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Oxidation of Elemental Selenium to Selenite by *Bacillus megaterium*

Abstract. *A strain of* Bacillus megaterium *isolated from soil has been found to oxidize elemental selenium in laboratory cultures to selenite and a trace of selenate (< 1 percent of the selenite). This observation represents an important but hitherto unreported oxidative step in the biological selenium cycle.*

This report, the first to our knowledge of a heterotrophic bacterium, *Bacillus megaterium*, oxidizing elemental selenium, provides evidence for the hitherto undocumented microbial oxidation of selenium (*1*). This oxidative step in the selenium cycle is significant in relation to the occurrence and control of the economically important and widespread selenium-responsive diseases of farm animals. According to Muth (*2*), problem areas exist in at least half the states in the United States, and diseases have been recorded in at least 20 other countries, which include most of the major livestock-producing areas of the world. They are of particular importance in New Zealand (*3*).

Microorganisms play an important role in the selenium cycle (*1*), and evidence for the reduction of inorganic forms of selenium is abundant (*1*, *4*). In contrast, the direct microbial oxidation of selenium compounds has received very limited attention. Shrift (*1*) quoted three brief reports of the microbial oxidation of selenium compounds, and in each case selenate is stated to have been produced, from sodium selenite by *Aspergillus niger* (*5*) and from elemental selenium by *Ectothiorhodospira mobile* (*6*) and by a soil bacterium (*7*). However, Bird *et al.* (*5*) did not investigate the oxidation of selenium compounds by molds, and the tabulation indicating selenate as a product of metabolism by *A. niger* with selenite arose from a printing error; "Na_2SeO_4" was misplaced from a position as a column heading to a position in the column under "Na_2SeO_3" (*8*). Lipman and Waksman (*7*) reported the isolation of an autotrophic selenium-oxidizing organism, similar to *Thiobacillus*. This report was later virtually retracted (*9*), and our attempts to isolate an autotrophic selenium-oxidizing organism were unsuccessful. The report on the oxidation of selenium by *E. mobile* (*6*), analogous to the oxidation of sulfur, is similarly without supporting experimental data.

The bacterium was isolated from topsoil from river alluvium, taken to a depth of 7.5 cm, under pasture containing a relatively high concentration of selenium, 1.1 μg/g. The sample was taken at a site in a paddock adjacent to, and 20 km from the mouth of, the Whangaehu River on the North Island of New Zealand. Freshly collected soil was enriched with 0.1 percent of the gray metallic form of elemental selenium and incubated aerobically at 25°C for 6 months. A 1-g sample of soil was then inoculated into 100 ml of growth medium A containing, per liter, yeast extract, 1.0 g; NH_4NO_3, 1.0 g; K_2HPO_4, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; NaCl, 0.3 g; and gray elemental selenium, 1.0 g at *p*H 7.0. Inoculated flasks were incubated at 28°C in an orbital shaker (150 rev/min) for 8 days, and the contents were subcultured (1 : 100 by volume) into medium B (see Fig. 1). Enrichment cultures were incubated for 14 days under similar conditions, centrifuged, filtered (0.45 μm), and analyzed for selenite (*10*), since selenite but not selenate was produced in measurable amounts (selenate < 2 percent of selenite) during the initial 6-month incubation period. The net increase of selenite in these cultures after 14 days was 0.46 μg of selenium per milliliter. Atmo-

Table 1. Oxidation of elemental selenium by *Bacillus megaterium*. Incubation periods with red and gray selenium were 45 and 42 days, respectively.

Treatment*	Selenite production rate† (μg of selenium per liter per day$^{1/2}$)	Selenate produced† (μg of selenium per liter)
Red selenium (0.01 percent) + organism	208 ± 43	13.1 ± 3.5
Red selenium (0.01 percent)	14 ± 4	1.2 ± 0.14
Gray selenium (0.1 percent) + organism	69 ± 10	7.1 ± 0.61
Gray selenium (0.1 percent)	8 ± 3	4.3 ± 1.5

*Four replicates. †Mean ± standard deviation.

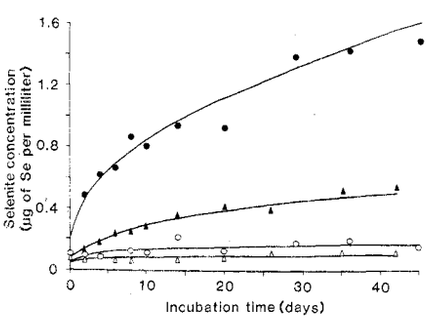

Fig. 1. Production of selenite from elemental selenium by *Bacillus megaterium* isolated from soil. Each experiment was carried out with four replicate flasks, each containing 50 ml of medium B inoculated with 1 ml of a culture 18 hours old (2.2×10^5 cells per milliliter) and incubated at 28°C in an orbital shaker (150 rev/min). Incubations were continued for 45 days (red selenium) and 42 days (gray selenium). Samples were removed periodically and analyzed for selenite (*10*). At the end of the incubation, all samples were centrifuged and filtered (0.22 μm); elemental selenium, selenite, and selenate were separated by anion-exchange chromatography, eluting with 1*M* HCl from a column of Dowex-1 × 8. Total selenium (*12*) was measured in each of 50 fractions per replicate. Medium B contained, per liter, yeast extract, 2.0 g; glucose, 1.0 g; NH_4NO_3, 1.0 g; K_2HPO_4, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; NaCl, 0.30 g; trace elements, 50 ml; elemental selenium, 1.0 g (gray selenium, analytical grade) or 0.1 g (red colloidal selenium); and Tween 80, 0.04 g. The trace element solution contained, per liter, the sodium salt of EDTA (ethylenediaminetetraacetic acid), 5 g; $CuSO_4 \cdot 5H_2O$, 0.02 g; $CaCl_2 \cdot 2H_2O$, 0.2 g; $FeCl_3$, 0.2 g; $Na_2MoO_4 \cdot 2H_2O$, 0.2 g; $ZnSO_4 \cdot 7H_2O$, 0.04 g; and $MnSO_4 \cdot 4H_2O$, 0.4 g. Red selenium was produced in colloidal form dispersed in Tween 80 by reducing selenite with thiosulfate and purifying by dialysis over 7 days. Open symbols designate the control flasks (showing atmospheric oxidation); closed symbols identify the inoculated flasks; triangles, gray selenium; circles, red colloidal selenium. Curves are the best-fit parabolic relations (Table 1). Each data point shown is the mean of four replicates.

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spheric oxidation to selenite in control flasks (without soil) amounted to less than 0.03 μg/ml. Enrichment cultures were then plated on medium B (containing 1.5 percent Difco Noble agar) and incubated at 28°C for 4 to 5 days. From these plates individual colonies were isolated, checked for purity, and again tested for their ability to oxidize elemental selenium to selenite as above.

A spore-forming bacterium capable of oxidizing elemental selenium was isolated by this procedure and identified as *Bacillus megaterium* (*11*). It oxidized elemental selenium to selenite (Fig. 1) at a rate that varied linearly with the square root of time to a close approximation [$R^2 = .67$ (multiple coefficient of determination), and the quadratic term was nonsignificant at the 5 percent level]. Conformity of the rate of production of selenite to a relation with the square root of time suggests that the rate-limiting step could be one of diffusion of particles through what would be effectively a semi-infinite medium. An analysis of variance was carried out on the parabolic coefficients of the straight line thus fitted for each of the four replicate flasks (Table 1).

Selenium was oxidized to selenite by atmospheric oxygen at an average rate of 0.011 ± 0.003 μg ml^{-1} $day^{-1/2}$ (95 percent confidence interval) in uninoculated control flasks containing the medium and elemental selenium. The organism increased the rate of oxidation ($P < .001$) (Table 1). The greater increase in rate for red than for gray selenium ($P < .01$, *t*-test) can probably be attributed to the difference in surface area rather than to the allotropic form. After incubation for 40 days, approximately 1.5 percent of the red selenium had been oxidized to selenite.

Trace amounts of selenate were found at the end of the incubation (Table 1) and were significantly ($P < .05$) different among the four treatments. Although this observation indicates that the organism is capable of oxidation to selenate, the amount of selenate produced represents less than 1 percent of the amount of selenite formed.

The production of only trace amounts of selenate and substantial amounts of selenite by this strain of *B. megaterium* is in contrast to the two earlier reports on the oxidation of elemental selenium by microorganisms (*6*, *7*). Both investigators claimed, but without supporting evidence, that selenium was oxidized to selenate and the formation of selenite was not discussed.

To prevent selenium deficiency, grazing ruminants require only 0.02 to 0.03 μg/g in pasture (*3*). Although the levels of oxidation reported here are small (≤ 1.5 percent of the added selenium), they represent a definite link in the selenium cycle. If achieved on natural or added selenium in soils, they could be of importance in providing sufficient concentrations of selenium in herbage to prevent animal deficiency of this element.

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Effects of Vasopressin on Human Memory Functions

Abstract. *Arginine vasopressin and a number of its synthetic analogs augment memory functions in experimental animals. One of these analogs, 1-desamino-8-D-arginine vasopressin (DDAVP), influences human learning and memory. Cognitively unimpaired, as well as cognitively impaired adults, treated with DDAVP for a period of several days, learn information more effectively, as measured by the completeness, organization, and consistency (reliability) of recall. DDAVP also appears to reverse partially the retrograde amnesia that follows electroconvulsive treatment.*

Arginine vasopressin (AVP) is one of the few peptides synthesized entirely within the central nervous system in several nuclei located in the medial hypothalamus. It is transported from magnocellular hypothalamic nuclei to the posterior pituitary, where it is stored and released into the systemic circulation to regulate renal free water clearance. It is also transported from the hypothalamus to the third ventricle. The cerebrospinal fluid is thought to mediate a variety of AVP's putative central nervous effects, including those related to information processing (*1*, *2*). The mechanisms by which AVP affects learning and memory have not been elucidated, but evidence suggests that it acts as a neuromodulator and that it can influence not only the functional activity of biogenic amines thought to be involved in memory processes but also the release of other peptide modulators, including the endorphins (*3*–*5*).

In a number of experiments, different small groups of informed and consenting subjects volunteered to be treated with 1-desamino-8-D-arginine vasopressin (DDAVP) (in doses of from 30 to 60 μg administered intranasally by a calibrated catheter three times a day), and placebo similarly administered. Placebo and DDAVP treatments were repeated each day over a period of from 2 to 3 weeks. Prior to placebo and drug treatment, all subjects had become familiar through practice with all cognitive procedures used in the study. During both the placebo and the active drug periods, subjects had their weight and serum electrolytes recorded regularly, and their fluid intake was regulated daily to give an average of 1400 ml in 24 hours. The manner in which treatments were administered was designed to ensure the double-blind nature of the study and protect subjects against DDAVP-induced water retention.

The cognitive response (learning and memory) after DDAVP treatment was contrasted with placebo effects (i) in six young unimpaired college students (four males and two females); (ii) in a similar group of young unimpaired subjects treated only with placebo for a period of 8 weeks; (iii) in four patients with histories of endogenous mood disturbance with accompanying cognitive impairment (all females between the ages of 36 and 47 years); and (iv) in two female mood disorder patients, who had been unresponsive to antidepressant medication, tested after electroconvulsive therapy (ECT), which would be expected to

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