacteria compared with control suspensions fed mercury-free bacteria. The phenomenon of acquired mercury tolerance was demonstrated within a single generation time. Ciliates fed mercury-free bacteria and subsequently exposed to increasing levels of mercury in solution showed an elevated tolerance to concentrations which, on initial testing, resulted in mortality of 83% of the ciliate population. The effect of ingesting mercury-laden bacteria on growth rate of *Uronema* was examined, and results showed no significant differences in growth rates of both 3- and 14-day-old cultures of protozoa that had been fed mercury-laden and mercury-free bacteria under controlled conditions.

Introduction

Bactivorous protozoa can be found in large numbers in sewage effluents rich in metal content and are considered to play a role in determining the quality of sewage effluents (6, 7). In ecosystem analyses, ciliates can contribute significantly to nutrient regeneration in the aquatic environment (14), as well as provide a source of food for marine zooplankton (1, 12). It has been suggested that ciliates can be useful indicators of heavy metal contamination (4, 10), because of sensitivity to metals demonstrated by some species of protozoa (4, 10, 20, 21, 24).

Resistance to toxic trace elements in protozoa can arise by a phenomenon known as dauermodification (15). For example, paramecia can acclimatize to toxic substances, such as arsenious acid, when concentrations of the substance are slowly increased. Once protozoa are resistant, the effect can be observed in organisms cultured in an arsenic-free solution for several months. Sartory and Lloyd (21) proposed that tolerance to mercury is acquired by populations of

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ciliates over extended periods of time, a conclusion also drawn by Tingle et al. (24), who suggested that repair mechanisms existed in mercury-damaged cells of *Tetrahymena pyriformis* which accounted for the phenomenon. In the study reported here, the levels of mercury to which a marine ciliate, *Uronema nigricans,* is sensitive were measured and studies were carried out to determine whether increased resistance to mercury was acquired by ingestion by the ciliate of bacteria laden with mercury and/or exposure to increasing concentrations of mercury in solution. The doubling time of ciliates feeding on Hg-free versus Hg-laden bacteria was also examined.

Material and Methods

Strains Used

Ciliated protozoa of the genus *Uronema* were isolated from the Rhode River subestuary of Chesapeake Bay. Cultures were maintained at room temperature in flasks containing filtered Rhode River water subsequently autoclaved and supplemented with washed suspensions of a *Pseudomonas* sp., strain P244, a bacterium also isolated from the Chesapeake Bay. The *Pseudomonas* sp. was grown at 25°C in a maintenance medium containing, per liter, 0.18 g KCl, 1.75 g $MgSO_4 \cdot 7H_2O$, 5.8 g NaCl, 3 g yeast extract, 10 g proteose peptone, adjusted to pH 7.2 to 7.4 prior to autoclaving.

Determination of Toxic Concentrations of Mercury

Two feeding regimes were followed for maintenance of cultures of *U. nigricans*. One group of ciliates was cultured for 9 days on a diet of washed suspensions of *Pseudomonas* sp. A second group of ciliates was fed washed suspensions of the bacterium, also grown in the maintenance medium, but with the addition of 20 ppm Hg^{2+}.

Both groups of ciliates were washed by repeated centrifugation at 900 × g for 3 minutes to remove uningested bacteria. Then 5-ml volumes of ciliates fed bacteria grown on mercury-containing or mercury-free media were suspended in solutions of 0, 10, 25, 40, 50, and 100 ppb Hg^{2+} in the form of $HgCl_2$. All experiments were conducted in triplicate. Suspensions of ciliates to which had been added 25, 40, 50, or 100 ppb mercury were observed through a dissecting microscope to determine the time required for 100% mortality of the ciliates. Next 50-μl aliquots of the suspension of ciliates in the solution containing 10 ppb mercury were withdrawn at selected time intervals and placed on a glass slide. The drop of suspension was allowed to stand approximately 2-minutes to allow dead ciliates to settle to the bottom. The entire drop was scanned at 100× magnification and dead cells were counted. Dead cells were distinguished from live ones on the basis of visible difference in refractive index, change in cell shape, and cessation of ciliary activity. A drop of acid Lugol's solution was added to fix the sample, and the cells were allowed to settle. A second scan was done to calculate the total number of cells.

Two sets of controls, i.e., tubes of ciliates with no $HgCl_2$ added, were included, and consisted of ciliates previously grown on Hg^{2+}-containing bacteria or on bacteria cultured without mercury. Immediately, and at 17 hours, samples were taken from each set of controls to count dead cells.

The protozoa were divided into two groups and allowed to feed for 6 hours, in order to determine whether acquired resistance to mercury arose within a single generation of ciliates. Each suspension of ciliates was amended with 15 ppb Hg^{2+}, and 10 minutes after exposure cell counts of total and dead cells were performed. Total viable counts of bacteria were made of the initial ciliate suspension by the spread plate method, using the maintenance medium solidified with 1.5% agar.

The effect of mercury in solution on mercury resistance was tested by exposing the ciliates to

$HgCl_2$ solutions whereby, prior to testing, one suspension was treated with increasing amounts of $HgCl_2$ and was exposed to each stepped-up concentration for 5 to 6 hours. The initial concentration of Hg^{2+} for this group of ciliates was 1 ppb, and subsequent additions of $HgCl_2$ yielded final concentrations of 2, 4, and 8 ppb Hg^{2+}. After 22 hours, suspensions of ciliates not exposed to mercury and suspensions of ciliates exposed to mercury were amended with an $HgCl_2$ solution to yield a final concentration of 10 ppb Hg^{2+} and allowed to stand for 5 hours, after which the suspensions were examined to determine numbers of total and dead ciliates, as described above. The ciliate suspensions were further amended with $HgCl_2$ to 20 ppb final concentration. After 2- and 12-hour exposure to 20 ppb, the ciliates in each treatment, as well as the controls, were counted and percent mortality calculated. Then 20 ppb Hg^{2+} was added to the controls (unexposed to Hg^{2+} for 42 hours) and an additional 10 ppb to the original suspensions that had been previously subjected to 20 ppb, yielding 30 ppb Hg^{2+} final concentration. These suspensions were examined for total viable and dead ciliates after 7 hours' exposure to the added mercury.

Chemicals

Studies of accumulation of mercury by bacteria were performed using radiolabeled ^{203}Hg in the form of $HgCl_2$ (New England Nuclear Corp., Boston, Mass.); specific acitvity 20 mCi/mg (99.000% purity). Stock solutions of Hg^{2+} were freshly prepared by adding 0.027 g (nonlabeled) $HgCl_2$ to 10.0 ml distilled water. The stock solutions were amended with 5 to 10 μl of the radioactive $HgCl_2$ solution, and the disintegrations per minute (dpm) were determined by liquid scintillation counting, using an Intertechnique Liquid Scintillation Counter Model SL-40 (Teledyne Corp., Westwood, N.J.). The ratio of dpm to μg of total mercury was calculated. Flasks containing sterile bacteriological growth media were amended with measured volumes of labeled mercuric chloride stock solutions to achieve the final desired concentrations. Scintillation cocktail used in the liquid scintillation analysis was prepared by adding 8.0 g Omnifluor (New England Nuclear) to 1 liter of scintillation grade *p*-dioxane (J.T. Baker Chemical Co., Phillipsburg, N.J.).

Mercury Accumulation by Pseudomonas *sp., Strain P244*

Freshly labeled (^{203}Hg) mercuric chloride stock solutions were prepared and the dpm/μg of total mercury was determined. Measured volumes of the stock solution were added to 250-ml screw cap side-arm flasks containing 50.0 ml sterile maintenance medium to attain a final mercuric ion concentration of 100 μg/ml. An additional culture flask containing the maintenance medium was supplemented with 100 μg/ml from a nonradiolabeled Hg^{2+} stock solution. Then 1-ml samples of *Pseudomonas* sp., strain P244, grown in 100 μg/ml mercury were added to each of the flasks. The inoculated flask cultures were incubated at 26° ± 2°C on a rotary shaker operated at 125 rpm. Optical densities of the cultures were measured at 620 nm with a Spectronic 20 spectrophotometer (Bausch and Lomb Inc., Rochester, N.Y.), and were recorded at regular intervals throughout the growth period. When each culture attained an optical density of 0.20 (early log phase of growth) and 0.65 (mid log phase), 25.0 ml of each culture was removed, and the bacteria were harvested from the culture medium by centrifugation and resuspension as described above. The cells were washed and centrifuged thrice to remove residual mercury. The washed bacteria were diluted to the original volume with sterile Chesapeake Bay water. Cell-associated radioactivity was measured by placing 0.1-ml samples of the washed cell suspension into 10.0 ml of scintillation cocktail, followed by radiometric measurement using the Intertechnique Liquid Scintillation Counter.

A total viable cell count of the washed bacterial suspension was also performed by dilution and spread plating. The total amount of cell-associated mercury was calculated from the ratio of dpm to total mercury. Bacteria grown in the nonlabeled mercury broth were harvested when the culture reached an optical density of 0.20. The cells were fixed for electron microscopy and comparison of the morphology of these cells with early log phase *Pseudomonas* sp. strain P244 cells grown in the absence of mercury was made.

Effects of Ingestion of Mercury-Laden Bacteria on the Growth Rate of U. nigricans

A 1.0-ml sample of *Pseudomonas* sp. strain P244 culture previously grown in 100 μg/ml Hg-supplemented maintenance medium was inoculated into a 250-ml side-arm flask containing 50.0 ml sterile maintenance medium with mercury added to a final concentration of 100 μg/ml. At a predetermined time, side-arm flasks containing maintenance medium without added mercury were inoculated with 1.0 ml of the *Pseudomonas* sp., strain P244. The times of inoculation for each culture were selected, based on the average observed length of the lag phase for *Pseudomonas* sp., strain P244, grown at the given mercury concentration, so that all cultures would simultaneously enter logarithmic phase of growth. The flasks were incubated at 25°C ± 2°C on a shaker set at 125 rpm, and the optical densities were monitored. When cultures attained an optical density of 0.20, the cells were harvested by centrifugation and resuspension, and diluted to a final optical density of 0.20 in filtered Chesapeake Bay water that had been autoclaved. Then 3-ml samples of each washed suspension of bacteria were placed in sterile test tubes. A measured volume of 3- or 14-day-old *U. nigricans* cultures was added to each tube to attain an initial protozoan density of 500 cells/ml. None of the protozoa used in the growth rate studies had been previously exposed to mercury or fed mercury-laden bacteria. Each tube was assigned a code at random to eliminate potential bias of the investigator. All cultures were incubated at 25°C, and at 6-hour intervals, until each protozoan culture reached stationary phase, the number of protozoan cells per milliliter of suspension was determined. After each tube was mixed on a vortex unit for 10 seconds, 50-μl aliquots were removed and placed onto a glass slide. A small drop of Lugol's Iodine solution was added to each drop of culture, fixing the cells for counting. The protozoa were allowed to settle and were counted by light microscopy at a magnification of 100×. As the numbers of protozoa increased with time, appropriate dilutions of the cultures were made in sterile, filtered Chesapeake Bay water to facilitate counting. The mean concentration of protozoa per milliliter, with respect to time, and the mean generation times for *U. nigricans* were calculated. An analysis of covariances test was performed, comparing the linear portions of the individual growth curves.

Results

Resistance of Ciliates Exposed to Hg^{2+} on Long-Term Basis

Ciliates cultured on mercury-laden bacteria exhibited an increased resistance to mercuric chloride in solution, as shown in Table 1. At all of the concentrations of Hg^{2+} tested, except 40 ppb, the time of total die-off of the mercury-acclimated ciliates was approximately twice that of ciliates cultured on bacteria grown

Table 1. Time (min) required for 100% mortality of *U. nigricans* exposed to Hg^{2+} at the indicated concentration

	Food source	
Concentration of Hg^{2+} (ppb)	*Pseudomonas* sp. Grown in the Absence of Hg	*Pseudomonas* sp. Grown in the Presence of Hg
100	2	4
50	5	10
40	8	9
25	17	30

without mercury. At 40 ppb $HgCl_2$, the time required for total die-off of the ciliates was only 1 minute longer than that required to kill ciliates fed Hg-grown bacteria, compared with ciliates fed Hg-free bacteria.

Upon exposure to 10 ppb Hg, the percent mortality was significantly higher for ciliates fed mercury-free bacteria compared with those fed Hg-laden bacteria (Table 2). A paired *t*-test conducted on the percent mortality for each treatment indicated significant differences at the 0.05% level.

Resistance of Ciliates Exposed to Hg^{2+} on Short-Term Basis

Ciliates allowed to feed for 6 hours on bacteria grown in the presence of mercury were washed and exposed for 10 minutes to 15 ppb Hg^{2+}. Initial and final enumeration of ciliates showed no increase in ciliate numbers under the given conditions, and no dividing stages were observed. After this treatment, 51.3% of the cells were dead. Ciliates treated in the same manner but fed bacteria grown in the absence of mercury yielded a mortality of 99%. Results of viable counts of bacteria remaining with the ciliates revealed approximately 1×10^4 cells/ml in the test solutions. Very few of the cells, however, appeared to be the *Pseudomonas* sp., strain P244 (distinguished by production of a fluorescent pigment), and therefore presumably would not contribute to removal of the mercury from solution, especially within 10 to 15 minutes.

Effects of Increasing Levels of Hg^{2+} on Resistance of Ciliates to Mercury

Resistance to mercury was amplified by gradually increasing the level of Hg^{2+} in solution into which were placed nondividing populations of the protozoa (Table 3). After the protozoa were exposed to increasing amounts of Hg^{2+} for 39 hours, the mortality at 20 ppb Hg was 0.4%, compared with 25.1% for cells not exposed to mercury. Ciliates previously exposed to mercury and subsequently suspended in 30 ppb mercury and ciliates not exposed to mercury but subsequently suspended in 20 ppb mercury yielded mortalities of 1.6 and 83.4%, respectively, after additional exposure for 7 hours.

Table 2. Effect of mercury (10 ppb) on *U. nigricans* fed bacteria grown in the presence and absence of Hg

Time of Exposure (hr)	Ciliates Fed *Pseudomonas* sp. Grown in the Absence of Hg[a]			Ciliates Fed *Pseudomonas* sp. Grown on Hg-Containing Media[a]		
	Total	Dead	Mortality (%)	Total	Dead	Mortality (%)
0	280	0	0	319	1	0.3
5.5	279	72	26	327	5	1.5
14.5	250	66	26	331	28	8.4

[a] Numbers given are averages of three 50-μl samples.

Table 3. Experimental design for testing the capability of static cultures of *U. nigricans* to acquire resistance to Hg

Time of Exposure (hr)	% Mortality of Controls	% Mortality of Ciliates Not Initially Exposed to Hg	% Mortality of Ciliates Exposed to Hg
0	0	0	0
22	0	0	0[a]
27	0	0[b]	0[b]
29	nd[c]	18.2	5.8
39	0.75	25.1	0.40
42	Mercury added to controls to a final concentration of 20 ppb	nd	10 ppb mercury added to a final concentration of 30 ppb
49	83.4	nd	1.6

[a] Mercury added in increments at T_0 to final concentrations of 10 ppb at 22 hr, and 10 ppb added to the ciliates not initially exposed to mercury.
[b] At this point, mercury was added to a final concentration of 20 ppb.
[c] Not determined.

Effects of Ingesting Hg-Laden Bacteria on Ciliate Growth Rates

The concentration of mercury associated with the bacterial cells during growth in the presence of 100 μg/ml mercury was found to be 2700 μg Hg^{2+}/g dry cell weight. *Pseudomonas* sp., strain P244, cells grown in a medium containing 100 μg Hg/ml accumulated almost 0.3% mercury, by weight, in early logarithmic phase of growth.

Examination by electron microscopy of *Pseudomonas* sp., strain P244, in early logarithmic phase, grown in 100 μg Hg/ml, revealed no apparent abnormalities compared with *Pseudomonas* sp., strain P244, in early log phase of growth in mercury-free medium. Electron-dense areas, indicative of mercury accumulation, were absent from both the cytoplasm and the periplasmic space. In general, the cell dimensions appeared unaltered (Fig. 1).

Growth of both 3- and 14-day-old *U. nigricans* cultures feeding on mercury-free and mercury-laden bacteria is shown in Fig. 2. No differences were observed in the length of lag phase, mean generation time, or final cell yield of 3-day-old *U. nigricans* feeding on *Pseudomonas* sp., strain P244, grown in 0, 10, 50, and 100 μg Hg/ml (Fig. 2 and Table 5). An 18- to 21-hour lag period was observed for all of the 14-day-old *U. nigricans* cultures, although the mean generation time and final cell yield for all 14-day-old protozoan cultures were comparable to 3-day-old cultures feeding on mercury-free and mercury-laden bacteria (Table 5). An analysis of covariances revealed no significant differences, at the $p > 0.01$ level, in the growth rate for different treatments within the same age group of protozoa, and when the growth rates of the two age groups were compared.

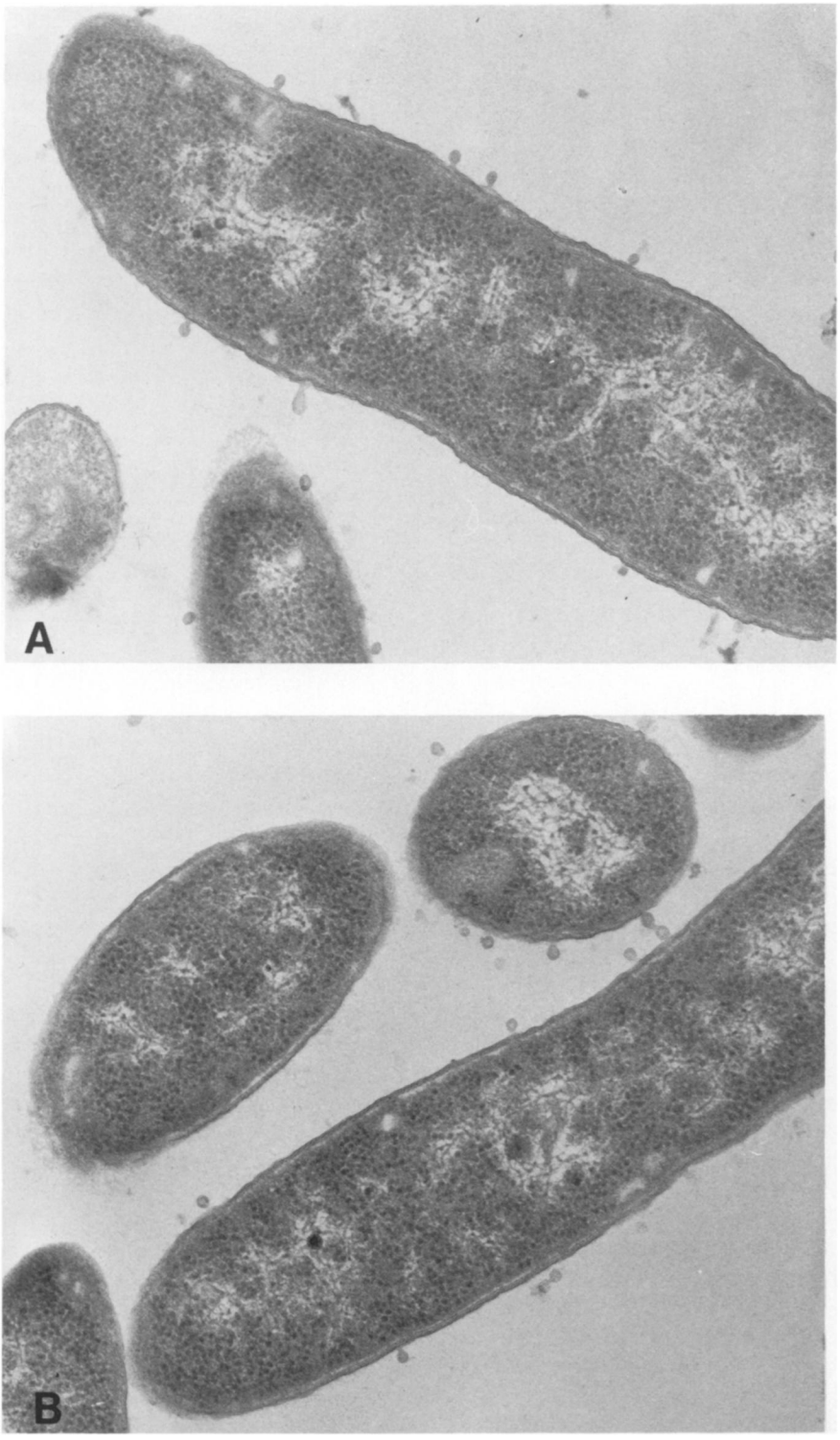

Fig. 1. Electron micrographs of *Pseudomonas* sp., strain P244, grown in the presence **(A)** and absence **(B)** of mercury. Magnification 67,000 ×.

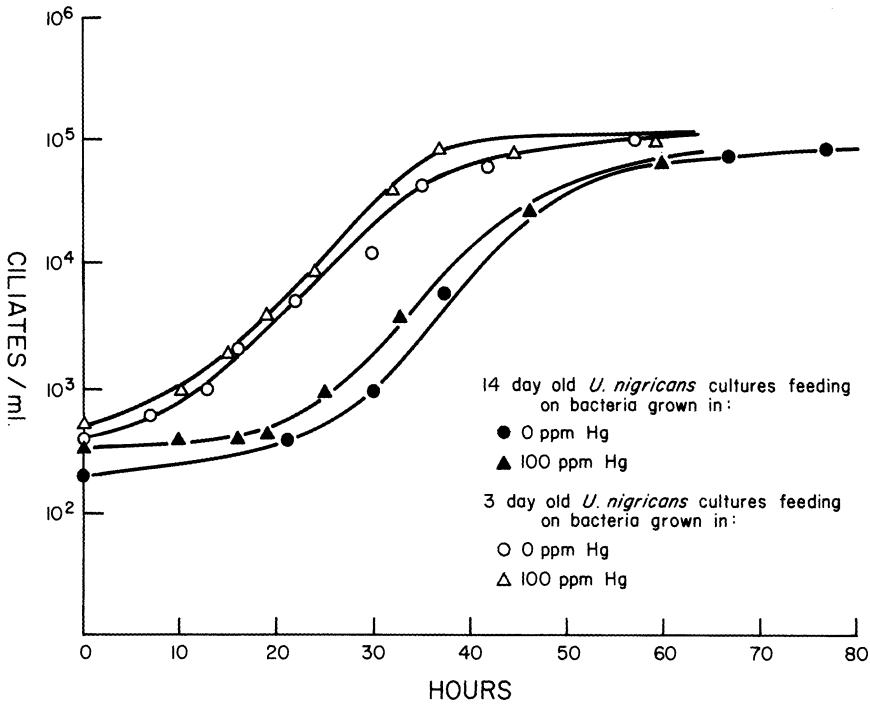

Fig. 2. Growth of *U. nigricans* fed *Pseudomonas* sp., strain P244, grown in presence of 100 μg Hg/ml and in the absence of mercury.

Discussion

Mercury concentrations toxic for *U. nigricans* in this study, when compared with lethal levels reported by other investigators for ciliates, were found to be within the same range (Table 4).

The geographical distribution and concentration of mercury in Chesapeake Bay water and sediment have been determined, with the data showing concentrations as high as 800 ppb occurring in sediment and 0.49 ppb in the water column of the most heavily polluted areas of Baltimore Harbor (2). The range of these concentrations of mercury found in Chesapeake Bay was selected for the study of *U. nigricans* reported here in order that the experiments be designed to simulate natural conditions of mercury pollution to which the organism would most likely be exposed.

Sartory and Lloyd (21) observed that populations of ciliates could be found in water samples containing concentrations of metal in solution in excess of levels found to be lethal in laboratory tests, suggesting that populations of protozoa can adapt to, or acquire a tolerance to, elevated concentrations of metals. Although the strain of *U. nigricans* examined in this study did not survive exposure to 10 ppb Hg^{2+} in solution without adverse effects, the organism could feed and grow on bacteria that contain approximately 1000 ppb Hg^{2+} *in toto*. Furthermore, when *U. nigricans* was fed Hg-laden bacteria for 1 week, the ciliate exhibited

Table 4. Tolerance of several species of ciliates to Hg[a]

Organism	Toxic Level of Hg^{2+}	Time Period of Exposure (hr)	Reference
Uronema nigricans	10–20 ppb	0.5	
Cristigera spp.	15 ppb = 100% mortality;	4	9
	3.7 ppb reduced growth 12%	4	10
Vorticella convallaria	5 ppb	48	21
Euplotes vannus	1 ppm	48	20
	100 ppb = no effect		
Tetrahymena pyriformis	500 ppb	24	24
Tetrahymena pyriformis	3.12 ppm = 50% mortality	0.5	4
Vorticella (6 species)			
Stentor (4 species)			
Campanella (1 species)	148–222 ppb = 50% mortality	3	
Carchesium (1 species)	74 ppb = no effect	48	3
Epistylis (2 species)			

[a] Toxic levels are those concentrations to which exposure resulted in 100% mortality of the protozoa.

increased tolerance to mercury in solution. In the latter case, it is possible that selection might have occurred, producing populations of ciliate tolerant to higher levels of mercury. It is highly unlikely that the ingested bacteria were responsible for detoxifying the mercury presented to the ciliates. One reason is that the bacteria were observed to be motile within the ciliate food vacuoles for less than 1 minute. The time between sealing of a vacuole and its arrival at the posterior end of the cell, where the bacteria are lysed, has been reported to be as short as 30 to 45 seconds for a *Uronema* sp. (11). More importantly, the mercury-free bacteria should have demonstrated the same degree of detoxification as the mercury-containing bacteria, since they were the same strain; yet upon exposure to solute mercury, there were dramatic differences in mortality of the ciliates.

One mechanism of mercury toxicity has been attributed to the binding of ionic mercury to sulfhydryl groups of proteins and enzymes (23). The effects of

Table 5. Mean generation time of *U. nigricans* fed *Pseudomonas* sp., strain P244, grown in 0, 10, 50, and 100 μg Hg/ml

Concentration (μg/ml) of Hg in the Medium Used to Grow Bacteria Fed to the Ciliates	Mean Generation Times (min)[a]	
	3-Day-Old Ciliates	14-Day-Old Ciliates
0	231.0 ± 14.5	250.9 ± 12.6
10	220.8 ± 18.2	263.3 ± 9.7
50	210.9 ± 22.9	291.1 ± 33.2
100	244.4 ± 11.0	260.0 ± 11.5

[a] Values represent the mean ± SD of 3 observations.

mercury toxicity have been examined for *Tetrahymena pyriformis* (23, 24), and it has been hypothesized that the concentrations of mercury associated with increased generation time may be acting at the level of DNA synthesis. Condensation of chromatin has been observed, suggesting direct interaction of ionic mercury with the DNA (24). *U. nigricans,* however, showed immediate stress upon exposure to elevated concentrations of mercury, indicating a target other than DNA. Electron microscopic examination of cell damage incurred by sublethal levels of $HgCl_2$ to *T. pyriformis* (24) revealed dramatic changes in the mitochondria that were correlated with functional changes in the cell. Decreased ciliary movement, probably due to a decrease in ATP production, and deficiency of osmoregulation, manifested by cellular swelling, were observed. In the study reported here, *U. nigricans* also exhibited cellular swelling and cessation of ciliary movement.

Resistance to mercury by *U. nigricans* is adaptable within a single generation, based on results of the short-term exposures. Yet adaptation of the population through natural selection may also occur during longer time exposure to Hg^{2+}. It is known that mercury resistance in bacteria is associated with an inducible mercury reductase, and the phenomenon is apparent within a single generation (5, 13, 22, 26). In the alga *Dunaliella,* mercury tolerance is related to a slow rate of mercury accumulation by this species but is largely due to detoxification of mercury within the cell by precipitation of a highly insoluble mercury compound (8). Binding of Hg^{2+} to large organic molecules or to bacteria and detritus may also account, in part, for survival of ciliates in natural waters containing large amounts of mercury. The question of how ingestion of mercury confers increased tolerance to Hg^{2+} ions in solution is extremely interesting and remains unresolved. The detailed biochemical mechanism(s) conferring mercury resistance on *U. nigricans* requires further investigation before an acceptable hypothesis can be offered.

Morphological abnormalities of mercury-resistant species of bacteria grown in mercuric chloride have been reported (25). It was therefore a concern that *Pseudomonas* sp., strain P244, might be morphologically altered during growth in mercury-containing media and consequently not be as readily recognized as food by the predator species. No apparent morphological abnormalities were observed for *Pseudomonas* sp., strain P244, grown in 100 μg Hg/ml. It is therefore concluded that *Pseudomonas* sp., strain P244, both mercury-laden and mercury-free, was recognized equally, on a morphological basis, as food by the protozoa in the feeding experiments.

The ingestion of mercury-laden prey by *U. nigricans* revealed no detectable effect on the growth rate of the ciliates. Significantly similar growth rates on equal numbers of bacteria indicate similar feeding rates by the ciliates, especially since the mercury-free and mercury-laden bacteria were not different in size or shape.

Mercury associated with the bacteria is probably complexed with organic cellular components, thus providing an explanation of the resistance demonstrated by *U. nigricans* to the potential lethal effects associated with ingestion of small, concentrated "packets" of mercury.

Dramatic differences in size, motility, and population density existed between 3- and 14-day-old *U. nigricans* cultures. Because it is not certain which

of the two age groups most closely resembles the condition of the majority of *Uronema* sp. in the natural environment of Chesapeake Bay, feeding studies were performed using both age groups of ciliates. Tolerance of ingested mercury by both age groups of ciliates was found to be approximately the same, indicating that resistance to ingested mercury in the protozoa is not age-dependent.

In a study similar to the one reported here, algae grown in the presence of 164 ng/ml of mercuric chloride were fed to planktonic copepods. Although the algae contained mercury accumulated during growth, no effects on the egg-laying rate of the feeding copepods or on the copepod progeny were observed (19). Thus in both an algae-copepod system and a bacteria-protozoa system, ingestion of mercury-laden food had no apparent effect on growth of the predators.

Natural selection in polluted environments results in bacterial populations possessing the ability to tolerate and accumulate metals (16, 18). The phenomenon of acquired resistance to metals via direct exposure of the protozoa to the metals may explain the presence of protozoa in water containing concentrations of metals otherwise lethal for the protozoa. Since ciliates can serve as food source for other zooplankton, acquired resistance to metals by the ciliates, coupled with unaltered growth rates after ingestion of mercury-containing food, provide mechanisms for passage of metals through the food chain. Clearly, the phenomenon of increased tolerance to mercury required that the use of ciliates as indicator organisms be reevaluated, since, from the results reported here, the presence of ciliates may not always reflect a healthy aquatic environment, as suggested by Carter and Cameron (4).

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