Table 1. Mean length data from chromosome arm measurements. AL, mean absolute lengths of standard pair; CI, centromeric indices of the standard pair. The short arms, long arms, and the total are the normalized mean lengths based upon the standard pair.

Test	Chromosome measurements		F
	Lemmon	Mingus	
AL standard	62.4	63.0	0.01
CI standard	0.25	0.25	0.2
Short arms	231.4	323.9	180.7*
Long arms	929.4	922.8	0.5
Total	1160.8	1246.6	56.6*

* Means differ at $P < .01$.

as well as some of the differences in karyotype between the two populations may be due to variations in the amount of constitutive heterochromatin present in the two karyotypes.

These results do not exclude the possibility of pericentric inversions contributing to chromosome variations in *Peromyscus*. They do indicate that another mechanism, addition or deletion of chromosomal material, is an important factor contributing to chromosomal variation in these organisms. If the differences in length and the differences in karyotype are due to differences in the amount of constitutive heterochromatin, then rather large amounts of karyotypic variations of the type reported in *Peromyscus* could occur with relatively few rearrangements within euchromatic portions of the genome.

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Microbial Uptake of Lead

Abstract. Micrococcus luteus *and* Azotobacter *sp. cells grown in broth in contact with a dialysis membrane containing lead bromide were found to immobilize 4.9 and 3.1 × 10^2 milligrams of lead per gram of whole cells, on a dry weight basis, respectively. Culture turbidity and cell count measurements on these and other cell cultures show that lead bromide, lead iodide, and lead bromochloride in concentrations approaching solubility limits have no detectable effect on overall growth rate and cell viability. Analyses of cellular subfractions reveal that fractions of cell wall plus membrane contain 99.3 and 99.1 percent of the lead found associated with* Micrococcus luteus *and* Azotobacter *sp., respectively. The remainder is found associated with the cytoplasmic fractions.*

We have studied effects of several inorganic lead salts on a number of microorganisms as part of an investigation of the possibility that there is a lead cycle in nature, for which animals are the focal point. Previous studies have indicated that lead causes a marked increase in the resistance of red cells to hypotonic saline solutions (*1*), is of questionable toxicity to *Azotobacter* (*2*), and does not affect specific cell functions (*3*). While it has been reported that lead can affect respiration in cell-free extracts of corn mitochondria (*4*), the effect of inorganic lead on whole cells might be quite different if lead must first penetrate the cell membrane. This report concerns the immobilization of inorganic lead by microbial cells and the fraction of immobilized lead that passes through the cell envelope into the cytoplasm.

Microbial isolates were obtained from samples of fresh lake water at Fort Collins Park and from various soil samples by standard techniques (*5*) for the following: photosynthetic bacteria; aerobic, nonsymbiotic, and nitrogen-fixing bacteria; sulfur-reducing bacteria; and selected fungi. Selected Gram-positive and Gram-negative bacteria in pure cultures were also studied. Each culture isolate was grown in broth in the presence of PbI_2, $PbBr_2$, and PbBrCl in concentrations of the salt ranging from 0 to 0.7 mg per milliliter of solution. *Azotobacter* and *Micrococcus* were also grown in media containing 2.5 mg of $PbBr_2$ per milliliter. Precautions were taken in inoculating the growing cultures to prevent their being killed by sudden increases in lead concentration. Both measurement of culture turbidity with a Beckman spectrophotometer and the viable cell count show that the three lead salts in the concentrations employed had no measurable effect on overall growth rate and cell viability. One can immediately raise the question of whether lead is passing through the membrane or cell wall.

The distribution of lead among cellular subfractions for *Azotobacter* sp. (soil isolate) and *Micrococcus luteus* FD 533 (*6*) was determined. *Azotobacter* sp. was grown in either 100-ml or 1000-ml quantities of Burk's nitrogen-free medium (*7*). *Micrococcus luteus* was grown in Trypticase soy broth (Baltimore Biological Laboratories). Lead salts were introduced through suspended dialysis membranes to keep salt crystals from direct contact with cells and thus prevent shock due to sudden localized increases in lead concentration. The preparations were sterilized in a steam autoclave and inoculated with biologically active cultures (5 ml per 100 ml of the medium); the inoculated preparations were incubated at 25°C with agitation in a rotary shaker. In the early stationary phase of growth, cells were harvested by centrifugation and

Table 1. Distribution of lead in bacterial cultures. Values are averages for four separate culture preparations for each species. Approximately 300 mg of $PbBr_2$ was placed in dialysis bags and submerged in the culture. Each culture was inoculated with 5 ml of biologically active culture per 100 ml of medium. Cells were harvested by centrifugation. Approximately 242 mg [0.12 mg total lipid phosphorus (*12*)] and 115 mg [0.93 mg total lipid phosphorus (*12*)] of cells, on a dry weight basis, were obtained for *Micrococcus luteus* and *Azotobacter* sp., respectively. The culture medium is the supernatant solution obtained after cell removal. Uninoculated preparations were used as controls.

Substrate	Total lead recovered			
	Micrococcus luteus		*Azotobacter* sp.	
	Micrograms	Percentage	Micrograms	Percentage
Whole cells	1,180	80.2	35,650	99.94
Culture medium	290	19.7	13	0.04
Whole cell wash	2	0.1	8	0.02
Control	520		1,700	

then washed with a salt solution (containing, per liter, 9 g of NaCl, 0.25 g of $MgSO_4$, 1.8 mg of $MnCl_2$, 0.22 mg of $ZnSO_4$, 0.08 mg of $CuSO_4$, and 0.005 mg of $FeCl_3$). Portions of the lyophilized cellular preparations were then converted to protoplast. Protoplast preparations of *M. luteus* were made in a hypertonic solution containing lysozymes, as described by Mitchell and Moyle (*8*) and Brown (*9*). *Azotobacter* sp. protoplast was prepared by the method of Jose and Wilson (*10*) in an aqueous solution of the following composition: 33 m*M* tris(hydroxymethyl)aminomethane, 0.06*M* sucrose, 13.3 mg of lysozyme per milliliter, and 5.32 mg of ethylenediaminetetraacetic acid tetrasodium salt per milliliter. Protoplast preparations were separated from cell wall digests by centrifugation and then washed and recentrifuged twice. The washings were added to the cell wall digests. Protoplast preparations were then lysed with cold distilled water (*8, 9*). The cytoplasmic fraction was separated from the membrane fractions by centrifugation, washed, and centrifuged again. These washings were added to the cytoplasmic fraction. Isolated cellular subfractions were chemically characterized by determining the amounts of protein (*11*), lipids (*12*), RNA (*9, 13*), and hexosamines (*14*). The quantities determined are typical for membrane preparations (*9*). Each cellular subfraction was then analyzed for lead by dissolution in concentrated nitric acid followed by atomic absorption spectrometric analysis with a Varian Techtron AA-5 spectrophotometer. The amounts of lead found in various cellular cultures and cellular fractions of *Micrococcus* and *Azotobacter* are listed in Table 1. *Azotobacter* cultures (*p*H 7.0 to 7.5) were incubated for 7 days, while *Micrococcus* cultures (*p*H 6.8 to 7.0) were incubated for 2 days to reach the same early stationary growth phase. Amounts of lead taken up by whole cells may thus be dependent upon both incubation period and culture *p*H. Considerably more lead is found in inoculated broth components than in uninoculated controls. Lead transport through the dialyzing membrane may be affected by both cellular structure and the composition of the culture medium. The distinctive feature of both sets of data is the appreciable uptake of lead by the microbial cells. The relatively greater effectiveness of *Azotobacter* for immobilizing lead may be related to the large quantity of capsular material surrounding the cells, which has a tendency to accumulate biological and nonbiological substances.

Table 2. Distribution of lead in cellular subfractions. Values are averages for four separate culture preparations for each species. Included in cell wall and cytoplasmic fractions are washes of the protoplast and membrane preparations, respectively.

Substrate	Total lead recovered			
	Micrococcus luteus		*Azotobacter* sp.	
	Micrograms	Percentage	Micrograms	Percentage
Cell wall fraction	111	9.5	11,505	37.6
Membrane fraction	1,048	89.8	18,833	61.5
Cytoplasmic fraction	8	0.7	273	0.9

The distribution of lead among cellular subfractions is reported in Table 2. The large amount of capsular material associated with *Azotobacter* may account for the difference between the amounts of lead found in the cell wall fractions of the two systems. Recovery of lead from the noncapsulated *Micrococcus* is essentially complete (Tables 1 and 2). Incomplete lead recovery for *Azotobacter* may be due to scavenging of the lead by the surface of the glass container during protoplast preparation. The striking feature of these data is the small amount of lead found in the cytoplasmic fractions for both systems. However, the amount found in the cytoplasmic fractions may be associated with finely fragmented membranes. Total lipid extraction of membrane preparations with methanol : chloroform : water, 10 : 5 : 4 by volume (*12*), results in extraction of the major portion of lead found in those fractions. Adjustment of the solvent mixture to 10 : 10 : 9 by volume (*12*) results in essentially all of the extracted lead being transferred to the methanol : water phase.

A major conclusion of this study is that the microbial systems investigated are highly capable of abstracting substantial quantities of inorganic lead. A second major finding is that lead thus immobilized is largely associated with cell membranes, and virtually none is found associated with the cytoplasmic fractions. Immobilization of lead by microbial systems may have importance in terms of transfer through the food chain.

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Dielectrophoresis of Macromolecules: Determination of the Diffusion Constant of Poly-γ-Benzyl-L-Glutamate

Abstract. *The translational diffusion constant of poly-γ-benzyl-L-glutamate in ethylene dichloride was measured by dielectrophoresis. The method is rapid, precise to about ± 5 percent, and yields values in good agreement with those obtained by standard methods.*

Dipolar molecules experience a net force in a strongly inhomogeneous electric field and migrate toward the region of maximum absolute field strength. This migration, called dielectrophoresis (*1*), will continue until a concentration gradient reflecting equilibrium with diffusion is attained. It is the dipolar