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Accumulation of Selenium in a Model Freshwater Microbial Food Web

ROBERT W. SANDERS[1]* AND CYNTHIA C. GILMOUR[2]

Academy of Natural Sciences, Patrick Center for Environmental Research, Philadelphia, Pennsylvania 19103,[1] and Academy of Natural Sciences, Benedict Estuarine Research Laboratory, Benedict, Maryland 20612[2]

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The transfer of selenium between bacteria and the ciliated protozoan, *Paramecium putrinum*, was examined in laboratory cultures. The population growth of the ciliate was not inhibited in the presence of the highest concentrations of dissolved selenite or selenate tested (10^3 μg liter^{-1}). Experiments with radioactive ^{75}selenite or ^{75}selenate indicated that accumulation of selenium by ciliates through time was low when feeding and metabolism were reduced by incubating at 0°C. However, selenium accumulated in ciliate biomass during incubation with dissolved ^{75}Se and bacteria at 24°C and also when bacteria prelabeled with ^{75}Se were offered as food in the absence of dissolved selenium. When ^{75}Se-labeled bacterial food was diluted by the addition of nonradioactive bacteria, the amount of selenite and selenate in ciliates decreased over time, indicating depuration by the ciliates. In longer-term (>5-day) fed-batch incubations with ^{75}selenite-labeled bacteria, the selenium concentration in ciliates equilibrated at approximately 1.4 μg of Se g (dry weight)$^{-1}$. The selenium content of ciliates was similar to that of their bacterial food on a dry-weight basis. These data indicate that selenium uptake by this ciliate occurred primarily during feeding and that biomagnification of selenium did not occur in this simple food chain.

Selenium is a nonmetallic element found in a variety of chemical forms in both marine and freshwaters. Selenate (Se VI) and selenite (Se IV), the common inorganic forms in natural waters, occur together with a poorly understood suite of Se-containing organic compounds. Selenium is required in trace amounts by organisms ranging from bacteria to phytoplankton to mammals. However, as a consequence of natural, agricultural, or industrial processes, selenium can reach levels of parts per billion and lead to death or reproductive failure of fish and waterfowl (14, 20, 22). The deleterious effects of selenium are attributed to body burdens accumulated from food or via uptake of dissolved selenium (19, 26).

The degree to which selenium is transferred through the food web and the mechanisms of transport are not well understood. However, it is likely that microorganisms contribute substantially to these processes. Bacteria are important in the transformation between the organic and inorganic forms of selenium (7, 10, 23, 24), and both bacteria and phytoplankton readily take up dissolved selenite, selenate, and organic selenium compounds via active and passive mechanisms (12, 30, 39, 42). Microorganisms incorporate selenium into amino acids and other macromolecules (9, 36), and reversible adsorption may also result in the accumulation of selenium (30). Regardless of the mechanism of microbial uptake, once selenium is associated with bacteria or algae, it is potentially available to bacterivorous and algivorous predators. Heterotrophic protists (protozoa) are important predators of both bacteria and phytoplankton in aquatic ecosystems (29, 31, 35, 40) and in turn are ingested by common freshwater zooplankton (33). Consequently, as intermediaries in aquatic food webs, heterotrophic protists may affect the transfer of selenium.

The major objectives of this study were to determine if selenium was accumulated by a bacterivorous protist feeding on bacteria and, if accumulated, whether it was bioconcentrated to levels exceeding those in the bacterial food and the water. We chose *Paramecium putrinum* as an experimental organism because ciliates in this genus are common in freshwaters and have previously been used as models for a variety of ecological and physiological processes. Additionally, preliminary tests indicated a tolerance of selenium by *P. putrinum*, their size allowed for efficient separation from bacterial prey, and their high feeding rates allowed for the accumulation of measurable amounts of radioactive selenium during short-term feeding experiments.

MATERIALS AND METHODS

Cultures and media. The ciliate *P. putrinum* (CCAP 1660/14) from the Culture Collection of Algae and Protozoa (Cumbria, United Kingdom) was maintained in a 0.1% Cerophyll ryegrass broth (media 802 [2]) with mixed bacteria as a food source. The ciliates were approximately 75 μm in length and had an average calculated biovolume of 1.4×10^5 μm^{-3}. For experiments in which bacteria and protozoa were added separately, unidentified bacteria from the *Paramecium* culture were grown in 0.25% Cerophyll broth or 0.2% yeast extract; cultures of the bacteria *Pasteurella* sp. (IHP#3 strain) (32) and *Corynebacterium* sp. were maintained on 0.2% yeast extract. All experiments were run in a synthetic growth medium (30) that otherwise simulated the major ion composition of a selenium-impacted lake (Hyco Lake, N.C.). The Hyco medium had the following composition (in milligrams per liter of distilled water): $CaCl_2 \cdot H_2O$, 32.2; $MgSO_4 \cdot 7H_2O$, 36.9; $NaHCO_3$, 25.2; K_2HPO_4, 8.7; $NaNO_3$, 85; $Na_2SiO_3 \cdot 9H_2O$, 28.4; KBr, 0.12; NaF, 0.4; FeEDTA, 4.3; $CuSO_4 \cdot 5H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.022; $CoCl_2 \cdot 6H_2O$, 0.01; $MnCl_2 \cdot 4H_2O$, 0.18; $Na_2MoO_4 \cdot 2H_2O$, 0.006; H_3BO_3, 0.62. HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer was used at 2.5 mM, and the pH was adjusted to 7.0 with NaOH.

Selenium toxicity. Chronic toxicity of selenium to *P. putrinum* was examined by comparing population growth in con-

* Corresponding author. Mailing address: Academy of Natural Sciences, 1900 Benjamin Franklin Parkway, Philadelphia, PA 19103-1195. Phone: (215) 299-1003. Fax: (215) 299-1028.

TABLE 1. Parameters of growth and preparation of ^{75}selenium-labeled *Pasteurella* sp. for ciliate feeding experiments shown in Fig. 3

Treatment	Selenium addition to bacterial cultures (μg liter^{-1})	Final bacterial density (cells ml^{-1})	Selenium accumulation by bacteria		Dissolved selenium carryover in ciliate cultures spiked with labeled bacteria (μg liter^{-1})
			ng cell^{-1}	μg g (dry wt)$^{-1}$	
Selenite	10	2.6×10^7	8.2×10^{-10}	2.33	0.004
Selenate	50	2.6×10^7	9.0×10^{-10}	2.58	0.011

trols with growth in a series of dissolved selenite or selenate concentrations. Selenite and selenate (Sigma Chemical Co., St. Louis, Mo.) were stored dry at room temperature until just prior to the start of the experiments, when stock solutions of selenite and selenate were made up in sterile Hyco medium. Stock solutions were made to take into account the volume of medium added with the microorganism inocula to give final concentrations of 10, 100, and 1,000 μg of selenite or selenate liter^{-1}. *Paramecium* and bacterial cultures were centrifuged at 425 $\times$ *g* for 30 min and 8,600 $\times$ *g* for 15 min, respectively, to separate the microorganisms from their growth medium. The pelleted *P. putrinum* and bacteria were resuspended in sterile Hyco medium, enumerated, and added to the solutions of selenium. Three replicates were run for controls and each concentration of selenite and selenate. The controls were identical to the treatment flasks, except selenium was not added. Samples were taken from each replicate and preserved with 2% Lugol's iodine (ciliates) or 10% formalin (bacteria) at approximately 24-h intervals for a period of 120 h, at which time the ciliates had reached stationary growth phase in all treatments. Bacterial samples were enumerated by epifluorescence microscopy (16). Ciliates from preserved samples were enumerated with phase-contrast microscopy at $\times$ 200 magnification. Specific growth rates were determined from the slopes of lines fit to ln(*Paramecium* abundance) versus time.

Bacterial uptake of ^{75}selenium. Uptake of selenium was determined for axenic cultures of *Pasteurella* sp. and *Corynebacterium* sp., using radioactive selenite and selenate ($Na_2{}^{75}SeO_3$ and $Na_2{}^{75}SeO_4$; Amersham Corp., Arlington Heights, Ill.) The specific activities of the selenite and selenate were 24 (3.29 μCi nmol^{-1}) and 385 (205 nCi nmol^{-1}) ng μCi^{-1}, respectively. Radioactive selenium was quantified with a Tennelec NaI(T1) gamma counting system equipped with a 7.6-cm well detector and multichannel analyzer. Experimental samples were calibrated against ^{75}Se stock isotope solutions.

Bacterial isolates in logarithmic growth were transferred to 0.2% yeast extract in Hyco medium, and incubations were run in duplicate at 22°C in the presence of 10 μg of selenium liter^{-1} as $Na_2{}^{75}SeO_3$ or $Na_2{}^{75}SeO_4$. Subsamples (5 ml) were removed at intervals, filtered onto 0.2-μm-pore-size membrane filters (Millipore GS), and washed with fresh Hyco medium prior to gamma counting. Growth for each species was monitored by using optical density (A_{660}) previously calibrated against cell abundance and dry weight measurements. Due to an interruption in availability of the isotope, the uptake of selenate by *Corynebacterium* sp. was not determined.

Se accumulation and depuration by *P. putrinum*. Gentle reverse filtration through a 5-μm-mesh sieve and copious rinsing with fresh medium were used to concentrate ciliates and reduce bacterial abundance for all accumulation experiments. This technique removed spent culture medium and most bacteria, while avoiding injury to ciliates from compression against a filter. Uptake of selenium by *P. putrinum* was examined in two ways. In the first type of experiment, dissolved $Na_2{}^{75}SeO_3$ or $Na_2{}^{75}SeO_4$ was added directly to mixed bacterial-ciliate cultures in Cerophyll-enriched (0.1%) Hyco medium. Bacterial abundances had been reduced to $\approx 10^5$ cells ml^{-1} in these cultures. Final concentrations of selenium in the cultures were 10 μg liter^{-1} for selenite and 50 μg liter^{-1} for selenate. For each selenium ion, duplicate samples were incubated at 24°C to examine active uptake of selenium by ciliates. Another set of samples was incubated at 0°C to reduce bacterivory and the metabolic rates of both ciliates and bacteria. Any uptake of ^{75}Se in the 0°C cultures was assumed to be due to adsorption rather than active uptake. Samples were taken at 0, 1, 2, 4, 8.5, 20, and 27 h for gamma counting and microscopic enumeration of ciliates and bacteria.

In a second type of experiment, bacteria were prelabeled with ^{75}selenium before addition to *P. putrinum* in unenriched Hyco medium. *Pasteurella* sp. was labeled by incubation with 10 μg of selenite liter^{-1} or 50 μg of selenate liter^{-1} for 24 h during exponential growth in 0.2% yeast extract (Table 1). Dissolved ^{75}selenium was separated from the bacteria by centrifugation and washing, and each of the ^{75}Se-labeled bacterial suspensions was added to duplicate cultures of bacteria-free ciliates in unenriched Hyco medium. Incidental transfer of dissolved selenium into the ciliate cultures was <0.02 μg liter^{-1} (Table 1). Cultures were maintained at 25°C in the dark, and samples for gamma counting and microscopic enumeration were taken immediately upon addition of the labeled bacteria and at 15 and 22 h. Accumulation of selenium in the ciliates was determined from the radioactivity retained on 5-μm-pore-size polycarbonate filters (Nuclepore Corp.). Preliminary tests with ^{75}Se-labeled bacteria indicated minimal retention of bacteria on these filters (3 to 6% retention on a 0.4-μm-pore-size filter). After sampling at 22 h, bacteria that had not been exposed to selenium were added to a final density of 5×10^7 cells ml^{-1}. This swamped the culture with unlabeled bacterial cells, and ciliate predation was then primarily on selenium-free bacteria. Selenium depuration rates by the ciliates were estimated from the reduction of ciliate-associated selenium during the next 23 h with the assumption that there was no further uptake during this time.

In a similar experiment, *P. putrinum* ciliates were maintained in fed-batch culture in Hyco medium for 6 days with ^{75}selenite-labeled *Corynebacterium* sp. as food. The initial addition of bacteria to the ciliates was from a *Corynebacterium* culture that had been incubated with ^{75}selenite for 100 h. The selenium content of bacterial cells was 1.9 μg of selenium g (dry weight)$^{-1}$ and the addition represented a spike of 5.7 ng of selenium liter^{-1} in bacterial cells. Additional ^{75}Se-labeled bacteria were added 21 and 44 h after the initial inoculation. Radioactivity in the ciliates was monitored over the time course to determine if selenite reached an equilibrium concentration in *P. putrinum*.

***Paramecium* feeding and egestion.** Ingestion rates for ciliates feeding on bacteria were estimated by the disappearance of bacteria from cultures in unenriched Hyco medium over time (15), assuming that the lack of added organic substrate precluded bacterial growth. In a separate experiment, *Parame-*

TABLE 2. Specific growth rates of *P. putrinum* during incubation with dissolved selenite and selenate[a]

Concn of added selenium (μg liter^{-1})	Specific daily growth rate (±SE)	
	Selenite	Selenate
0	0.32 ± 0.06	0.48 ± 0.05
10	0.39 ± 0.02	0.43 ± 0.10
100	0.39 ± 0.05	0.44 ± 0.08
1,000	0.38 ± 0.05	0.49 ± 0.09

[a] Growth rates in treatments were not significantly different from those in controls with no added selenium ($P > 0.05$; analysis of covariance).

cium egestion rates were determined by a modification of the method of Sherr et al. (34). Briefly, tracer amounts of nondigestible 0.9-μm Fluoresbrite carboxylated microspheres (Polysciences, Warrington, Pa.) were added to cultures of *P. putrinum* and bacteria. Aliquots were fixed over a time course, and microspheres within individual ciliates were counted by epifluorescence microscopy. Microsphere accumulation in food vacuoles was monitored until the number of microspheres per cell plateaued. The microsphere concentration was then reduced by dilution with a large volume of Hyco medium containing bacteria, so that microspheres were rarely encountered and ingested. The time required (after dilution) for the average number of microspheres per ciliate to reach a constant background level was considered the retention time for any undigestible portion of the bacterial biomass.

RESULTS

Toxicity. Neither acute nor chronic toxicity was observed for *P. putrinum* exposed to dissolved selenite and selenate at concentrations of up to 1,000 μg of Se liter^{-1}. Microscopically observed swimming behavior was normal in all selenium treatments, and ciliate population growth was at least as high in selenium-exposed cultures as in unexposed controls (Table 2). Differences in ciliate growth between treatments and the controls were not statistically significant (Table 2) ($P > 0.05$; analysis of covariance). The growth of *Pasteurella* sp. and *Corynebacterium* sp. in the presence of 10 μg of Se liter^{-1} (Fig. 1) was similar to growth in the absence of added selenium (data not shown).

Bacterial uptake of Se. Accumulation of selenium by *Pasteurella* sp. occurred in two phases. There was an initial rapid and comparable uptake in the presence of both selenite and selenate during the first hour, after which accumulation continued, but at lower rates (Fig. 1A). Selenate uptake was not as rapid as cell growth, so that the selenate concentration per unit biomass decreased after the first hour of incubation (Fig. 1A). The relatively constant amount of selenate (≈0.5 μg of Se g [dry weight]$^{-1}$) associated with *Pasteurella* sp. during the final portion of the growth phase indicated that uptake matched growth. Accumulation of selenite by *Pasteurella* sp. was more rapid than cell growth and about 10 times more rapid than selenate uptake. *Corynebacterium* sp. accumulated selenite throughout the growth phase. After 8 h, selenite reached a concentration (7.6 μg of Se g [dry weight]$^{-1}$) that was more than double that observed for *Pasteurella* sp. after 8 h of incubation (2.9 μg of Se g [dry weight]$^{-1}$) (Fig. 1).

Accumulation of dissolved selenium by *P. putrinum*. Incubation at 0°C was used as an indicator of nonactive uptake (i.e., by adsorption and diffusion) due to low metabolisms of ciliates and bacteria at this temperature. Ciliate and bacterial abundances remained constant at this temperature, and sorption by

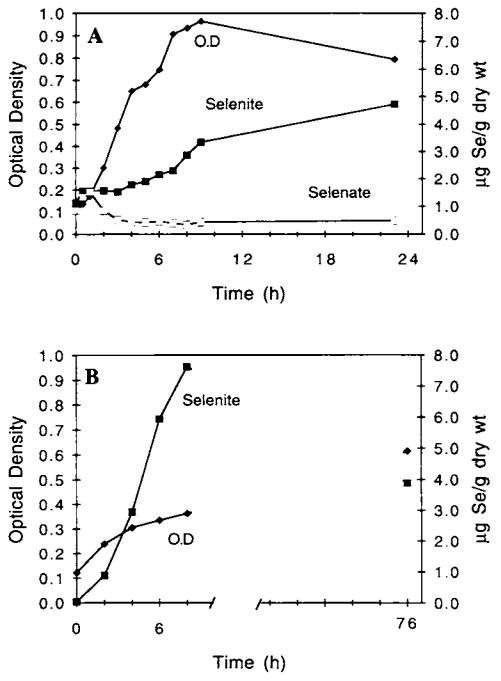

FIG. 1. Weight-specific selenium contents and optical densities of bacterial cultures incubated with dissolved ^{75}selenite or ^{75}selenate (10 μg liter^{-1}). (A) *Pasteurella* sp. Optical density was plotted only for the selenate incubation, but optical density in the selenite incubation was nearly identical at every sampling. (B) *Corynebacterium* sp. ^{75}Selenate was not available at the time of the experiment.

ciliates equilibrated after 2 to 4 h at approximately 0.1 and 0.2 μg of Se g (dry weight)$^{-1}$ for selenite and selenate, respectively (Fig. 2). In the incubations at 24°C, *Paramecium* abundances remained constant (approximately 200 ciliates ml^{-1}), but bacteria increased from an initial density of 1.3×10^5 to 1.5×10^7 cells ml^{-1} after 20 h. Uptake of selenium by ciliates at 24°C was initially similar to uptake at 0°C (Fig. 2). However, after 8.5 h, concentrations of selenium in ciliates from 24°C incubations exceeded those from 0°C incubations. At 20 h, concentrations of selenite and selenate in ciliates from the 24°C

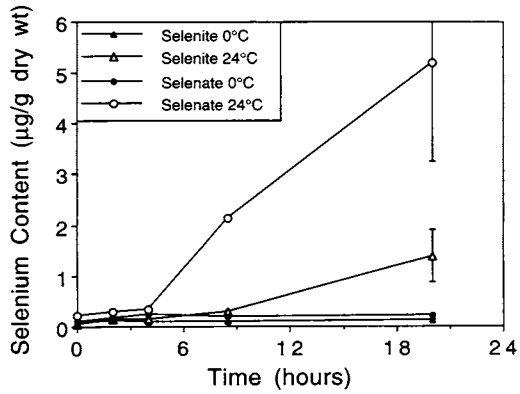

FIG. 2. Selenium contents of *P. putrinum* (means ± 1 standard error) during incubation at 0 and 24°C in the presence of dissolved ^{75}selenite (10 μg liter^{-1}) or ^{75}selenate (50 μg liter^{-1}). Live bacteria were present in all incubations.

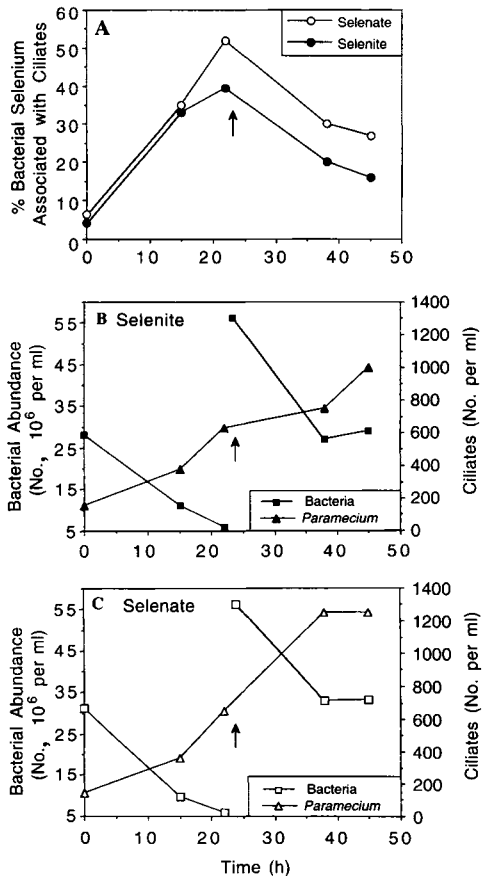

FIG. 3. (A) Selenium content in *Paramecium* biomass as a percentage of that originally associated with prelabeled bacteria added as a food source. Arrow indicates addition of unlabeled bacteria (5×10^7 cells ml^{-1}). The addition of a high concentration of unlabeled bacteria minimized uptake of remaining prelabeled bacteria, and the percentage of selenium associated with ciliates declined as selenium was depurated. (B) Abundances of bacteria and *P. putrinum* in the selenite treatment. (C) Abundances of bacteria and *P. putrinum* in the selenate treatment.

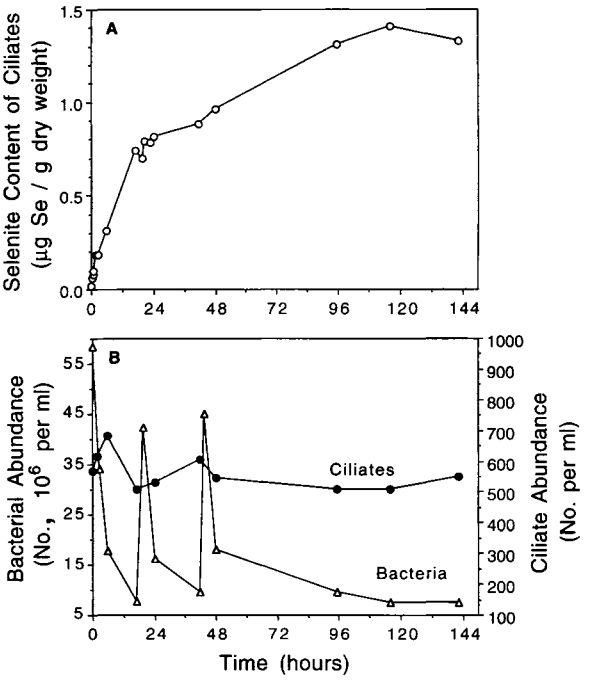

FIG. 4. (A) Selenite content of *P. putrinum* versus time in a fed-batch culture of ^{75}selenite-labeled bacteria. (B) Abundances of *P. putrinum* and bacteria in the fed-batch cultures.

treatments were 1.39 and 5.19 µg of Se g (dry weight)$^{-1}$, respectively (Fig. 2).

Accumulation of bacterium-associated selenium by *P. putrinum*. Within 15 h of the addition of bacteria preexposed to ^{75}selenite or ^{75}selenate, *Paramecium* abundance increased approximately twofold and bacterial abundance was reduced by 80% in both treatments (Fig. 3). *P. putrinum* ingested an average of 4,600 and 5,400 bacteria h^{-1} in the selenite and selenate treatments, respectively, and by 22 h approximately 40 and 50% of the selenium originally associated with bacteria in the treatments were in the ciliate size fractions (Fig. 3A). The maximum selenium content of the ciliates (0.80 µg g [dry weight]$^{-1}$ with added selenite and 0.86 µg g [dry weight]$^{-1}$ with selenate) was much less than the biomass specific content of the bacterial food (Table 1).

Within 17 h after the addition of unlabeled bacteria, selenium content of the ciliates decreased to 20% (selenite treatment) and to 30% (selenate treatment) of that originally associated with bacteria (Fig. 3A). Assuming negligible ingestion of Se-labeled bacteria during this period, the loss of selenium from ciliates was equivalent to a depuration rate of 0.022 µg g (dry weight)$^{-1}$ h^{-1} in both treatments. In a longer-term experiment in which ciliates were fed ^{75}selenite-labeled *Corynebacterium* sp. daily for 3 days, *P. putrinum* accumulated selenium from ingested bacteria for a period of 6 days (Fig. 4A). The selenium content of the *Corynebacterium* sp. in this experiment was 1.86 µg of Se g (dry weight)$^{-1}$. Selenium content of the ciliates reached 0.81 µg of Se g (dry weight)$^{-1}$ within 24 h and equilibrated at approximately 1.35 µg of Se g (dry weight)$^{-1}$ after 4 days.

Egestion and digestion rates of *P. putrinum*. The loss of selenium from ciliates ingesting Se-labeled bacteria may have been due to excretion of dissolved selenium or from egestion of selenium-laced bacterial cell debris from the ciliates' digestive vacuoles. Fluorescent microspheres were used as nondigestible visual tracers of egestion by the ciliates to quantify the time scale of potential losses due to egestion of incompletely digested bacteria. Disappearance of microspheres from ciliates indicated that, at 25°C, any undigested material in vacuoles would be egested within 1 h of ingestion. Thus, losses of selenium with bacterial cell debris would have occurred soon after the addition of unlabeled bacteria (arrows in Fig. 3).

DISCUSSION

Toxicity of selenium to microorganisms. Selenium toxicity has been determined for only a few heterotrophic protozoa. A concentration of 10,000 µg of selenite $liter^{-1}$ (the lowest concentration tested) inhibited the growth of the heterotrophic dinoflagellate, *Crypthecodinium cohnii* (28). Growth of another heterotrophic flagellate, *Entosiphon sulcatum*, was affected in the presence of only 3 µg of selenite $liter^{-1}$ (4). The addition of 20 to 160 µg of selenite $liter^{-1}$ to freshwater laboratory microcosms had no effect on protozoan biomass accumulation but did result in reduced protozoan diversity after 21 days of exposure (27). In outdoor experimental streams, protozoan species richness was unaffected relative to controls after 10

days of exposure to 30 μg of selenite liter^{-1} (27). The current study indicated that the concentrations of selenite and selenate found in any but the most highly selenium-contaminated waters (8, 18, 37, 38) are unlikely to be directly toxic to *P. putrinum*. Indirect effects such as changes in community structure, however, could still affect populations of selenium-tolerant species such as *P. putrinum*.

The effect of selenium on other microorganisms was examined in several studies. Consistent with the data for *P. putrinum*, inorganic selenium compounds were not highly toxic to cultured and naturally occurring bacteria (4, 5, 14a) or to phytoplankton (4, 30, 41). But some algae are negatively affected by relatively low concentrations of selenium. The growth of the green alga, *Selenastrum capricornutum*, was inhibited at ≤125 μg of selenite liter^{-1}, and that of *Scenedesmus* sp. was inhibited at 840 μg of selenite liter^{-1} (4, 13). Conversely, benthic diatom growth was inhibited at concentrations as low as 1,000 μg of selenate liter^{-1}, but diatoms grew well, albeit with reduced diversity, in the presence of selenite up to a concentration of 40,000 μg liter^{-1} (25). It appears that protozoa and algae, at least, show species-specific ranges of sensitivity to selenium and that the form of dissolved selenium may be an important factor in its toxicity.

Bacterial uptake. The initial rates of selenium uptake by the bacterium *Pasteurella* sp. in the presence of selenite or selenate were rapid and comparable, but uptake was much slower after 2 h (Fig. 1). This biphasic accumulation of selenium by *Pasteurella* sp. resembled a pattern previously observed for algal cultures (30). Riedel et al. (30) found that the initial rapid accumulation of selenium, especially selenite, was similar for both active and heat-killed phytoplankton cells and concluded that most initial uptake by algae was due to abiotic sorption. A slower, but apparently active, process dominated uptake by phytoplankton during the remainder of their growth (30). Rapid initial uptake of selenite by heat-killed ruminal bacteria (17) and by natural populations of freshwater bacteria after heat killing (14a) indicates that strong abiotic sorption of dissolved selenite occurs in bacteria as well as in algae. The slower accumulation of selenium by *Pasteurella* sp. in the second stage of uptake (i.e., after 2 h) is also consistent with the phytoplankton model and may represent active accumulation. In any case, the uptake of selenite after 2 h was more rapid than that of selenate by a factor of 10 (per unit of biomass). Preferential accumulation of selenite was also reported for marine bacteria (12), algae (30, 39, 41), zooplankton, and periphyton (3) and is consistent with shorter selenite residence times in coastal waters and the ocean (9, 43).

Dissolved selenium and *P. putrinum*. The processes that could contribute to the accumulation of dissolved selenium by ciliates (Fig. 2) include adsorption to the cell surface, passive diffusion, active uptake across the membrane, and uptake with water engulfed during the formation of food vacuoles. Selenium contents of *P. putrinum* at the end of the 0°C incubations were 2.1 and 4.1 fg of selenate ciliate^{-1}, which was about equal to, or much less than, the concentration dissolved in the media for selenite and selenate, respectively. Thus, in contrast to algal and bacterial cells (14a, 17, 30), passive accumulation of selenium by *P. putrinum* via adsorption and diffusion was negligible compared with active processes. This may have been due in part to differences in cell size since adsorption is presumably a surface phenomenon and the surface/volume ratio of the bacteria and phytoplankton are greater than that of *Paramecium* spp.

Active uptake appears to account for most of the accumulation of selenium observed at 24°C. The data are consistent with incidental uptake of dissolved selenite and selenate during the formation of food vacuoles and ingestion of bacteria. Water is typically taken into food vacuoles as they form at the cytostome (mouth) and pinch off internally during feeding by ciliates. Loss of the fluid to the cytoplasm during digestion reduces the size of the food vacuoles (1, 21). Although osmoregulatory organelles remove water from the cell, the selenium content of the excreted water is not necessarily the same as that which enters in the food vacuole. Particulate matter (i.e., bacteria) is important in inducing food vacuole formation in ciliates, and more food vacuoles are formed at higher food concentrations (21). Consequently, as bacterial abundance increased during the course of the incubation, ingestion rates and vacuole formation also would have increased. The net result would be a larger influx of food- and water-associated selenium later in the experiment, as was observed for *P. putrinum*.

The importance of feeding for selenium accumulation by *Paramecium* spp. is further suggested by the relative degree that ciliates accumulated dissolved selenite and selenate. When abiotic sorption was examined in phytoplankton and bacteria, the accumulation of selenite was approximately an order of magnitude greater than that of selenate (14a, 28). However, the concentrations of selenite and selenate in *P. putrinum* relative to the water were similar (140- and 104-fold, respectively). The factor of 10 difference observed for the abiotic uptake of selenite versus selenate in bacteria and phytoplankton, but not in *P. putrinum*, suggests that sorption of selenite may be a less important mechanism of accumulation in phagotrophic cells such as ciliates.

The accumulation due to uptake of dissolved selenium by the ciliates versus that from ingested bacteria could not be directly determined from this experiment because the bacteria took up selenium at an unknown rate. However, the uptake of selenium by ciliates in the presence of bacteria and dissolved selenite and selenate (26.0 and 97.0 fg ciliate^{-1}, respectively) was more rapid and accumulation was greater than when washed ^{75}Se-labeled bacteria were added to cultures (see below) (Fig. 4). This indicates that direct accumulation from water via feeding may be a significant pathway for ciliates when dissolved selenium is present at high concentrations.

Accumulation and loss of food-associated selenium. The differences between the removal of bacterial biomass (>60%; Fig. 3) and the transfer of bacterium-associated selenium into *Paramecium* biomass (≤35%) suggest that much of the selenium ingested by the ciliates was not assimilated. In the absence of dissolved selenium, selenium per unit weight actually decreased in *P. putrinum* relative to its content in the bacterial food sources. The maximum concentration of selenium in *P. putrinum* during the 2-day experiment (Fig. 3) was <0.9 μg of Se g (dry weight)$^{-1}$ compared with approximately 2.6 μg of Se g (dry weight)$^{-1}$ in the bacteria (Table 1). Incorporation efficiency of selenium during the first 15 h of this experiment ranged from 28 to 34% as calculated from the total amount ingested with bacterial food and the selenium content of ciliates at the end of that time. In the experiment in which ^{75}selenite-labeled bacteria were replenished at regular intervals over 3 days, selenite continued to increase in *P. putrinum* until concentration per unit weight equilibrated 2 days after the final addition of Se-labeled bacteria (Fig. 4). The concentration of selenite in this bacterial food (*Corynebacterium* sp.) was 1.9 μg of Se g (dry weight)$^{-1}$, and selenite in *P. putrinum* reached equilibrium at about 1.4 μg of Se g (dry weight)$^{-1}$. It appeared that selenium was not biomagnified in ciliates feeding on bacteria in these experiments. Rather, the equilibrium concentration of selenium in the ciliates was lower than, but generally reflected, the concentration in their food.

The loss of selenium ingested by ciliates was calculated during two phases of the pulse-chase experiment (Fig. 3). The total amounts of selenite and selenate ingested per ciliate during the first 15 h of this experiment (calculated from the selenium content of bacteria and their disappearance over time) were 44.1 and 56.9 fg, respectively. Subtracting the selenium content of ciliates at 15 h from the total amount ingested, the maximum loss rates during that time were 1.9 (0.102 μg of Se g [dry weight]$^{-1}$ h^{-1}) and 2.7 (0.144 μg of Se g [dry weight]$^{-1}$ h^{-1}) fg of selenate ciliate^{-1} h^{-1}. These rates were considerably greater than the depuration (0.022 μg of Se g [dry weight]$^{-1}$ h^{-1}) determined later in the same experiment from the decrease in ^{75}Se associated with ciliate biomass after ingestion of ^{75}Se-labeled bacteria was minimized. A similar pattern of selenite loss from *P. putrinum* (calculated by subtracting accumulated selenite from ingested selenite) was observed in the 6-day experiment with labeled *Corynebacterium* sp. (Fig. 4). A loss of 0.243 μg of Se g (dry weight)$^{-1}$ h^{-1} was determined from samples taken early in the experiment when large numbers of bacteria were ingested. A much lower depuration rate (0.050 μg of Se g [dry weight]$^{-1}$ h^{-1}) was calculated for the period between days 2 and 4 when labeled bacteria had been reduced by grazing and ciliate ingestion rates were lower. These results could reflect a direct correspondence between the amount of selenium ingested and the amount excreted. However, it is also probable that a proportion of the selenium loss was from defecation of partially digested bacteria, i.e., that all of the ingested selenium was not assimilated. Egestion of any selenium-laced bacterial cell debris from the digestive vacuoles would be much less as labeled bacteria were depleted in the later stages of these experiments. Consequently, the lower rates of selenium loss reported here are probably more representative of the depuration of assimilated material.

General considerations of selenium in the microbial food web. Selenium is incorporated into amino acids, and its transfer and depuration in the microbial food web may be analogous to those of nitrogen in some ways. Food web transfer and remineralization of nitrogen and other nutrients by protozoa can be predicted from the carbon/nutrient ratios of the predator and prey if the respiration and gross growth efficiency are known (6). However, further study is needed before this model can be adapted to selenium. The degree to which selenite and selenate were biotransformed during accumulation is unknown for any of the experiments of the present study. Additionally, several factors may differentiate incorporation of selenium from that of nitrogen. These include the high proportion of selenium that binds abiotically to bacteria and phytoplankton food and the possibility that some seleno-amino acids synthesized by algae cannot be incorporated into proteins (11).

On the bases of this study, our related unpublished field work, and our colleagues' work with phytoplankton (30), our working hypotheses are that most selenium accumulation occurs at the base of the food web and selenium concentration in predators generally reflects the concentration in their prey. Because bacteria are not grazed efficiently by most metazoans, passage of selenium in the aquatic food web may be primarily via phytoplankton and protozoa because they are ingested at high rates by zooplankton and benthic microfauna (11, 33) and also occur as epibionts. Zooplankton and microfauna, in turn, are ingested by fish and waterfowl. Consequently, factors that determine the rate and amount of selenium accumulation in microorganisms may in large part determine the eventual accumulation of selenium in predators such as fish, whose reproductive cycles may be strongly affected at selenium levels that are not toxic to many microorganisms.

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