

Effects of Mercuric Chloride on Growth and Morphology of Selected Strains of Mercury-Resistant Bacteria

Z. VAITUZIS, J. D. NELSON, JR.,¹ L. W. WAN,² AND R. R. COLWELL*

Department of Microbiology, University of Maryland, College Park, Maryland 20742

Received for publication 6 September 1974

A survey of the comparative cytological effects of growth in the presence of mercury by a group of mercury-resistant bacterial cultures and a characterization of the process of bacterial adaptation to Hg^{2+} ion was accomplished. Mercury resistance was found to be dependent upon the ability to volatilize mercury from the medium and upon the amount of mercury accumulated by the cells. The results indicate that most cultures which adapt to growth in the presence of $HgCl_2$, exhibit extensive morphological abnormalities. Significant effects are delay in the onset of growth and cell division and numerous structural irregularities associated with cell wall and cytoplasmic membrane synthesis and function. A detailed analysis of the adaptation process and the resulting effects on morphology was performed on an *Enterobacter* sp. During the period preceding active multiplication, a selection for mercury-resistant mutants occurred. It was also demonstrated that growth commenced only at a specific threshold concentration of Hg^{2+} .

Phytoplankton (1) and various bacterial species (9, 12, 20) are capable of adapting to growth in the presence of mercury. Investigations of mercury resistance in microorganisms have revealed several possible mechanisms by which this process takes place. In some bacteria, resistance is attained through the acquisition of a plasmid, an extrachromosomal element which can be transferred intra- and intergenerically (9, 10, 13, 17, 19). Generally, microorganisms have been shown to detoxify mercurial compounds metabolically by the formation of volatile mercury (1, 4, 9, 12, 20) or mercury mercaptides (16, 18).

Comparatively little information is available concerning the morphological effects of mercury upon microorganisms. Tingle et al. (21) reported mitochondrial damage and swelling, pellicular membrane disruption, and loss of motility in *Tetrahymena pyriformis* incubated in a medium containing 0.5 μg of $HgCl_2$ per ml. Exposure of *Pseudomonas aeruginosa* cells to $HgCl_2$ was shown to cause swelling which could be reversed by addition of sulfhydryl compounds (2). Also, extremely low levels of phenylmercuric acetate (0.9 to 3 ng/ml) have been reported to cause gross changes in the cellular form of growing cultures of *Phaeodactylum*

tricornutum and *Chlorella* (14). Thin sections of a fungus (15) and a yeast (3) exposed to $HgCl_2$, were shown to have electron-dense inclusions associated with the nucleus and cytoplasm, respectively, indicating possible mercury accumulation.

Quantitative evidence linking mercury-resistant bacteria in a mercury-contaminated habitat with in situ generation of Hg^0 has been obtained (J. D. Nelson and R. R. Colwell, *J. Microbiol. Ecol.*, in press). The positive correlation observed between numbers of aerobic, heterotrophic bacteria resistant to $HgCl_2$, and ambient mercury concentration found in Chesapeake Bay sediments suggests that environmental mercury contamination can exert an effect upon the population structure of the estuarine microflora. To elucidate possible mechanisms by which mercury manifests selective pressures, we examined the effects of inorganic mercury upon growth and morphology of representative strains of bacteria isolated from the Chesapeake Bay. (Preliminary results of this investigation were presented at the 73rd Annual Meeting of the American Society for Microbiology, Miami Beach, Fla., 1973.)

MATERIALS AND METHODS

Organisms and cultural methods. Microorganisms used in this study were isolated by spreading suitable dilutions of water and sediment on an artificial estuarine salts nutrient broth and agar medium

¹Present address: Biology Department, University of Bridgeport, Bridgeport, Conn. 06602.

²Present address: Department of Civil Engineering, Howard University, Washington, D.C. 20059.

consisting of 0.2% glucose, 1.0% Casamino Acids (Difco), 0.1% yeast extract (Difco), 1.0% NaCl, 0.23% $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and 0.03% KCl (pH 7.2). The medium was solidified with 2.0% agar (Difco) and supplemented with varying concentrations of HgCl_2 . The identity of the isolates was determined to the genus level by established cultural and biochemical procedures. A listing of the microorganisms, together with their strain designations, appears in Table 1.

Assay for Hg^{2+} content. Portions (0.1 ml) of mid-log phase *Enterobacter* 85, grown without mercury, were inoculated into cotton-stoppered flasks containing 5 ml of fresh estuarine salts nutrient broth. One group of flasks, containing the medium alone, was used as uninoculated controls. The assays were made under two sets of conditions. In the first, both inoculated and control flasks contained 2 μg of ^{203}Hg -labeled HgCl_2 per ml (Amersham/Searle, Arlington Heights, Ill.). In the second set, the amount of labeled HgCl_2 was increased to 4 $\mu\text{g}/\text{ml}$. All flasks were placed in a shaker-incubator at room temperature, and the rate of growth was monitored on a Klett-Summerson photoelectric colorimeter (Klett Manufacturing Co., New York, N.Y.) and expressed as optical density relative to the uninoculated control. Duplicate 0.1-ml volumes were periodically removed from all flasks and either added directly to 10 ml of preblended liquid scintillation mixture 3a70 (Research Products International, Elk Grove Village, Ill.) or filtered through a 0.45- μm membrane filter. The filter membranes were washed with three 1-ml volumes of buffered three salts solution (see assay for HgCl_2 metabolism below) and were placed in 10 ml of liquid scintillation mixture. An ABAC SL40 liquid scintillation spectrometer was used for determination of total and nonfilterable radioactivity. Portions of cells grown in medium containing unlabeled HgCl_2 were removed aseptically at varying time intervals (see arrows, Fig. 1) for electron microscopy or plating for viable count determination.

Assay for HgCl_2 metabolism. One milliliter of each culture, previously grown in HgCl_2 -containing broth, was inoculated into 5 ml of fresh medium containing a previously determined threshold concentration of HgCl_2 for each particular culture (Table 2). The flasks were incubated with shaking for 2 h at 25 C. The cultures were then centrifuged and suspended in 1 ml of 0.01 M potassium phosphate-buffered (pH 7.0) "three salts" solution (1.0% NaCl, 0.23% $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and 0.03% KCl). The cells were centrifuged again and resuspended in 5 ml of buffered three salts with 1 μg of ^{203}Hg -labeled HgCl_2 per ml. The suspensions were aerated by bubbling moist air through capillary tubes to ensure uniformity of suspension and to enhance the evolution of volatile mercury. Portions (0.1 ml) of the suspensions were removed at 0, 15, and 30 min and either added directly to 10 ml of liquid scintillation mixture or filtered through a 0.45- μm membrane filter. The filter membranes were washed with three 1-ml volumes of buffered three salts and were placed in 10 ml of liquid scintillation mixture to determine the percentage of mercury accumulated by the cells. Mercury metabolism was measured by the loss of ^{203}Hg from the

aerated cell suspension. The decrease in radioactivity after a 30-min incubation was expressed as the percentage of mercury metabolized relative to the remaining concentration of HgCl_2 in an uninoculated control tube containing 1 μg of ^{203}Hg -labeled HgCl_2 per ml.

Electron microscopy. Cells taken from late log-phase cultures were transferred to fresh broth (0.1 ml of inoculum per 5 ml of broth) in flasks with and without sublethal concentrations of HgCl_2 . These were incubated at 25 C with shaking for three to four generation times or until equal turbidities were obtained during early log phase of growth. The cultures were fixed according to the procedure described by Kellenberger and Ryter (7). After a stepwise dehydration through a 25, 50, 75, 85, 95, and 100% ethanol and propylene oxide series, the specimens were embedded in Epon (11) and were thin sectioned by using an LKB 800 Ultratome III fitted with a diamond knife. The sections were collected on collodion-coated, 200- or 300-mesh copper grids and poststained with saturated aqueous uranyl acetate (25) and alkaline lead citrate (24). The specimens were viewed and photographed in an RCA EMU 3F or Hitachi HU-11A electron microscope operating at an accelerating voltage of 50 kV.

RESULTS AND DISCUSSION

Survey of morphological effects. Electron microscopic examination of thin sections of the bacterial cultures grown at sublethal or threshold concentrations of HgCl_2 showed a wide range of morphological abnormalities. Growth in the presence of mercury commenced after varying lengths of time, with an array of morphological effects which did not appear in control cultures grown without mercury of the same physiological age (Table 1). In gram-negative bacteria, the most frequently observed defect was an apparent loss of regulation in the cell wall synthesizing process. This was suggested by the appearance of irregular cell wall contours, elongated pleomorphic as well as giant cells and spheroplasts, and the development of cross-walls and branching in lieu of cell division by constriction. In gram-positive bacteria, the major defect occurred in cross-wall formation. Thin sections showed cross-walls with irregular contours, and a failure of some cells to divide resulted in elongated pleomorphic forms with a number of septa within them at apparently random irregular locations within the bacteria. A number of cultures also showed plasmolysis and changes in mesosomal structure, indicating possible interference by the mercury with cytoplasmic membrane synthesis and function. These effects may be related to the finding that most of the mercury bound to bacterial cells is associated with either the cell wall or cytoplasmic membrane (unpublished data; 22, 23).

TABLE 1. Survey of morphological changes in bacteria grown in the presence of HgCl₂

Culture		HgCl ₂ concn (μg/ml)	Lag phase (h)	Morphological effects ^a
Genus	Strain			
<i>Arthrobacter</i>	72	5	24	Irregular septum formation Formation of irregular and multiple septa
<i>Bacillus</i>	394	15	41	
<i>Citrobacter</i>	132	20	70	Plasmolysis, membrane damage Pleomorphic cells, outgrowth of cell wall, plasmolysis
<i>Enterobacter</i>	85 ^b	1	0	
<i>Flavobacterium</i>	119	10	40	Convoluted cell wall, plasmolysis, elongated cells, irregular mesosomes
<i>Pseudomonas</i>	244	25	0	None
<i>Pseudomonas</i>	94	10	24	Convoluted cell wall
<i>Pseudomonas</i>	B-16 ^c	15	6 days	Small spherical cells bounded by common outer membrane
<i>Vibrio</i>	639	5	72	Giant, pleomorphic cells, spheroplasts, elongated cells

^a Four generation times after the lag phase as determined by turbidimetric comparison with mercury-free control cultures.

^b See Fig. 4 to 9 for detailed morphological changes.

^c Supplied by R. A. MacLeod, McGill University, Montreal, Que., Canada.

Further examination of Table 1 reveals a varying lag period prior to onset of growth. Furthermore, some organisms are capable of adapting to growth in the presence of mercury more rapidly than others. The initial concentration of mercury that any given culture will adapt to was also found to be different. The most resistant culture, *Pseudomonas* strain 244, did not exhibit any appreciable lag at all before the onset of growth. Thus, both the reasons for resistance to varying amounts of mercury and the transpiration of events during the characteristically prolonged lag phase were investigated by monitoring the amount of mercury in the medium and within the cells.

Metabolism and accumulation of HgCl₂.

The comparative metabolism of Hg²⁺ to volatile mercury is presented in Table 2. Generally, the more resistant cultures metabolized a large proportion of the Hg²⁺ (70 to 81%) and relatively less Hg²⁺ was cell associated (16 to 37%). Likewise, electron microscopic examination of thin sections showed a direct correlation between mercury accumulation by the cells and the amount of electron-dense clusters of ribosomes in the cytoplasm. These data suggest that the ability to convert Hg²⁺ to volatile mercury is part of the basis for bacterial resistance. This trait, which is presumably responsible for the reduction in Hg accumulation by the cells, is observed after the induction of mercury-metabolizing enzyme(s) (5, 8, 12). During our survey of morphological effects of growth in the presence of mercury, the cells had not been

TABLE 2. Comparative metabolism and accumulation of HgCl₂ in a buffer solution containing 1 μg of HgCl₂ per ml

Culture	HgCl ₂ (μg/ml) in growth medium	Dry weight of cells (mg/ml)	HgCl ₂ metab- olized (%)	Cell- associated HgCl ₂ (%)
<i>Arthrobacter</i> 72	1.2	0.24	0.0	36.8
<i>Bacillus</i> 394	3.0	0.28	36.6	64.2
<i>Citrobacter</i> 132	6.0	0.42	80.4	15.6
<i>Enterobacter</i> 85	1.2	0.51	10.5	90.6
<i>Pseudomonas</i> 94	3.0	0.42	80.9	23.4
<i>Pseudomonas</i> 244	6.0	0.44	70.6	37.1
<i>Vibrio</i> 639	1.2	0.51	1.5	99.8

previously exposed to mercury and thus displayed characteristic lag phases of varying length. It appears, then, that the onset of growth and cell division in the presence of Hg is contingent upon the induction of the necessary enzyme(s) for the detoxification of the growth medium. The following experiments were designed to examine this process of adaptation to mercury, and to correlate it with morphological changes.

Growth and morphological changes during adaptation to HgCl₂. A gram-negative species was selected for a detailed investigation to clarify events occurring while bacteria were apparently quiescent in mercury-containing broth and to relate these events to cytological changes associated with adaptation. *Enterobacter* strain 85 was serially transferred several times in the absence of mercury before

inoculation into flasks with and without 2 μg of ^{203}Hg -labeled or unlabeled HgCl_2 per ml. After a 24-h lag phase, the mercury-containing culture grew at a rate comparable to that of the control culture (Fig. 1). Although the turbidity of the HgCl_2 -containing culture remained constant, an initial 3-log drop in viable count occurred. Viable count and turbidity increased at the end of the lag phase. An "isotope effect" was suggested by the slightly shorter lag phase in the unlabeled mercury-containing culture. During the initial phase of incubation, radioactivity was rapidly lost from both the inoculated broth and from the sterile control. Other investigators have shown that reducing agents such as glucose (6) and yeast extract (A. D. Murray, and D. K. Kidby, Abstr. Annu. Meet. Am. Soc. Microbiol. 1972, E98, p. 17) in the growth media promote the reduction of Hg^{2+} . The loss of radioactivity from the control broth represented the volatilization of mercury and did not arise

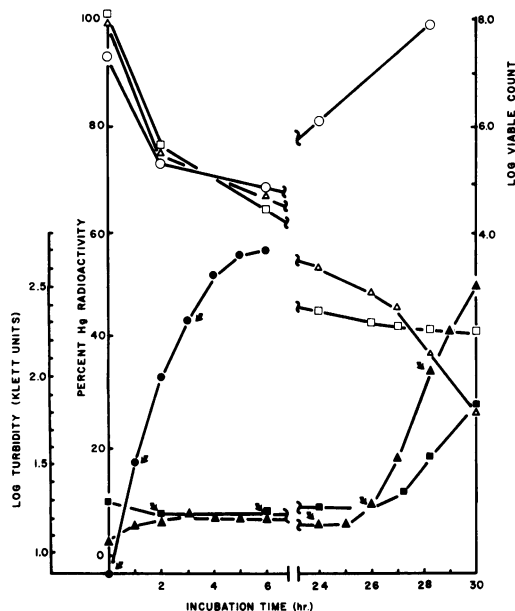


FIG. 1. Metabolism and uptake of 2 μg of HgCl_2 per ml by *Enterobacter* strain 85. Shaker flasks containing broth with and without ^{203}Hg and unlabeled HgCl_2 (2 $\mu\text{g}/\text{ml}$) were inoculated with an 18-h culture. Turbidity of the culture without mercury (\bullet) and the culture with labeled mercury (\blacktriangle) were measured. Total (Δ) and nonfilterable (\blacksquare) activity of the latter were also measured. An uninoculated, ^{203}Hg -labeled control flask (\square) was also assayed for total radioactivity. Portions were removed from flasks without and with unlabeled mercury at intervals for examination by electron microscopy (arrows). The open circles (\circ) represent total viable counts in the unlabeled mercury culture.

from adsorption to the glass walls of the flask, since only 8.9% of the radioactivity was recovered in sequential washes of the vessel with water, 0.5 N HNO_3 , and 10% Radiac wash (Atomic Products Corp., Center Moriches, N.Y.). An initial, small loss of cell-bound mercury coincided with the drop in viable count, indicating that lysis of the cells may have occurred. When the mercury concentration dropped approximately 45.0%, to 1.1 $\mu\text{g}/\text{ml}$, growth was initiated. At this point, the rate of mercury loss from the broth accelerated in the inoculated flask, and the rate of cellular uptake per unit volume of broth increased.

After incubation of the spread plates prepared for determination of total viable counts, small colonial variants (85-S) of the HgCl_2 -containing culture began to appear (Fig. 2). These colonies contained nonmotile, pleomorphic cells, in contrast to the large "parent"-like colonies (85-L) comprising the remainder of the population. The comparative properties of the two types are discussed in a subsequent section.

The growth experiment was repeated by using an elevated concentration of mercury (4 $\mu\text{g}/\text{ml}$) to test the hypothesis that the length of the lag phase demonstrated by this organism was dependent upon reduction of mercury concentration to a specific threshold concentration of 1.1 $\mu\text{g}/\text{ml}$. The pattern of growth and loss of mercury was as observed with 2 μg of HgCl_2 per ml, except that the lag phase was found to be almost 3 days long (Fig. 3). During the lag phase, the mercury concentration dropped by 70%, to a level of 1.2 $\mu\text{g}/\text{ml}$, a concentration closely approximating the concentration of 1.1 μg of HgCl_2 per ml at which growth commenced in the first experiment. An increased isotope effect was observed in the second experiment, where the difference between the lag phase for labeled and unlabeled cultures was nearly 10 h. Again, a drop in viable count was accompanied by a drop in cell-bound mercury. As in the first experiment, the rate of loss of mercury from the inoculated flask accelerated at the end of lag phase. Numbers of small colonial variants comprised approximately 1% of the total viable count, and these small colony variants increased in parallel with the total population (Fig. 3).

An electron microscopic examination of thin sections of cells harvested from the unlabeled broth with and without mercury was made. Control cells examined at the early, mid, and late logarithmic phases of growth were found to be normal in all respects, namely, array and morphology of the deoxyribonucleic acid fibrils, ribosomes, and cell envelope, as well as mode of

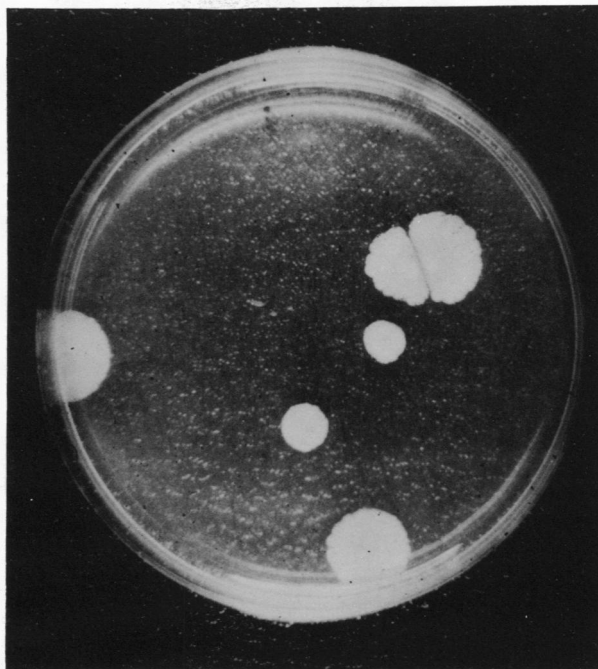


FIG. 2. Colonial variation in *Enterobacter* strain 85. Samples of culture grown in $2 \mu\text{g}$ of unlabeled HgCl_2 per ml containing broth were diluted and plated on basal nutrient medium and incubated at 25°C for 7 days. Small (85-S) and large (85-L) colonial forms were observed.

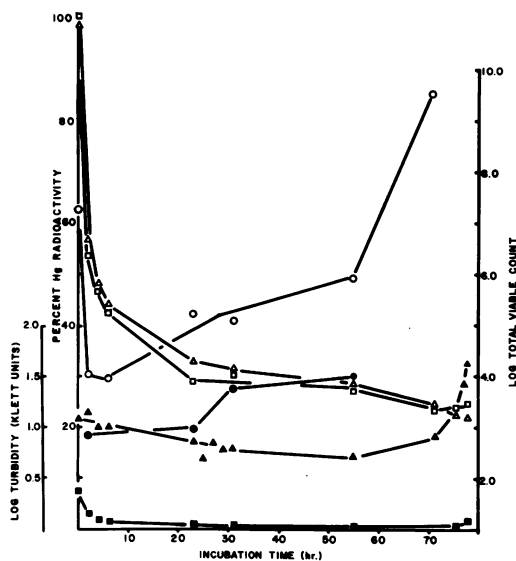


FIG. 3. Metabolism and uptake of $4 \mu\text{g}$ of HgCl_2 per ml by *Enterobacter* strain 85. Shaker flasks containing broth with ^{203}Hg -labeled and unlabeled HgCl_2 ($4 \mu\text{g}/\text{ml}$) were inoculated with an 18-h culture. Turbidity (\blacktriangle) and total (\triangle) and nonfilterable (\blacksquare) radioactivity in the labeled culture were measured. An uninoculated, labeled control flask (\square) was also assayed for total radioactivity. Total viable counts

division. At 0 h, the mercury-containing culture was found to be identical to the 0-h control (Fig. 4). After 2 h (Fig. 5), the cells appeared normal in overall morphology. The deoxyribonucleic acid fibrils in some cells appeared condensed, rather than dispersed throughout the cytoplasm, and electron-dense areas among the ribosomes were seen. At 6 h, intact cells similar to those at 2 h were accompanied by swollen and plasmolyzed cells (Fig. 6). Approximately one-third of all the cells at this stage showed signs of lysis. At 24 h (Fig. 7), the culture was characterized by lysed cell debris as well as viable, but pleomorphic, cells with abnormal cross-wall formation. Electron-dense clusters of ribosomes were more frequently seen in these cells. As growth progressed through the 26th and 28th h, the cells became relatively more rod shaped, although irregular cell walls and electron-dense ribosome clusters were still present (Fig. 8, 9). The latter clusters are similar to electron-dense structures seen in other microorganisms exposed to mercury (3, 15). A high-resolution analytical technique, however, such as electron probe microanalysis, would be required to determine whether the density is due to preferen-

(○) and numbers of small colonial variants (85-S) (●) were obtained.

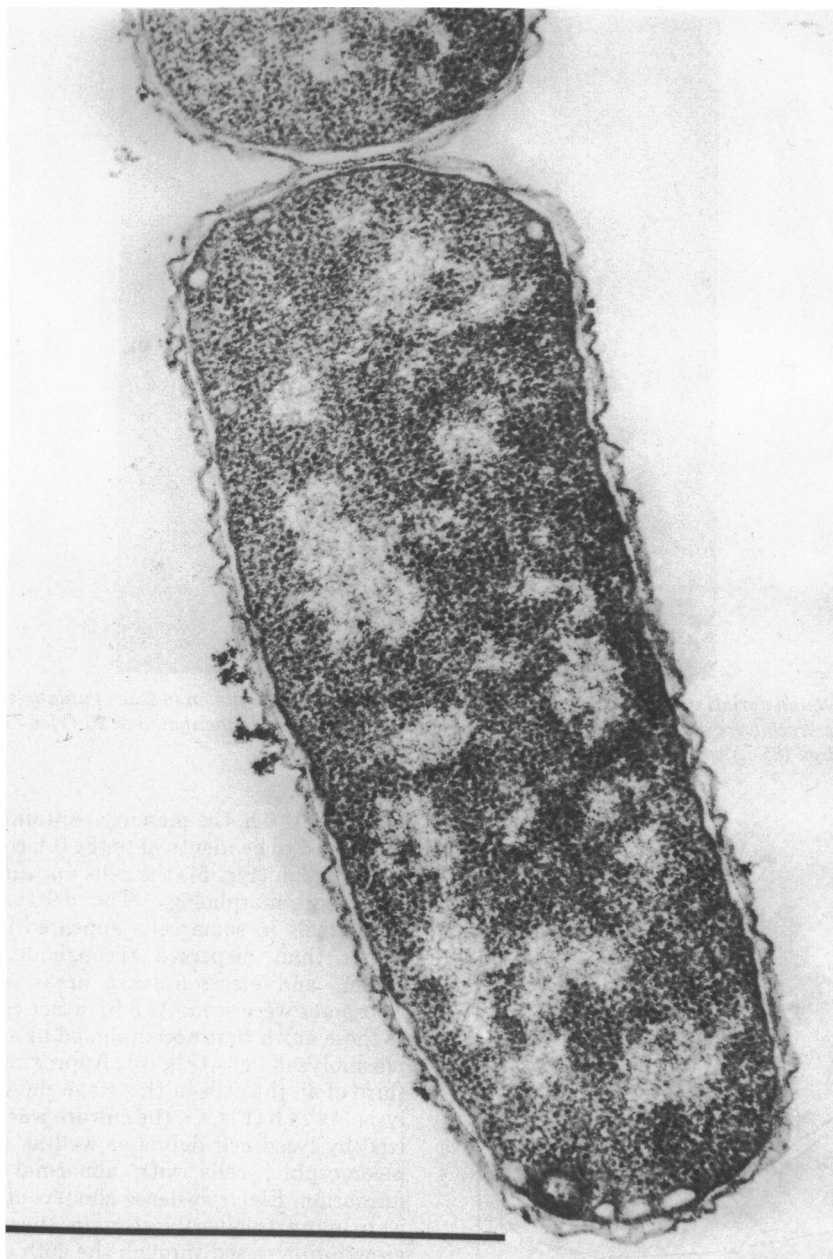


FIG. 4. Thin section of *Enterobacter 85* immediately after addition to broth containing $2 \mu\text{g}$ of HgCl_2 per ml. The cells show dispersed chromatin, normal ribosomes, and cell envelope constituents, as well as typical gram-negative cell division by constriction. The bar in all electron micrographs represents $1 \mu\text{m}$.

tial intracellular binding of Hg.

Properties of mercury-selected colonial variants. After repeated serial transfers in mercury-free medium, the small colonial variants (85-S) lost their pleomorphism and regained motility, but retained their colonial

morphology. The variants were examined to see if they differed from the parent stock, with respect to mercury resistance and/or the ability to reduce Hg^{2+} (Tables 3 and 4). It is evident that the small colonies selected by growth in the presence of HgCl_2 represent a stable mutation

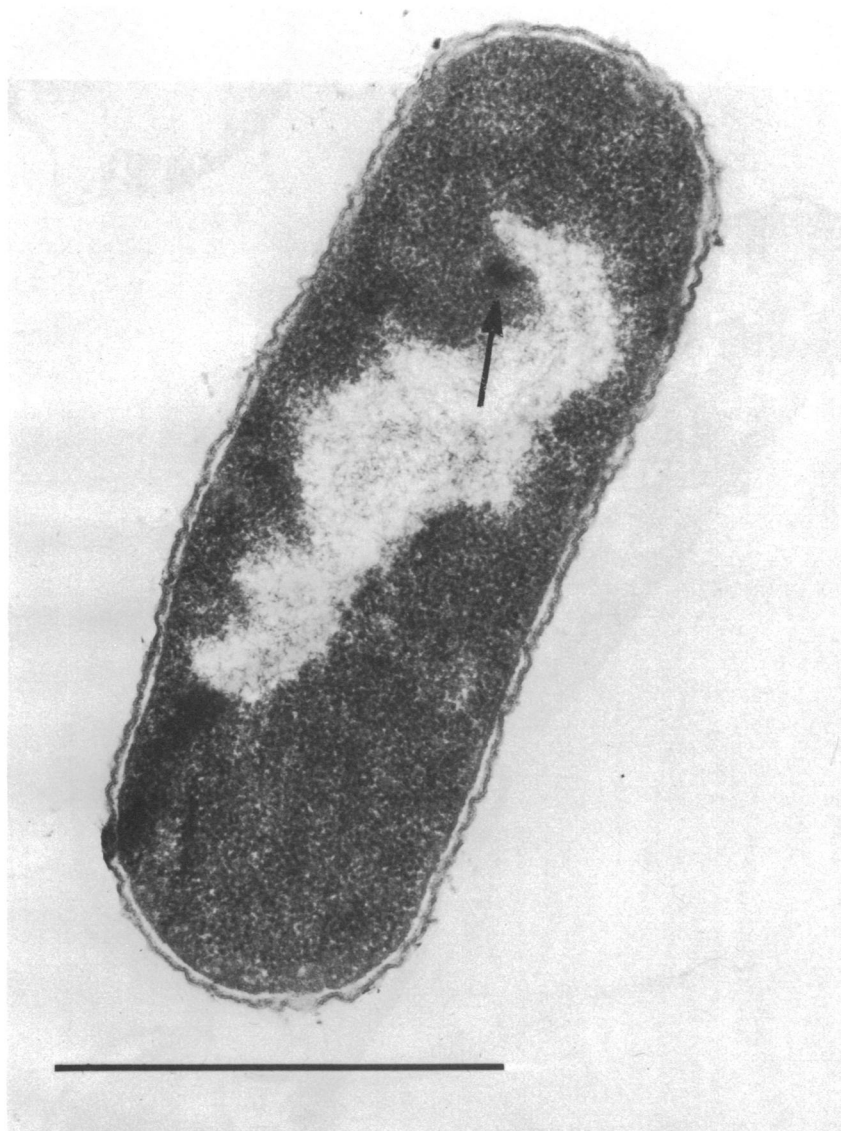


FIG. 5. Thin section of *Enterobacter* 85 after a 2-h incubation in broth containing $2 \mu\text{g}$ of HgCl_2 per ml. The overall morphology is similar to that of cells at zero time, except for condensed chromatin and the appearance of electron-dense groups of ribosomes (arrow).

to mercury resistance (Table 3). The basis for increased resistance is unclear from the results of assays of mercury metabolism in the parent and variant cultures (Table 4). Culture 85-S showed only a slight increase in metabolism of HgCl_2 . The mechanism of resistance might be due to a lowered mercury accumulation. This, however, does not appear to be an acceptable explanation, because each of the cultures accumulated a similar amount of labeled Hg^{2+} .

In the case of the *Enterobacter* strain studied,

it appears that during the lag phase most of the cells are lysed and mercury-resistant mutants are selected, and, when the mercury concentration is reduced to ca. $1 \mu\text{g}/\text{ml}$ via chemical and biological reduction of Hg^{2+} , the surviving cells initiate growth at a normal rate. Whereas the exact mechanism of resistance to HgCl_2 for this particular organism is unclear, we have shown that resistance in other organisms is related to the ability to reduce Hg^{2+} ion to Hg^0 (12). Our results suggest that in the presence of toxic

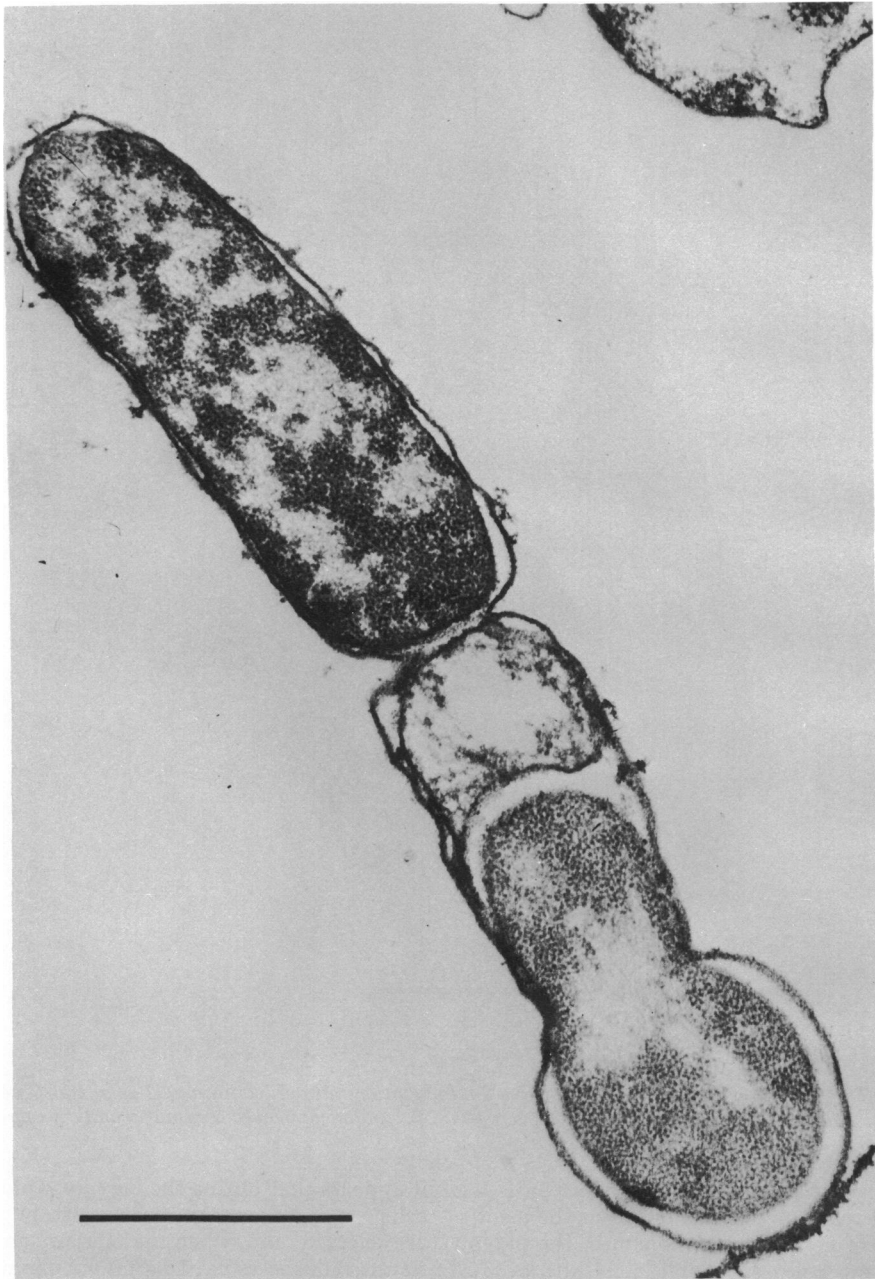


FIG. 6. Thin section of *Enterobacter* 85 after a 6-h incubation in broth containing $2 \mu\text{g}$ of HgCl_2 per ml. This figure shows the typical morphology of the intact cells. The major portion of the cells is lysed at this stage.

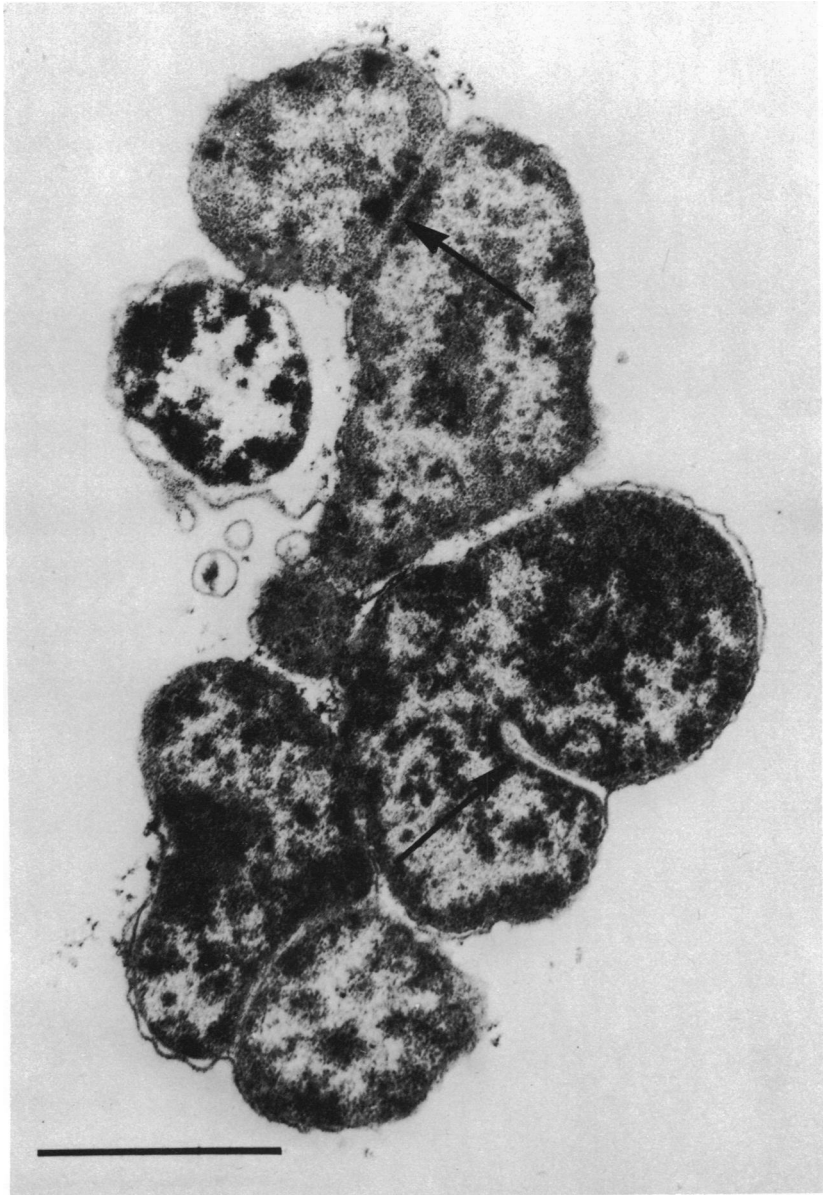


FIG. 7. Thin section of *Enterobacter 85* after a 24-h incubation in broth containing $2 \mu\text{g}$ of HgCl_2 per ml. Viable, pleomorphic cells and fragments of lysed cells are seen. Cellular division occurs by cross-wall formation (arrows) rather than by normal constriction. Numerous electron-dense groups of ribosomes are also seen.

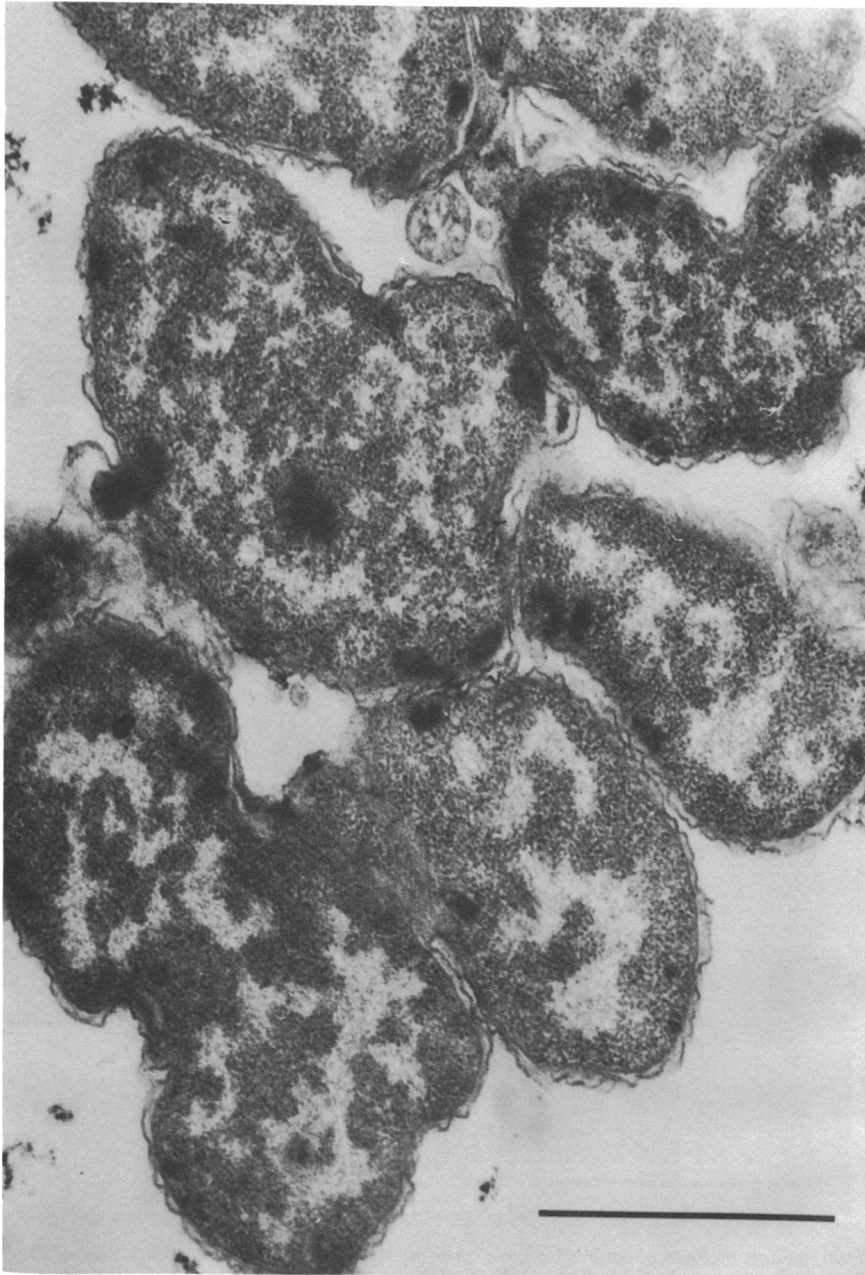


FIG. 8. Thin section of *Enterobacter* 85 after a 26-h incubation in broth containing $2 \mu\text{g}$ of HgCl_2 per ml. Viable, pleomorphic cells predominate. Electron-dense clusters of ribosomes are present in all cells.



FIG. 9. Thin section of *Enterobacter 85* after a 28-h incubation in broth containing $2 \mu\text{g}$ of HgCl_2 per ml. The cells show reversion to rod-shaped morphology, although cell wall contours are still somewhat irregular. Electron-dense clusters of ribosomes are present in fewer numbers.

levels of mercury, reduction of the mercury concentration to a definite threshold level, either by metabolic or chemical volatilization, permits growth of the organism. In the early

stages of growth the cells appear pleomorphic and contain dense inclusions. As the concentration of mercury in the medium continues to decrease, the bacteria revert to normal mor-

TABLE 3. Mercury resistance^a by *Enterobacter 85* before and after growth in the presence of HgCl₂ (strains 85-L and 85-S)

Culture	Growth in HgCl ₂ concn (μg/ml)				
	2	4	6	8	10
85	+	+	-	-	-
85-L	+	+	+	-	-
85-S	+	+	+	+	+

^aThe cultures were serially subcultured in the absence of mercury. Each tube of broth containing the concentrations of mercury indicated above was inoculated with 3 drops of a log-phase culture and incubated, with shaking, at 25 C for 4 days.

TABLE 4. Mercury metabolism by *Enterobacter 85* before and after growth in the presence of HgCl₂ (strains 85-L and 85-S)^a

Culture	Dry wt of cells (mg/ml)	HgCl ₂ metabolized (%)	Cell-associated HgCl ₂ (%)
85	0.41	9.8	92.3
85-L	0.30	7.1	93.0
85-S	0.38	11.3	91.7

^aSee footnote to Table 3.

phology. Experiments are now in progress to detect the involvement of plasmids in the mercury resistance of this particular group of organisms.

ACKNOWLEDGMENT

This work was supported by Environmental Protection Agency grant RE-02529-01.

LITERATURE CITED

- Ben-Bassat, D., G. Shelef, N. Gruner, and H. Shual. 1972. Growth of *Chlamydomonas* in a medium containing mercury. *Nature (London)* **240**:43-44.
- Bernheim, F. 1971. The effect of cyanogen iodide and mercuric chloride on the permeability of cells of *Pseudomonas aeruginosa* and the antagonistic action of sulfhydryl compounds. *Proc. Soc. Exp. Biol. Med.* **138**:444-447.
- Brunker, R. L., and T. L. Bott. 1974. Reduction of mercury to the elemental state by a yeast. *Appl. Microbiol.* **27**:870-873.
- Furukawa, K., T. Suzuki, and K. Tonomura. 1969. Decomposition of organic mercurial compounds by mercury-resistant bacteria. *Agric. Biol. Chem.* **33**:128-130.
- Furukawa, K., and K. Tonomura. 1972. Induction of metallic mercury-releasing enzyme in mercury resistant *Pseudomonas*. *Agric. Biol. Chem.* **36**:2441-2448.
- Gillespie, D. C. 1972. Mobilization of mercury from sediments into guppies (*Poecilia reticulata*). *J. Fish. Res. Board. Can.* **29**:1035-1041.
- Kellenberger, E., and R. Ryter. 1958. Cell wall and cytoplasmic membrane of *Escherichia coli*. *J. Biophys. Biochem. Cytol.* **4**:323-326.
- Komura, I., T. Funaba, and K. Izaki. 1971. Mechanism of mercuric chloride resistance in microorganisms. II. NADPH-dependent reduction of mercuric chloride and vaporization of mercury from mercuric chloride by a multiple drug resistant strain of *Escherichia coli*. *J. Biochem. (Tokyo)* **70**:895-901.
- Komura, I., and K. Izaki. 1971. Mechanism of mercuric chloride resistance in microorganisms. I. Vaporization of a mercury compound from mercuric chloride by multiple drug resistant strains of *Escherichia coli*. *J. Biochem. (Tokyo)* **70**:885-893.
- Kondo, I., T. Ishikawa, and H. Nakahara. 1974. Mercury and cadmium resistances mediated by the penicillinase plasmid in *Staphylococcus aureus*. *J. Bacteriol.* **117**:1-7.
- Luft, J. H. 1961. Improvement in epoxy resin embedding material. *J. Biophys. Biochem. Cytol.* **9**:409-414.
- Nelson, J. D., and R. R. Colwell. 1973. Metabolism of mercury compounds by bacteria in Chesapeake Bay, p. 767-777. In R. F. Acker, B. F. Brown, J. R. DePalma, and W. P. Iverson (ed.), *Third International Congress on Marine Corrosion and Fouling*. Northwestern University Press, Evanston, Ill.
- Novick, R. P., and C. Roth. 1968. Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. *J. Bacteriol.* **95**:1335-1342.
- Nuzzi, R. 1972. Toxicity of mercury to phytoplankton. *Nature (London)* **237**:38-40.
- Ross, I. S., and K. M. Old. 1973. Mercuric chloride resistance of *Pyrenophora avenae*. *Trans. Br. Mycol. Soc.* **60**:293-300.
- Ross, I. S., and K. M. Old. 1973. Thiol compounds and resistance of *Pyrenophora avenae* to mercury. *Trans. Br. Mycol. Soc.* **60**:301-310.
- Smith, D. 1967. R factors mediate resistance to mercury, nickel, cobalt. *Science* **156**:1114-1115.
- Stutzenberger, F. J., and E. O. Bennett. 1965. Sensitivity of mixed populations of *Staphylococcus aureus* and *Escherichia coli* to mercurials. *Appl. Microbiol.* **13**:570-574.
- Summers, A. O., and E. Lewis. 1973. Volatilization of mercuric chloride by mercury-resistant plasmid-bearing strains of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. *J. Bacteriol.* **113**:1070-1072.
- Summers, A. O., and S. Silver. 1972. Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. *J. Bacteriol.* **112**:1228-1236.
- Tingle, L. E., W. A. Pavlat, and I. L. Cameron. 1973. Sublethal cytotoxic effects of mercuric chloride on the ciliate *Tetrahymena pyriformis*. *J. Protozool.* **20**:301-304.
- Tonomura, K., K. Maeda, F. Futai, T. Nakagami, and M. Yamada. 1968. Stimulative vaporization of phenylmercuric acetate by mercury resistant bacteria. *Nature (London)* **217**:644-646.
- Troger, R. 1959. Über den Metallnachweis in quecksilber- oder kupferbehandelten Bakterien. *Arch. Mikrobiol.* **33**:186-190.
- Venalle, J. H., and R. Coggeshall. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**:407-408.
- Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* **4**:475-478.