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Mechanisms of Microbial Resistance and Detoxification of Mercury and Organomercury Compounds: Physiological, Biochemical, and Genetic Analyses

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INTRODUCTION

The presence of heavy metals in the environment has received a great deal of attention due to their highly toxic nature and translocation through the food chain. The problem of mercury pollution came into focus after the discovery of high levels of methylmercury in fish and shellfish in Minamata Bay, Japan, that resulted in 46 deaths (18). The source of mercury was found to be a fertilizer plant that used mercury as a catalyst in the production of vinyl chloride, and discharged mercury was shown to accumulate at various stages of the food chain (75). In Sweden the use of phenylmercuric acetate (PMA) and methylmercury in fungicidal agents in seed dressings resulted in a significant decrease in the populations of seed-feeding birds (32). High levels of methylmercury have also been detected in fish from the Great Lakes region of North America.

Mercury and organomercurial compounds are highly toxic. Methylmercury is 100 times more toxic than inorganic mercury and has been found to be mutagenic under experimental conditions (26). The solubility of inorganic and organic mercury compounds in lipids as well as their binding to sulfhydryl groups of proteins in membranes and enzymes (4) account for their cytotoxicity.

Sources of mercury in the environment are both natural and anthropogenic in origin. In nature, cinnabar (red HgS) and metacinnabar (black HgS) are the most important mercury-containing ores. Livingstonite ($HgSb_4S_7$) and mercury-containing sulfide minerals such as tetrahydrite ($6Cu_2S \cdot Sb_2S_3$) are also considered important sources (18).

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Concentrations between 5 and 100 $\mu g\ kg^{-1}$ are common in rocks, and the level of mercury in the air above rocks and minerals high in mercury ranges from 1.6 to 16 μg $liter^{-1}$ (ppb) (26, 59).

Understanding the chemistry of mercury is important in understanding how mercury cycles through the environment. Mercury is the only metal that occurs in liquid form in its elemental state at ordinary earth temperatures. Inorganic mercury exists in three valence states: (i) Hg^0 (metallic mercury); (ii) Hg^{2+} (mercuric mercury); and (iii) Hg^+ (mercurous mercury). These forms exist in equilibrium by chemical dismutation as follows (65): $Hg_2^{2+} \rightleftharpoons Hg^0 + Hg^{2+}$. Mercury is widely distributed in rocks, soils, air, and water due to its volatility, adsorption to surfaces, and ability to form complexes.

In surface waters $Hg(OH)_2$ and $HgCl_2$ are the most common species, and reported levels in unpolluted waters are generally <0.1 μg $liter^{-1}$ (21). The most common species of mercury in sediments is HgS due to the low redox potential (32). Most atmospheric mercury exists as Hg^0 or methylmercury, whereas much lower levels of dimethylmercury are reported. In unpolluted air the levels of mercury are generally 1 to 10 ppb and the distribution is highly variable depending on levels of mercury in the soil, water, and mineral deposits in the area (51). Mercury released into the atmosphere due to natural degassing of the earth's crust is estimated at 2.5×10^4 to 5.0×10^5 tons $year^{-1}$, whereas total levels of mercury in the ocean are estimated at 2×10^8 tons (2×10^{11} kg) (119).

Anthropogenic sources of mercury include those associated with its use in the chlor-alkali, paint, agriculture, pharmaceutical, and paper and pulp industries as disinfectants, catalysts, and fungicidal agents. Consumption of >9 million tons worldwide is estimated. Over 12,500 tons of mercury per year are released into the environment from industrial mining activities (18). The burning of fossil fuels is believed to be a major source of mercury released into the environment. Although the content of mercury in fuels is relatively low (on the order of 180 ppb), over 3,000 tons of mercury $year^{-1}$ are released into the environment through the burning of coal, and an additional 10,000 to 60,000 tons are released from crude oils (50). Therefore, human activities are estimated to account for 2×10^4 to 7×10^4 tons of mercury $year^{-1}$ being released into the atmosphere and water supply (10^3).

Sewage treatment facilities constitute a widespread source of both inorganic and organic mercury compounds (Hg^0, Hg^{2+}, methylmercuric chloride [MMC], and dimethylmercury) with values ranging from 0.5 to 105 ppb of Hg. Levels of mercury were found to be highest close to the facility and to fall off rapidly within several miles (93). Similar concentration-distance dependency is found for airborne mercury fallout from chlor-alkali plants in Sweden which release levels of 100 to 400 kg $year^{-1}$ (49).

An increase in the deposition of mercury in the Greenland ice sheet in recent years has been noted. The levels have increased from an average of 60 ± 17 ng kg of $water^{-1}$ before 1952 to an average of 125 ± 52 ng kg of $water^{-1}$ between 1952 and 1965. This rise in total mercury content is taken as an indication of an increased input of mercury from human sources (119). Thus, it appears that although humans have contributed to the levels of mercury in the environment, it remains a relatively small percentage of the total mercury present in the biosphere.

The biological cycle of mercury in the environment has received a great deal of study to determine the contributions made by the activity of microorganisms. The roles of a number of different bacteria in the transformations of mercury have been documented (103). A positive correlation between the distribution of mercury compounds and that of resistant microorganisms in metal-contaminated sediments has been reported (111). In addition, there is a strong positive correlation between antibiotic resistance and heavy-metal resistance among both clinical and environmental isolates (73, 91, 101, 103).

Heavy-metal toxicity is influenced by a number of environmental factors such as (i) binding to environmental constituents, (ii) pH, and (iii) ion interactions, which influence the form and availability of mercury to microorganisms (20, 31).

The reduction of Hg^{2+} to Hg^0 by bacteria has been reported. In one anomalous case the activity was also associated with a culture filtrate (39). The methylation of mercury by microorganisms from soil, sediments, and even the human intestinal tract has been reported (26, 47, 48, 122, 124, 125). The methylmercury produced by microorganisms is believed to account for the elevated levels present in aquatic organisms (122).

The reduction of Hg^{2+} to Hg^0 and the decomposition of organomercurial compounds by mercury-resistant bacteria have been demonstrated among a wide range of bacterial genera isolated from soil, sediments, and clinical sources (63, 103, 116). The detoxification of mercury and organomercury compounds involves an inducible plasmid-encoded enzyme system that is believed to be under positive regulation.

With the widespread range of microorganisms and mercury compounds involved in the transformations outlined above, it is evident that they play an important role in the cycling of mercury in the environment. The purpose of this paper is to examine the mercury detoxification systems present in microorganisms. A detailed survey of the organisms involved and ranges and patterns of resistance, as well as the biochemical and genetic bases of detoxification, is presented.

METHYLATION OF MERCURY BY MICROORGANISMS

The methylation of mercury has received a great deal of attention since the discovery that methylmercury is present at relatively high levels in aquatic organisms despite a lack of input of organomercury compounds into the aquatic environment. The major form of mercury in fresh- and seawater is Hg^{2+}, whereas the predominant form in fish is methylmercury (32, 52, 120). Methylmercury and dimethylmercury have a high solubility in lipids and solvents and a high affinity for the sulfhydryl groups on proteins. Methylmercury is a potent neurotoxin and may be accumulated in the food chain, making it a potential health problem (4). The toxicity of methylmercury to microorganisms depends on its residence time and stability in the water system (8, 31, 84). Biological methylation of mercury by microorganisms is believed to play a role in the formation of methylmercury in aquatic organisms and sediments and may represent an important link in the mercury cycle. Although methylmercury is more toxic than inorganic mercury, it is more volatile, and therefore methylation may actually be a detoxification mechanism.

Mechanism of Methylation of Mercury

Three pathways involving the methylation of mercury have received attention: (i) abiotic or photochemical methyl-

ation of Hg^{2+}; (ii) the methylation of Hg^{2+} in sediments by bacteria that excrete methylcobalamin which can act as a methyl donor; and (iii) the methylation of mercury by bacterial flora of aquatic organisms also perhaps utilizing methylcobalamin (103).

The photochemical methylation of mercury is believed to account for as much as a 3% conversion of mercuric acetate day^{-1} which is reported to be >2 orders of magnitude greater than rates reported for microbial activities (103). This process is inhibited 99.9% if $HgCl_2$ and acetic acid are used in place of mercuric acetate, which may discount the role of photochemical methylation in seawater.

The biological methylation of mercury has been demonstrated under anaerobic conditions by bacteria in river and lake sediments and rotting fish, as well as by cell-free extracts of methanogenic bacteria (47, 78, 124–127). Methylmercury formation has been reported to be affected by the growth stage of the microorganisms (84). Formation of methylmercury under aerobic conditions has also been demonstrated by soil and sediment organisms and even by bacteria isolated from the human intestinal tract (36, 80, 89, 114). The conditions under which methylmercury formation by bacteria occurs, the organisms involved, and the mechanism of methylation are discussed below.

The mechanism of methylation of mercury remains unclear but appears to involve the nonenzymatic transfer of methyl groups from methylcobalamin to Hg^{2+} (5, 17, 42, 86, 122). Three major coenzymes are known to be involved in methyl transfer: (i) *N*5-methyltetrahydrofolate derivatives; (ii) *S*-adenosylmethionine; and (iii) vitamin B_{12} (methylcobalamin). The last is believed to be responsible for methylation of inorganic Hg^{2+} salts because they are the only agents capable of transferring carbanion methyl groups (5, 17). The methylation reaction is believed to proceed via electrophilic attack of the mercuric ion on the carbanion species which is stabilized by the cobalt atom (17). The overall reaction proceeds as follows:

$$Hg^{2+} \xrightarrow{CH_3B_{12}} (CH_3)Hg^+ \xrightarrow{CH_3B_{12}} (CH_3)_2Hg$$

The first methylation reaction proceeds 6,000 times faster than the second (122).

Methylcobalamin reacts rapidly with $HgCl_2$ but at slower rates with organic mercury compounds in aqueous solutions. The products of this reaction have been found to be hydroxycobalamin and methylmercury as detected by absorbance spectra and gas-liquid chromatography, respectively (5, 42). An increase in absorbance at 351 nm and a concomitant decrease at 381 nm occur during the formation of hydroxycobalamin from methylcobalamin.

Enzymatic transfer of methyl groups to mercury has also been proposed but has not been clearly demonstrated (124). Methylcobalamin is involved in the synthesis of methionine via methylation of homocysteine in bacteria, making plausible the possibility that it serves as a methyl donor to mercury. Although Hg^{2+} appears to be the most likely direct precursor of methylmercury, soil and aquatic microorganisms have been reported (62) that are capable of producing dimethylmercury from PMA.

Methylmercury Formation Under Anaerobic Conditions

Bottom sediments from freshwater aquaria and putrescent homogenates of fish have been shown to produce methylmercury from Hg^{2+} and dimethylmercury from methylmercury, respectively (47). Autoclaved sediments and blank controls were shown to contain 40 ng of methylmercury g of $sediment^{-1}$. The experimental samples that were incubated with $HgCl_2$ contained 180 and 440 ppb of methylmercury at 5 and 10 days, respectively. After 7 days approximately 125 ng of methylmercury had been formed g of $sediment^{-1}$ from the initial 100 μg of $HgCl_2$ added g^{-1}. Methylmercury could be quantitatively recovered from the controls. Dimethylmercury was formed from methylmercury by fish homogenates during 4 to 7 weeks of incubation under anaerobic conditions and from Hg^{2+} within 4 days.

The formation of mono- and dimethylmercury from Hg^{2+} under anaerobic conditions has been demonstrated in cell extracts of methanogenic bacteria isolated from canal mud in Delft, Holland (124). Methylcobalamin was present at substrate concentrations and the reaction was shown to require ATP and hydrogen as the source of electrons. The enzymatic transfer of methyl groups from Co^{2+} to Hg^{2+} was proposed as the mechanism of methylation of mercury. However, rapid methylation at higher Hg^{2+} levels suggested that the methyl transfer from Co^{2+} to Hg^{2+} may also proceed via a nonenzymatic pathway. The constituents of the reaction mixture make it difficult to determine whether the reaction proceeds via an enzymatic or a nonenzymatic mechanism.

Methylmercury is formed from $HgCl_2$, HgI_2, HgO, $Hg(NO_3)_2$, $Hg(SO_4)_2$, and $Hg(CH_3COO)_2$ but not from HgS by the anaerobic bacterium *Clostridium cochlearium*. The formation of methylmercury was confirmed by thin-layer chromatography and by the degradation of the product by the *Pseudomonas* sp. K62 soil strain capable of degrading methylmercury (125). The addition of 0.1% glucose and cysteine was found to enhance the formation of methylmercury. The role of cysteine may be to reduce the toxicity of Hg^{2+} by reducing it to Hg^+. Methylcobalamin was produced by cell extracts of this strain as determined by the absorbance spectrum. Exogenous vitamin B_{12} was shown to stimulate methylmercury formation. The formation of methylmercury correlation with the formation of hydroxycobalamin measured as an increase in absorbance at 351 nm and a concomitant decrease at 380 nm and led the investigators to conclude that methylcobalamin is responsible for the methylation of mercury by these strains. A positive correlation between methylmercury formation and sporulation by *Clostridium* spp. was also observed (126).

Methylmercury Formation Under Aerobic Conditions

Methylmercury is also reportedly produced in aerobic sediments and by pure cultures of aerobic microorganisms (36, 60, 84, 94, 114). A comparison of aerobic and anaerobic methylation of $HgCl_2$ in San Francisco Bay sediments indicated that methylmercury formation was faster and resulted in higher net levels under anaerobic conditions and in samples with the highest organic content (78). Autoclaved sediments and those receiving no $HgCl_2$ did not produce methylmercury. The methylmercury formed under anaerobic conditions was found to be stable due to the absence of methylmercury-degrading bacteria. However, 21 of 30 methylmercury-degrading bacterial isolates from Lake St. Clair have been found to degrade methylmercury under anaerobic conditions (94).

Methylmercury has been shown to be formed from added $HgCl_2$ in lake sediments incubated under aerobic conditions. The organisms involved were identified as *Pseudomonas* spp. Methylmercury, identified by electron capture gas chromatography, increased from 0 to 0.31 $\mu g\ g^{-1}$ in sediments during the first 50 days and appeared to be produced in cycles. Periods of methylmercury production were fol-

lowed by a decrease in the amount of methylmercury and a concomitant increase in Hg^0, the product of microbial reduction of Hg^{2+} (94).

A mercury-resistant strain of *Enterobacter aerogenes* isolated from river sediments was capable of methylating mercury, but it was unable to reduce Hg^{2+} to metallic mercury (36). Aerobic growth conditions stimulated methylmercury production by this isolate, the process also appearing to be cyclic in nature. Methylmercury formation was found to decrease in the presence of L-cysteine in the culture filtrates. When methylcobalamin was added to the cultures, methylmercury was formed in both the cell cultures and the uninoculated controls at 372 and 339 ng ml^{-1}, respectively. Cultures of *Pseudomonas fluorescens* and *Escherichia coli* were shown to both degrade methylmercuric chloride to mercuric chloride and methylate mercury to form methylmercury in Ottawa River water (84).

Pure cultures of *Pseudomonas fluorescens*, *Bacillus megaterium*, *Escherichia coli*, and *Enterobacter aerogenes* have been found to methylate Hg^{2+} at higher levels under aerobic conditions, producing 240 to 865 ng of methylmercury $liter^{-1}$ from 20 mg of added $HgCl_2$ $liter^{-1}$. Addition of methylcobalamin to *Escherichia coli* cultures had no significant effect, whereas no detectable levels of methylmercury were produced by *Enterobacter aerogenes* in the absence of this compound (114). Fungal cultures of *Aspergillus niger*, *Scopulariopsis brevicaulis*, and *Saccharomyces cerevisiae* produced methylmercury up to 240 mg g (dry weight) of $cells^{-1}$ after 28 days, results similar to those reported for *Neurospora crassa* (60). None of the test strains were able to degrade methylmercury. Landner (60) suggested that methylation of mercury may be an ancillary reaction in the synthesis of methionine.

Bacterial isolates from the human intestine have also been shown to be capable of methylating mercury. Pure cultures of *Escherichia coli*, streptococci, staphylococci, lactobacilli, bacteroides, bifidobacteria, and yeasts were examined for the ability to form methylmercury from $HgCl_2$. Methylmercury was shown to be produced by a large percentage of the streptococci, staphylococci, yeasts, and *Escherichia coli* (approximately 60%) isolates, whereas only a small percentage of the obligate anaerobes, i.e., the bacteroides, bifidobacteria, and lactobacilli, were able to form this compound. In addition, the anaerobic bacteria produced less methylmercury than the facultative anaerobes under similar conditions (89).

Effects of HgS on Methylation of Mercury

In natural environments hydrogen sulfide may be evolved in anoxic sulfur-containing sediments. Mercuric sulfide is formed when divalent mercury ions and sulfide ions are simultaneously present due to the extremely low solubility in water (12, 19, 32). Therefore, the question of the availability of mercury for methylation in sediments is of interest. Methylmercury is formed from mercuric sulfide by aerobic organic sediments but at much lower rates (100 to 1,000 times slower) than those observed for $HgCl_2$. No methylmercury was formed under anaerobic conditions, presumably because of the low redox potential. Under aerobic conditions sulfide is oxidized to sulfate, resulting in an increased solubility of Hg^{2+} and hence a greater availability of the Hg^{2+} for methylation (19). Methylmercury was not produced from HgS by cultures of *Clostridium cochlearium* or by chemical methylation with methylcobalamin under anaerobic conditions in another study (18). Only during the formation of methane, when the total available HgS had been exhausted, was the production of methylmercury detected.

In this context, it is also of interest to note that hydrogen sulfide aids the volatilization of mercury (88). This has been demonstrated in laboratory studies with H_2S and water-soluble CH_3HgCl. An intermediate product $[(CH_3Hg)_2S]$ is first formed which then decomposes to β-HgS and $(CH_3)_2Hg$ (12).

Therefore, it is evident that microorganisms can methylate mercury under both aerobic and anaerobic conditions, thus contributing to the mobilization of mercury from sediments and perhaps the accumulation of methylmercury in aquatic organisms. Methylcobalamin is a known methyl donor and is produced in many microorganisms. It may very well serve as the source of methyl groups. However, whether the biological mechanism involves an enzymatic or a nonenzymatic mechanism remains unanswered. The rate of synthesis of methylmercury is dependent upon a number of variables including the concentration and availability of Hg^{2+}, composition of the microbial population, pH, temperature, redox potential, and synergistic or antagonistic effects of chemical and biological processes.

MICROBIAL RESISTANCE TO MERCURY AND ORGANOMERCURIALS

Mercury- and organomercurial-resistant bacteria were first isolated from mercury-contaminated soil in Japan (108, 110). They have since been isolated from sediments of the New York Bight heavily polluted for years by a variety of domestic and industrial wastes containing high concentrations of mercury and other heavy metals (111). Mercury-resistant isolates have also been obtained from the Chesapeake Bay area, where high concentrations of mercury exist. These bacteria are capable of degrading petroleum in addition to decomposing PMA and mercuric chloride (116). However, more attention has been paid to the frequency of mercury resistance among clinical isolates (67–69, 77, 91, 117, 118). Mercury-resistant enteric bacteria, staphylococci, and *Pseudomonas* spp. have all been isolated from clinical settings.

Bacterial resistance to mercury and organomercurials is determined by plasmids, which in many instances also encode resistance to other heavy metals and antibiotics (43, 57, 77, 91, 95, 97, 103). The plasmid-determined nature of resistance to mercury compounds was established by determining the ability for cotransduction (with other plasmid-encoded determinants) and high frequency of conjugal transfer of the Hg^r determinant. Isolation of covalently closed circular DNA from the Hg^r strains and its ability to transform Hg^s recipients to the Hg^r phenotype provided further evidence that the Hg^r determinant is plasmid encoded, as did curing Hg^r strains with agents such as ethyl methanesulfonate (67–69, 77).

The relationship between resistance to mercury and other heavy metals and antibiotics in the hospital environment has been explored in numerous studies. There appears to be a strong correlation between antibiotic resistance and resistance to mercury and several other metals (67–69, 77, 91). In most instances, the frequency of heavy-metal resistance is the same as or higher than that of antibiotic resistance.

The frequency of mercury resistance among clinical isolates of the Hammersmith Hospital collection is about 25%. This represents a collection of 800 plasmids of enteric origin that have been transferred into a common host, *Escherichia coli* K-12. A wide range of genera, including *Proteus*, *Providentia*, *Salmonella*, *Shigella*, *Klebsiella*, and *Serratia*,

are represented (91). Of a total of 787 clinical isolates of *Pseudomonas aeruginosa*, 99.8% were found to be metal resistant, with 99.5% exhibiting multiple resistance. The frequency of mercury resistance among these isolates was 75.1%. Only 53.2% of these metal-resistant isolates were also multiply antibiotic resistant (68). These results suggest that the frequency of resistance to metals is greater than resistance to antibiotics and that most of the metal-resistant strains are multiply resistant.

An investigation of the frequency of drug and heavy-metal resistance in clinical isolates of *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* revealed that metal ion resistance occurred at frequencies equal to or higher than resistance to antibiotics (69). The frequencies of mercury resistance were 57.3, 65.9, 75.1, and 36.3% for the organisms listed above. The Hg^r determinant was transferred in bacterial matings 89.9% of the time and could be cured at a high frequency by treatment with acriflavin or growth at 48°C (69). Among 338 isolates of *Escherichia coli* from hospital patients, 58.6% were found to be Hg^r (67).

The penicillinase plasmids of *Staphylococcus aureus* carry determinants for resistance to mercury as well as arsenate, lead, cadmium, and bismuth ions all grouped in one region (57). A frequency of Hg^r among hospital populations of staphylococci is reported to be 32%. There is a close association of the Hg^r phenotype with tetracycline resistance, a lack of mannitol fermentation, and coagulase-positive phenotype (35). Staphylococci isolated from rural and urban populations of Iraq exposed and not exposed to heavy metals or antibiotics gave different results. Over 90% of the isolates from both rural and urban populations not known to be exposed to either metals or antibiotics were resistant to one or more antibiotics. In contrast, resistance to metals occurred in only 39% of the isolates. Populations exposed to methylmercury from grains coated with the compound exhibited no significant increase in the incidence of mercury resistance. However, a higher incidence of Hg^r isolates was found in the urban than in the rural populations (34). In all cases cited above, a clear distinction between mercury-susceptible and -resistant populations is demonstrated, with MIC levels of 10 to 50 $\mu g\ ml^{-1}$.

It seems unlikely that metal-resistant microorganisms would arise merely by chance. The association of mercury resistance with antibiotic resistance on R factors and the frequency of occurrence in clinical situations raised questions as to what the selective forces might be. The increase in frequency of mercury-resistant strains is believed to be relatively recent and concomitant with the increase in frequency of antibiotic resistance. Recently, it has been noted that a reduction in the use of mercurial compounds as diuretics and disinfectants has resulted in a 66% decrease in frequency of mercury-resistant *Staphylococcus aureus* isolates from hospitals in Tokyo and down to 2% of the isolates in a St. Louis hospital (83, 92). The evolution of multiply resistant metal and antibiotic strains may be the result of the sequential acquisition of individual transposons to form composite transposable units. In this case, the entire transposon or simply one of the components may be lost (37). This would explain the loss of mercury resistance without a loss of resistance to antibiotics and the increase in heavy-metal-resistant bacteria concomitant with the increase in antibiotic resistance.

The frequency of plasmids encoding resistance to metals in natural settings has received little study. Untreated Boston sewage, sewage from a hospital, and that from an industrial plant that reprocesses used photographic film were analyzed for Ag-, Hg-, and tetracycline-resistant organisms (97). The frequency of metal-resistant isolates was found to be much higher (virtually all isolates were Ag^r, Hg^r, and Tet^r) than among a collection of standard plasmid-bearing strains in a hospital bacteriology laboratory. *Klebsiella pneumoniae* comprised 85% of the multiply resistant city sewage isolates, whereas 80% of the film-processing isolates were identified as *Citrobacter freundii*. In both city and hospital sewage sludge 25 to 35% of the antibiotic-resistant strains were also resistant to Ag and Hg. In contrast, 8% of the metal-resistant isolates of the city sewage, 40 from the hospital sewage, and only 1% from the film-processing sludge were resistant to antibiotics. The transferability of the Hg^r plasmids among the city sewage, hospital sewage, and film-processing sludge isolates was found to be 28, 39, and 69%, respectively. The frequency of metal ion-resistant isolates suggests that metal resistance is associated with resistance to antibiotics in nonclinical isolates and that the high silver concentration of the film-processing sludge provides a strong selective force for metal-resistant bacteria.

Range of Resistance to Mercury and Organomercury Compounds

More detailed studies have provided information on the range of resistance of Hg^r plasmid-bearing bacteria to mercury and organomercury compounds (Table 1). It appears that all Hg^r bacteria confer resistance to Hg^{2+} and that all gram-negative Hg^r bacteria also confer resistance to merbromin and fluorescein mercuric acetate (FMA) (118).

The Hg^r plasmids of *Escherichia coli* and other enterics fall into two classes of resistance: (i) "narrow-spectrum" resistance plasmids that are resistant to Hg^{2+}, merbromin, and FMA; and (ii) "broad-spectrum" resistance plasmids which confer resistance to PMA and thimerosal in addition to Hg^{2+}, merbromin, and FMA (117, 118). The broad-spectrum resistance plasmids have only been identified in the A–C, L, and H2 incompatibility groups.

The Hg^r plasmids of *Pseudomonas aeruginosa* also fall into narrow- and broad-spectrum classes. The narrow-spectrum plasmids confer low-level resistance to *p*-hydroxymercuribenzoate (pHMB) in addition to Hg^{2+}, merbromin, and FMA. Broad-spectrum resistance plasmids differ from those of *Escherichia coli* by also conferring resistance to ethylmercuric chloride (EMC), MMC, and pHMB (13, 82, 117, 118). The *Pseudomonas aeruginosa* plasmids conferring resistance to pHMB can be maintained in *Escherichia coli* but do not confer pHMB resistance (92). The Hg^r plasmids of *Staphylococcus aureus* are all considered to belong to the broad-spectrum resistance class. These plasmids confer resistance to Hg^{2+}, PMA, pHMB, and FMA, but are sensitive to EMC, MMC, merbromin, and thimerosal (117, 118). It is of interest that a catabolic plasmid (pWW17) in *Pseudomonas* sp. strain MT14 soil isolate encodes ability to grow on phenyl acetate and resistance to mercuric chloride (81).

Mechanism of Resistance to Mercury and Organomercury Compounds

Detoxification of Hg^{2+}. The mechanism of resistance to mercuric ions and organomercurials involves the elimination of the metals from the growth medium. Two additional mechanisms have been proposed: (i) the synthesis of thiols that bind the mercury compound, thereby reducing its toxicity to the cell; and (ii) the existence of a permeability

TABLE 1. Summary of plasmid resistance toward Hg^{2+} and organomercurials and inducibility by these compounds (modified from reference 118)[a]

Plasmid	Hg^{2+}	PMA	Thimerosal	EMC	MMC	pHMB	Merbromin	FMA
Escherichia coli[b]								
Narrow spectrum	R^i	S	S	S	S	S	R^i	R^i
Broad spectrum	R^i	R^i	R^{ND}	S	S	S	R^i	R^i
Pseudomonas spp.								
Narrow spectrum	R^i	S^{ip}	S^{ip}	S^{ND}	S^{ND}	R^i	R^i	R^{ND}
Broad spectrum	R^i	R^i	R^i	R^{ND}	R^{ND}	R^i	R^i	R^{ND}
Staphylococcus aureus	R	R^{ip}	S	S	S	R^i	S^i	R^i

[a] Boxed positions denote volatilization of Hg^0 from Hg^{2+} or volatilization of Hg^0 after hydrolysis of the organomercurial and subsequent reduction to Hg^0. Unboxed resistances do not involve hydrolysis or volatilization. Narrow-spectrum plasmids confer the ability to volatilize mercury only from inorganic Hg^{2+}; broad-spectrum plasmids confer the ability to volatilize mercury from both Hg^{2+} and organomercurials. R, Resistance; S, sensitivity; i, ability of compound to induce volatilization of Hg^0 from Hg^{2+} or various organomercurials; ip, poor inducer; ND, not determined.

[b] *E. coli* volatilizes Hg from EMC, MMC, and pHMB at such low levels that it does not confer resistance.

barrier that would limit access of the mercury to the cell (103).

In all cases studied to date, involving more than 100 Hg^r organisms, mercury has been shown to be converted to a volatile form which is eliminated from the growth medium (Fig. 1). The volatilization of mercury is the action of the inducible mercuric reductase enzyme. Assays of the mercury-volatilizing activity routinely use washed cell suspensions of Hg^{2+}-induced cultures to which ^{203}Hg-labeled substrate has been added. A closed system is used in which the vapor phase or liquid phase or both are analyzed to determine the loss of ^{203}Hg from the growth medium or its appearance in the vapor phase or both (100). The major difficulty in assessing the levels of ^{203}Hg volatilized lies in the inability to quantitatively recover the radiolabeled metal. Losses of up to 25% of the radiolabeled mercury compounds have been reported and are attributed to nonbiological factors such as sorption to the surface of the vessel or to leaky seals (100).

Numerous strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, as well as *Pseudomonas putida* and *Thiobacillus ferrooxidans*, have all been found to volatilize mercury from added Hg^{2+} (7a, 11, 28–30, 38, 43, 100, 102, 117, 118). Over 30 Hg(II)-resistant strains from the Hammersmith Hospital collection were tested, and all were found to volatilize added $^{203}HgCl_2$ when induced by prior exposure to Hg^{2+} (91). The distinction between sensitive and resistant strains is absolute with respect to conversion of Hg^{2+} to a volatile form. Sensitive plasmidless strains show no detectable loss of $^{203}HgCl_2$ or ^{203}Hg-PMA, whereas Hg^{2+}-resistant strains volatilize added $HgCl_2$ and strains resistant to both Hg^{2+} and PMA are capable of volatilizing both (91). The broad-spectrum Hg^r *Pseudomonas* sp. K62 strain, the *Pseudomonas putida* strain harboring the *mer* plasmid, and *Pseudomonas aeruginosa* strains harboring the narrow-spectrum resistance plasmids pMH1, pMG2, R26, R933, R93-1, and pVS1 all have been shown to confer resistance to Hg^{2+} via the enzymatic reduction and subsequent volatilization of the added mercury (11, 28–30).

Several mercury-resistant *Staphylococcus aureus* strains originally shown to be non-volatilizing exhibited an increased uptake and binding of Hg^{2+} believed to account for the resistance phenotype. Subsequently this strain was found to be able to volatilize mercury (57). The discrepancy is believed to be due to poor induction conditions and the bacteria have since been shown to volatilize mercury, albeit at lower rates than mercury-resistant volatilizing strains of *Escherichia coli* and *Pseudomonas* spp. (100, 117). *T. ferrooxidans* strain BA-4 has recently been found to convert added ^{203}Hg to a volatile form (80).

Purified enzyme preparations from *Pseudomonas* sp. K62, *Escherichia coli* J53-1(R831), and *Staphylococcus aureus* strains have also been found to convert Hg^{2+} to a volatile form, as was the case for whole-cell suspensions of these strains (29, 30, 43, 55, 56, 109, 110, 117).

The nature of the volatile mercury proved to be a critical question, as both elemental mercury (Hg^0) and organomercury compounds such as methyl- and dimethylmercury are volatile. In most, but not all, cases, it has been demonstrated that metallic mercury (Hg^0) is the volatile end product of mercury detoxification (Table 2), such that mercury resistance and volatilization of Hg^0 are essentially synonymous. Atomic absorption spectrophotometry is the most popular and widely used method of detecting mercury in biological materials; however, chemical speciation is not possible by this method (18). The Hatch-Ott method is routinely used to determine levels of metallic mercury in samples containing as little as 1 ppb. Total mercury can be determined by a procedure involving acid hydrolysis, oxidation, and reduc-

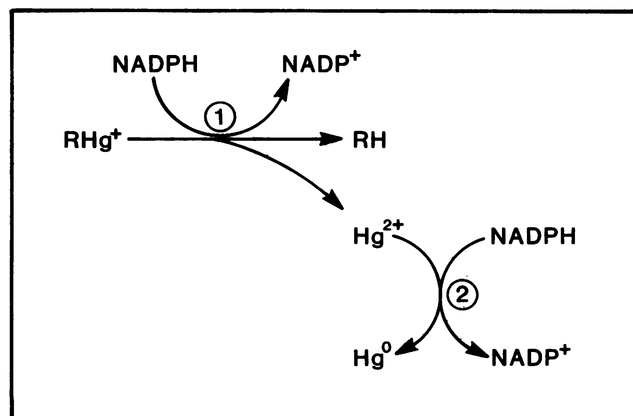

FIG. 1. Detoxification of mercury by organomercurial lyase (1) and mercuric reductase (2) enzymes.

TABLE 2. Products of mercury and organomercurial detoxification by intact cells and purified enzymes

Strain(plasmid)	Substrate	Rate of volatilization	Assay mixture	Assay method	Chemical nature of volatile mercury	Method of detection[a]	Reference(s)
Escherichia coli W2252 (R factor)	Hg^{2+}	ND[b]	1. Induced washed cell suspensions 2. Enzyme prep	1. ^{203}Hg volatilization trapped in aqua regia 2. NADPH oxidation	ND	NA	55, 56
E. coli AB1932-1 (RTF JJ1)	Hg^{2+}	4–5 nmol min^{-1} 10^8 $cells^{-1}$ (maximum)	Induced washed cell suspensions	Chloroform or toluene extracts of cell suspensions exposed to $^{203}HgCl_2$	Hg^0	Coupled GC-MS	102
Pseudomonas sp. K62 (no plasmid identified)	PMA MMC	ND	Growing bacterial cultures	Vaporized Hg collected in cold trap	Hg^0 Benzene Methane	Cold trap GC	28
Pseudomonas sp. K62 (no plasmid identified)	MMC	ND	Purified enzyme	^{203}Hg vaporization	Hg^0 Methane	GC	29
Pseudomonas spp., Hg^r estuarine isolates	PMA	ND	Growing bacterial plate cultures	Closed-system bioreactor—measured volatilized Hg; measured Hg^0 total	Hg^0 Benzene	AA[c] GC	73
Pseudomonas aeruginosa (pMG1, pMG2, R26, R933, R93-1, pVS1)	Hg^{2+} PMA	3–6 nmol of Hg^{2+} min^{-1} mg of $cells^{-1}$	Induced washed cell suspensions	Sampling of vapor-phase flask culture	Hg^0 Benzene	AA[c]	11
Staphylococcus aureus RN23(pI258)	Hg^{2+} PMA Thimerosal	1.8 nmol of Hg^{2+} min^{-1} mg of $cells^{-1}$; 0.7 nmol of PMA min^{-1} mg of $cells^{-1}$	Induced washed cell suspensions	Sampling of vapor-phase flask culture	ND	AA[c]	117
Pseudomonas spp.	Methyl-mercury	ND	Growing bacterial cultures	Volatilized gas trapped from reactor flasks	Hg^0	GC-MS	95
Pseudomonas sp. K62	PMA pHMB	ND	Purified enzyme	1. Extraction of enzyme reaction mixture 2. Analysis of vapor	Hg^0 + benzoic acid Benzene	GC TLC	109, 110

[a] AA, Atomic absorption spectroscopy; GC, gas chromatography; TLC, thin-layer chromatography; MS, mass spectroscopy; NA, not applicable.
[b] ND, Not determined.
[c] Hatch-Ott procedure for measuring inorganic and organic mercury by AA.

tion to Hg^0 of the remaining mercury compounds (38). Thin-layer chromatography has also been used to detect inorganic mercury and organic decomposition products of PMA and pHMB (109, 110). Identification of the species of volatilized mercury is accomplished by combined gas-liquid chromatography and mass spectroscopy (102).

Initially, the chemical nature of volatile mercury was determined with the Hg^r *Pseudomonas* sp. K62 strain by collecting the volatilized mercury in a cold trap and measuring the formation of metallic liquid mercury (28). Purified enzyme preparations from the above strain proved to possess the same activity, indicating that the reduction of Hg^{2+} to Hg^0 results in the volatilization of added mercury at both cellular and subcellular levels (29, 30).

Both intact cells and enzyme preparations of the Hg^r *Escherichia coli* strain W2252 have been shown to be capable of converting added $HgCl_2$ to a volatile form (55, 56). The enzyme preparation catalyzed the Hg^{2+}-dependent

oxidation of NADPH, which led these investigators to conclude that the Hg^{2+} was concomitantly reduced to metallic mercury.

The most convincing evidence to date that metallic mercury is the volatile form of mercury produced upon detoxification of Hg^{2+} comes from the combined gas chromatography-mass spectroscopy analysis of the volatilized mercury. The volatile form of mercury produced by narrow-spectrum Hg^r *Escherichia coli* strain AB1932-1 was found to be soluble in organic solvents such as toluene, chloroform, benzene, and cyclohexane (102). Metallic mercury is soluble in organic solvents at 10^{-5} M (65, 102). However, because organomercurials such as methylmercury are also soluble in these organic solvents, the speciation of the volatile mercury proved critical to rule out the possibility that methylmercury could be the end product of detoxification.

Toluene extracts of bacterial cultures exposed to $^{203}HgCl_2$ for 7 min at 37°C were analyzed by coupled gas-liquid chromatography-mass spectroscopy. These extracts showed a single peak containing mercury in the gas chromatographs, with a retention time characteristic of metallic mercury. No higher-molecular-weight compounds containing mercury clusters were observed, such as the characteristic mercury isotope clusters at 198 to 204 *m/e* (Hg^{2+}), 213 to 219 *m/e* (CH_3Hg^+), and 228 to 234 *m/e* (CH_3HgCH_3) (102).

Thus, the volatilization of mercury by Hg^r bacteria has become synonymous with the production of metallic mercury. This is somewhat misleading because, although the mechanism of resistance to Hg^{2+} appears to be similar among several bacterial genera, the speciation of the volatilized mercury from only a limited number of strains has been conducted. However, this proposed mechanism is supported by enzyme studies indicating the NADPH-dependent reduction of Hg^{2+}. Since many bacteria are capable of converting Hg^{2+} to other volatile forms of mercury including methylmercury, the possibility exists that under some conditions these strains may produce other volatile mercury compounds.

Detoxification of organomercury compounds. The detoxification of organomercurials is believed to result from the cleavage of the carbon-mercury linkage by the organomercurial lyase enzyme followed by the reduction of Hg^{2+} to Hg^0 by the mercuric reductase enzyme. Both of these enzymes have been purified from Hg^r bacteria and are discussed in detail in this paper. The resistance and volatilization patterns of broad-spectrum mercury-resistant bacteria are presented in Table 1.

The decomposition of organomercury compounds by mercury-resistant bacteria was first detected in the *Pseudomonas* sp. K62 soil isolate. This strain was shown to catalyze the degradation of PMA to Hg^0 and benzene by using ^{203}Hg- or ^{14}C-labeled PMA (28). Approximately 70% of the ^{203}Hg or ^{14}C label was shown to disappear from the medium in 2 h. The products of the reaction could be separated by adsorption to activated carbon and eluted with toluene, thus indicating that the carbon-mercury bond had been cleaved. The phenyl radical of PMA was shown to be converted to benzene as identified by gas chromatography, whereas the vaporized mercury was found to be metallic mercury. In similar experiments, the products of methylmercury were found to be methane and metallic mercury (28). Purified enzyme preparations from this strain were subsequently found to be capable of degrading pHMB to Hg^0 and benzoic acid as detected by thin-layer chromatography (109, 110).

Broad-spectrum mercury-resistance plasmids of *Pseudomonas aeruginosa* encode resistance to PMA, MMC, EMC, thimerosal, merbromin, FMA, and pHMB, but the bacterium is capable of degrading only the first four compounds (Table 1). In contrast, the broad-spectrum mercury-resistance plasmids of *Escherichia coli* encode volatilization of mercury from PMA and thimerosal. The broad-spectrum strains of *Escherichia coli* are sensitive to EMC, MMC, and pHMB, but they are able to volatilize mercury from these compounds at very low rates. *Escherichia coli*, like *Pseudomonas aeruginosa*, is resistant to merbromin and FMA but does not decompose either. The mercury-resistant strains of *Staphylococcus aureus* are capable of decomposing PMA, pHMB, and thimerosal while being sensitive to the latter (118). In all cases, the decomposition of the organomercury compound was measured by the volatilization of mercury.

The decomposition of PMA by Hg^r estuarine isolates exhibiting varying levels of resistance to the compound revealed Hg^0 and benzene as products. Over 60% of the isolates were identified as *Pseudomonas* spp. Metallic and total mercury was measured by atomic absorption spectrophotometry, and benzene was identified by gas chromatography as described above. The molar ratio of benzene to Hg^0 varied from 4.4 up to 389 over a 48-h period among the strains tested. In one case the ratio increased from 5.5 at 48 h to 105 at 143 h. This accumulation of benzene is attributed to a loss of Hg^0 observed in control experiments, presumably due to its volatility and ability to adsorb to glass (73). These results suggested again that the PMA was cleaved to produce Hg^0 and benzene.

Bacterial degradation of methylmercury has been demonstrated with both mixed and pure cultures from lake sediments. Four isolates were able to degrade between 15 and 55% of added (^{203}Hg)CH_3HgBr to the methane and Hg^0 in 20 h. After 170 h methylmercury was completely recovered from the control flask (95). Methane was detected in the head space of the reaction flask by flame ionization gas-liquid chromatography. All four pure cultures were identified as *Pseudomonas* spp. and were very similar to the *Pseudomonas* sp. K62 strain described previously (109).

Thus, it appears that bacterial resistance to organomercury compounds involves the degradation of methylmercury, ethylmercury, PMA, and pHMB to Hg^0 and methane, ethane, benzene, and benzoic acid, respectively. The mechanism in all instances appears to be the same; however, the degradation products have not been speciated for all broad-spectrum mercury-resistant strains isolated. It is interesting that purified enzyme preparations of the organomercurial lyase enzyme from the *Pseudomonas* sp. K62 strain can decompose pHMB, whereas intact cells of *Pseudomonas aeruginosa* cannot degrade pHMB although they are resistant to it. This may represent an alternate mechanism such as a permeability barrier, which may be clarified by uptake studies with the pHMB-resistant *Pseudomonas aeruginosa* strain in coordination with enzyme studies.

INDUCIBILITY OF THE MERCURY AND ORGANOMERCURIAL DETOXIFICATION SYSTEMS

The mechanism of resistance to inorganic and organomercurials involves the inducible synthesis of the mercuric reductase and organomercurial lyase enzymes by subtoxic levels of Hg^{2+} or various organomercurials. The inducible nature of mercury resistance systems has been well documented, with all mercury resistance systems described for *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* spp. comprising over 100 Mer^r strains that possess inducible enzyme systems (3, 28–30, 54–57, 90, 91, 100, 101, 104, 117, 118).

In all cases tested, the mercuric reductase or organomercurial lyase detoxifying enzymes or both are produced only in response to the presence of inorganic mercury or a variety of organic mercury compounds, some of which are not themselves substrates for degradation, thus acting as gratuitous inducers. Only one case, involving a *T. ferrooxidans* strain, has been reported to be constitutive for the production of the mercuric reductase enzyme, although initial reports indicated that it too was an inducible system (79, 80). This case bears further study, as it is reported that the strain lost the mercury-volatilizing ability after five passages without added Hg^{2+}. This may indicate the loss of the *mer* genes or it may represent a different regulatory mechanism, thus warranting further study. Cross-induction of the mercury resistance system has been demonstrated, and it appears that induction by an organomercurial or Hg^{2+}, to which the strain is resistant, will induce resistance to other Hg compounds. Gratuitous inducers also have been found, such as merbromin and FMA, that are not themselves substrates for the mercury-detoxifying enzymes yet are capable of inducing the system. However, evidence exists that indicates that resistance to merbromin and FMA is also inducible, although these compounds are not volatilized (117, 118). Both the mercuric reductase and the organomercurial lyase enzymes have been shown to be inducible, although not always in a coordinate fashion.

Growth Inhibition Studies

The original evidence for the inducible nature of the mercury resistance system came from growth studies of mercury-resistant and mercury-sensitive strains in the presence of inhibitory concentrations of Hg^{2+} or organomercurials. It has been observed that after a variable lag period, lasting from several minutes to several hours, growth of the resistant strains resumed at normal rates (101). The length of the lag period varied with the strain and the amount of the mercurial compound added. The maximal concentration of $HgCl_2$ that a *mer* strain of *Staphylococcus aureus* was able to tolerate on solid medium was 20 $\mu g\ ml^{-1}$. However, 10 $\mu g\ ml^{-1}$ in broth culture was found to be inhibitory to growth, giving rise to a rapid and steep decline in cell numbers during the first 8 h. Cell numbers started to increase after 12 h of exposure, and by 30 h the cell density increased to the same level as the control grown in the absence of $HgCl_2$ (57). In another case, using *Escherichia coli* Hg^r strain W2252, a lag period of 5 h was observed before normal growth in the presence of 0.02 mM $HgCl_2$, compared to a 1-h lag in medium without added $HgCl_2$ (44). When 0.02 mM $HgCl_2$ was added to cultures grown previously with and without $HgCl_2$ during early growth (optical density = 0.05), there was no detectable effect on the cells previously grown in the presence of $HgCl_2$. The cultures previously grown in the absence of $HgCl_2$ demonstrated the same lag of 5 to 6 h before resuming normal growth (55, 56). In contrast, a mercury-sensitive strain of *Escherichia coli*, W2252, grew only after a 60-h lag period in medium containing 0.01 mM $HgCl_2$, whereas immediate growth was observed in the medium without $HgCl_2$. In addition, it was noted that cells of the sensitive cultures appeared to lyse upon the addition of 0.01 mM $HgCl_2$ at an optical density of 0.5, whereas the resistant cells were not susceptible to lysis. The effects of $HgCl_2$ on the growth of other sensitive and resistant strains including *Escherichia coli* and *Enterobacter aerogenes* were similar (55, 56). Only the resistant strains grew with almost the same numbers of colonies on plates with and without 0.04 mM $HgCl_2$. These results indicated that the resistant population was homogeneous and that mercury resistance was not being selected from a heterologous population. Previous growth in the presence of Hg^{2+} or an organomercurial compound at subinhibitory levels eliminates the lag phase observed with mercury-resistant strains.

Induction of Mercuric Reductase and Organomercurial Lyase Detoxifying Enzymes

Hg^r and Hg^s strains can be distinguished in that the sensitive strains are not capable of producing either one of the enzymes responsible for the detoxification of mercury compounds. Because detoxification of Hg^{2+} or organomercurials ultimately results in the volatilization of metallic mercury from the growth medium, the measurement of enzyme levels or volatilization activity or both in response to added ^{203}Hg compounds may be used to confirm the inducible nature of the system (11, 22, 23, 28, 30, 90, 91, 102).

The *Pseudomonas* sp. K62 strain is resistant to both organic and inorganic mercurials. Prior exposure to PMA, MMC, or EMC at levels of 20, 10, and 5 mg $liter^{-1}$, respectively, was required for the bacteria to be able to decompose ^{14}C-phenyl-labeled PMA (28). Later studies demonstrated that the formation of the enzymes depended upon or were induced only when the organism was grown in the presence of PMA, mercuric chloride, *p*-chloromercuric benzoate (pCMB), merzonin, or metallic mercury. Furthermore, the enzymes were not induced by HgS or other metal ions; in fact, Cd^{2+} and Cu^{2+} were inhibitory (30). The relationship between PMA and mercuric chloride concentrations and the formation of the mercuric reductase and organomercurial lyase enzymes was studied in depth. Induction constants were calculated for both PMA and mercuric chloride from Lineweaver-Burk plots in which 1/PMA or 1/mercuric chloride were plotted against $1/v$ (where v = velocity of decomposition of ^{14}C-phenyl-labeled PMA, as determined by volatilization). The PMA-decomposing activity reached a maximum when the organism was grown with 6×10^{-5} M PMA or 7.4×10^{-5} M mercuric chloride, with induction constants of 3.8×10^{-5} M for PMA and 4.5×10^{-5} M for $HgCl_2$. Similar results were reported with pCMB, merzonin, or metallic mercury as inducing agent (30). Additional experiments on the effect of other metal ions on enzyme induction led to the conclusion that the induction is specific for mercury. The ability to produce the enzyme was lost when the organism was subsequently grown without mercurials.

In a study (102) with various Hg(II)-resistant, plasmid-bearing strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* the volatilization activity was only found to be induced by exposure to $HgCl_2$ at 10^{-5} M for not less than 3 h. Other mercurials were not tested in this study. Only the resistant strains were found to volatilize mercury, and in all cases the rate of loss of ^{203}Hg was much higher when cells had been exposed to 10^{-5} M $HgCl_2$ before determination of mercury-volatilizing activity (102). These results are summarized in Table 3.

It is apparent that in all cases there is a significant increase in the rate of volatilization of ^{203}Hg between uninduced and induced resistant strains. The *Staphylococcus aureus* strains do not exhibit as marked a difference between sensitive and resistant cells or between uninduced and induced resistant strains as seen with the *Escherichia coli* and *Pseudomonas aeruginosa* test strains (102). These results were reported to be reproducible. Exposure to 10 μM Hg^{2+} was shown to cause a 10-fold increase in the rate of ^{203}Hg volatilization by

TABLE 3. Comparative rates of ^{203}Hg volatilization upon induction (100)

Strain	Rate of ^{203}Hg volatilization (nmol min^{-1} ml of $cells^{-1}$)		Increase in rate (fold)
	Uninduced	Induced	
Escherichia coli			
Sensitive	0.01	—[a]	
Resistant	0.03–0.05	1.75–2.55	18–25
Staphylococcus aureus			
Sensitive	0.00–0.01	—	
Resistant	0.05	0.15	3
Pseudomonas aeruginosa			
Sensitive	0.01	—	
Resistant	0.22–0.85	1.9–2.35	2–10

[a] No values were given, presumably as the sensitive strains would be killed at concentrations of $HgCl_2$ used to induce the resistant strains.

a narrow-spectrum mercury-resistant strain of *Pseudomonas putida* (11).

Over 30 Hg^r strains from the Hammersmith Hospital collection resistant to mercurials were tested for ability to volatilize $^{203}Hg(II)$ and PMA. Based on the initial rate of loss of $^{203}Hg(II)$, the volatilization was found to be 5- to 10-fold more rapid by those strains that had prior exposure to 10 μM $Hg(NO_3)_2$ or 1 μM PMA (91). In another instance, *Escherichia coli* strain AB1932-1 containing Hg^r plasmid JJ1 was able to volatilize >90% of added ^{203}Hg after 10 min, whereas with uninduced cells 68% of the ^{203}Hg remained after this time period (102). With *Escherichia coli* strain DU1040(pDU202), a Hg^r wild-type strain, mercury volatilization was found to be completely inducible. Less than 0.1% of the maximum induced activity of the resistant strain (7 μmol min^{-1} g of $cells^{-1}$ when induced by 4 μM Hg^{2+}) was observed in the uninduced resistant cells (22). *Pseudomonas aeruginosa* PAO9501 carrying plasmid pVS1, which is the *mer* operon on transposon Tn*501*, has been shown to have up to 6% of the soluble cellular protein comprised of mercuric reductase enzyme upon induction by 10 μM merbromin (24). In contrast, the mercuric reductase of an *Escherichia coli* strain containing the cloned fragments of the *mer* operon on plasmid pRR130 constitutes only 0.1% of the soluble protein upon induction (87).

In a study with lambda transducing phages prepared from cointegrate isolates of lambda and plasmid R100, all lysogens exhibited only inducible synthesis of the mercuric reductase enzyme. During infection of one lambda-*mer* cointegrate into a lambda-sensitive strain, Hg(II) reductase activity was observed without induction by Hg^{2+}. This result would be consistent with the reductase gene being transcribed from the lambda p_L promoter. A time course study of inducible synthesis of Hg(II) reductase after infection of *Escherichia coli* by one of the lambda-*mer* cointegrates showed a maximum activity of 0.6 nmol of Hg^{2+} volatilized min^{-1} 10^{-8} cells within 45 min after induction, whereas uninduced activity was essentially undetectable (15).

It is interesting that in both induced Hg^r strains of *Escherichia coli* and *Pseudomonas aeruginosa*, rates of volatilization of mercury from organomercurials were lower than those observed for Hg^{2+} under comparable conditions. This may represent a difference in the effectiveness of induction by the inorganic and organomercurial substrates.

Thus, mercury resistance is inducible and is paralleled by the induction of the mercury-detoxifying enzymes. With the exception of the reported constitutive nature of the *T. ferrooxidans* mercuric reductase system, plasmid-determined mercury volatilization activity increases upon induction by exposure to mercurials. The induction by exposure to Hg^{2+} or an organomercurial is a standard experimental procedure, usually involving two steps to minimize toxic effects while maximizing enzyme function (104). However, there are reports of "microconstitutive" activity among some plasmid-bearing strains of *Escherichia coli*, *Pseudomonas putida* (Mer), and *Pseudomonas aeruginosa* strains. This activity ranges from barely detectable in *Escherichia coli* strains to a few percent of the maximum induced activity in *Pseudomonas putida* (Mer) strains up to a high of 15 to 20% of the maximum induced activity of *Pseudomonas aeruginosa* PU21. These results have been shown to be reproducible over a period of several years (11).

Range and Efficiency of Inducers

Although the mechanism of detoxification appears to be the same, the range of substrates and inducers varies from strain to strain with patterns emerging and conserved along species lines. Resistance and induction patterns among *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* spp. are summarized in Table 1 (11, 91, 117, 118).

It should be noted that some compounds that serve as inducers are not themselves substrates of the system (marked S or R and not boxed in; see Table 1). These gratuitous inducers appear to be widespread, existing for all mercury-resistant strains studied to date. Cross-induction is also characteristic of the mercury resistance system. The induction by Hg^{2+} or an organomercurial generally confers resistance to and volatilization activity towards all compounds to which that strain is resistant. Variations exist in the effectiveness of inducers and the patterns of induction and volatilization.

Induction and volatilization among the 47 Hg^r strains derived from the Hammersmith Hospital collection illustrate the cross-inducibility of the mercury resistance system. Eight different Hg^r originating in *Serratia marcescens*, transferred into the common host *Escherichia coli* J53-1, were shown to confer activity toward both Hg^{2+} and PMA, with growth on either substrate inducing the immediate and rapid volatilization of both (91). These results indicated the existence of a single operon or cross-induction of one or two systems. A second, less common induction/volatilization pattern was observed in 38 of the remaining Hg^{2+}-resistant test strains. With these strains growth in the presence of PMA induced rapid volatilization of ^{203}Hg even though PMA was not utilized as a substrate, thus acting as a gratuitous inducer. This group included plasmids from *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and several *Proteus* spp. as well as from *Escherichia coli*, *Shigella*, *Salmonella*, and *Serratia* spp. (91). Schottel et al. (91) also observed some unusual induction patterns. For example, two plasmids from *Serratia marcescens* were shown not to be resistant to PMA, but upon induction with Hg^{2+} or PMA the strain was able to volatilize PMA. In another strain resistant to Hg^{2+} only, induction by PMA strongly inhibited (<20% of the uninduced level) the ability of the cells to volatilize $^{203}Hg^{2+}$.

In a study with a large number of *Staphylococcus aureus* Hg^r strains, all were found to be inducible by 10 μM Hg^{2+}. The ability to volatilize mercury from Hg^{2+} or PMA was inducible; the inducers included Hg^{2+}, PMA, and several

organomercurials that do not serve as substrates (90, 91). *Staphylococcus aureus* RN23 harboring the broad-spectrum mercury resistance plasmid PI258 was induced most effectively by Hg^{2+}, as compared to PMA, pHMB, MMC, and merbromin, based on measuring rate of $^{203}Hg^{2+}$ volatilization. However, in other experiments merbromin was a comparable or better inducer, and although PMA is a substrate it proved to be a poor inducer. MMC was found to be neither a substrate nor an inducer of the system (117). Mutant variants of *Staphylococcus aureus* plasmids PI258 and PI147 were induced to low levels of activity (10% of the wild type) with an optimum inducer level of 2.5 μM Hg^{2+} as compared to 10 μM for the wild type. This may indicate that an uptake system is still intact while the mercuric reductase is being produced at low levels. In this case, 10 μM Hg^{2+} would now prove to be toxic. Therefore, only Hg^{2+}, thimerosal, and PMA are substrates for volatilization of ^{203}Hg, with PMA as a poor inducer and pHMB and merbromin effective as gratuitous inducers.

The same patterns of induction/volatilization do not exist in *Pseudomonas aeruginosa* and *Pseudomonas putida* mercury-resistant strains. MMC and EMC are additional substrates for the volatilization of mercury by *Pseudomonas* spp. broad-spectrum resistance plasmids (Table 1). A low-level resistance to pHMB was observed but the cells are unable to hydrolyze this compound (11). Both PMA and thimerosal were found to be poor but significant inducers of mercury volatilization activity, measured as ^{203}Hg volatilization from $^{203}Hg^{2+}$ by strains of *Pseudomonas aeruginosa* and *Pseudomonas putida* harboring narrow-spectrum resistance plasmids. The same compounds were much more effective inducers with the broad-spectrum organomercurial resistance plasmids, with volatilization rates comparable to those observed with Hg^{2+} as the inducer. Narrow-spectrum plasmid-bearing strains were found to be preferentially induced by Hg^{2+}, whereas merbromin and pHMB were effective inducers of both narrow- and broad-spectrum resistant strains of both *Pseudomonas aeruginosa* and *Pseudomonas putida* (11).

Coordinate Induction of Mercuric Reductase and Organomercurial Lyase Activity

Separate enzymes appear to be involved in the detoxification of mercury and organomercurial compounds. The organomercurial lyase enzyme hydrolyzes the organomercurial such as PMA to produce benzene and Hg^{2+}, which is followed by the reduction of Hg^{2+} to Hg^{0} by mercuric reductase. The question of coordinate induction of both mercuric reductase and organomercurial lyase activities has been addressed in studies with *Staphylococcus aureus* and several *Pseudomonas* sp. strains (11, 117). Coordinate induction of activity has been considered indicative of genes under common regulatory control, constituting an operon.

With the broad-spectrum mercury-resistant *Pseudomonas aeruginosa* strains PU21 (FP2) and PU21 (R3108), organomercurial lyase activity (as percentage of the maximum PMA volatilization activity) was plotted against mercuric reductase activity (as percentage of Hg^{2+} volatilized), using Hg^{2+}, PMA, thimerosal, pHMB, and merbromin as inducers. Both activities were found to be inducible by these compounds, but induction was not strictly coordinate. There was significant scatter from the line drawn at 45° representing coordinate induction, with all experimental points falling below the line for both strains. In particular, pHMB appeared to induce volatilization from Hg^{2+} preferentially, whereas under some conditions (not specified) thimerosal and merbromin induced PMA degradation preferentially (11). There were also differences observed between the strains tested, with all inducers tested giving higher Hg^{2+}-volatilizing activity in strain PU21, although the maximum volatilization activities of PMA were higher with this strain.

Similar experiments were conducted with *Staphylococcus aureus* wild-type, mercury-resistant, plasmid-bearing strain RN23 and an Hg^{s} mutant, RN987, exhibiting high lyase activity but low reductase activity (18% of the wild-type levels). With strain RN23, induction of lyase and reductase activities approximately coordinate at the whole-cell level. However, the scatter observed was considered to be greater than could be attributed to experimental error, indicating that some induction conditions give rise to disproportionate levels of the two activities, but not to the extent observed with *Pseudomonas* sp. strains. With the mutant strain RN987, noncoordinate induction was observed. Merbromin was shown to induce preferentially lyase activity, whereas pHMB preferentially induced reductase activity, as was also observed with strain PU21 (118). These results suggest that the organomercurial lyase and mercuric reductase enzymes may be transcribed from separate operator-promoter regions that are cross-induced under some conditions, but not always in a coordinate fashion. In general, studies indicate that the lyase activity is the limiting step in the detoxification of organomercurial compounds. This may account for what appears to be greater volatilization of mercury from Hg^{2+} than from organomercurial substrates such as PMA, as well as why some compounds appear to preferentially induce Hg^{2+} volatilization activity. This does not explain preferential induction of PMA under some conditions as cited above. More detailed studies of coordinate induction are required, including a comparison of induction/volatilization patterns among Hg^{r} plasmids from different sources in a common host strain. This line of research would determine to what extent, if any, the host cell background affects these patterns.

TRANSPOSABILITY OF *mer* GENES

The mercury resistance genes, as for other resistance genes carried by plasmids, often occur on transposons (3, 96). These transposable elements are specific DNA sequences that can insert more or less randomly into other DNA sequences in the absence of host cell-mediated recombination functions. It is generally accepted that these elements play an important role in evolution (53). Many different transposable elements have been identified and characterized, and they are believed to be widespread in nature. Plasmid-borne genes for resistance to ampicillin, chloramphenicol, kanamycin, streptomycin, and trimethoprim have all been shown to exist on transposons singly or in combination (58). These elements have demonstrated the ability to transpose from plasmid to plasmid and from plasmid to chromosome, carrying genes for antibiotic resistance, as well as a variety of functions including fusion of unrelated DNA molecules, deletions, inversions, excisions, and as functional transcriptional start and stop signals (53). Several such transposons associated with the acquisition of mercuric resistance have been described (Table 4) (3–5, 7, 8, 13, 27, 33, 96, 105, 107). The transposable element Tn*501*, associated with mercuric resistance, has been well characterized (3, 8, 96). Tn*501* has been described as a discrete piece of DNA, 5.2 megadaltons (Mdal) in size, as determined by intramolecular heteroduplex analysis of hybrid plasmid

TABLE 4. Hg^r transposons

Strain/plasmid	Mer^r transposon (markers)	Mol wt	Terminal sequences (bp)	Transpositional frequency (Hg^r)	Reference(s)
Pseudomonas aeruginosa pVS1	Tn*501* (Su, Hg)	5.2×10^6	38	2.9×10^{-3} (RR1-1) 5.9×10^{-3} (RP1) (integrates in 5 sites)	3, 8, 96
P. putida (originally isolated from pVS1)	Tn*1861* (same serotype as Tn*501*)	5.5×10^6	NC[a]	$1.5–2.0 \times 10^{-1}$	27
Pseudomonas sp.	Tn*502* (different serotype from Tn*501*); Tn*503*	$>5.2 \times 10^6$	NC	NR[b]	92
Escherichia coli K-12 derivatives R100.1 IncFII	Tn*2670* Tn*2671* Tn*21*	13.3×10^6 (20 kb[c])	38 (30/38 homologous to Tn*501*)	3.0×10^{-3} to 6.0×10^{-4}	37
	Tn*2603*	20 kb	200 (inverted repeats)		
E. coli K-12 R861	Not designated	$8.5–11.2 \times 10^6$	NC	ND[d] (insertional inactivation of TC gene)	105

[a] NC, Not characterized or sequenced.
[b] NR, Transposition frequency not reported/data not available for calculation.
[c] kb, Kilobases.
[d] ND, Not determined.

RP1-Tn*501* carrying two copies of the putative transposon and by comparison of ColE1 (4.2 Mdal) with hybrid plasmid pUB781(ColE1::Tn*501*), 9.4 Mdal (3). Tn*501* was originally discovered on the small nonconjugative *Pseudomonas* sp. plasmid pVS1 and can function in *Escherichia coli* (96). In transduction studies with derivatives of *Pseudomonas aeruginosa* PAO or PAT strains, mercury-resistant (Hg^r) transductants were recovered from both Rec^+ and Rec^- recipients at frequencies of 2.3×10^{-8} to 29×10^{-8} PFU^{-1}. This indicates that inheritance of the *mer* genes present on plasmid pVS1 is not dependent on the host recombination system (96). It has been demonstrated that Tn*501* is able to translocate into RP1, as well as to other broad-host-range conjugative plasmids. This occurs in either orientation at many sites in both *Escherichia coli* and *Pseudomonas* sp. host strains, as determined by restriction endonuclease analysis of RP1::Tn*501* recombinant plasmids, and is not dependent on host-mediated recombination mechanisms (3). Furthermore, Tn*501* insertion into a number of RP1 sites has been demonstrated to lead to the loss of RP1-borne resistance determinants of RP1::Tn*501* recombinants, using Hg^r transconjugants generated from *Pseudomonas* sp.-*Escherichia coli* matings. The loss of the Tra^+ phenotype occurred in 1 to 8% of the transconjugants, whereas 1 to 4% of the Tn*501* insertions resulted in the Cb^s or Km^s RP1 phenotype. All RP1::Tn*501* hybrids, except Cb^s/Amp^s mutants, showed a constant increase in size, indicative of integration of Tn*501* (5.2×10^6). Mutational events leading to Amp^s strains may have occurred by a deletion in Tn*A* (Amp^r gene) as the result of Tn*501* transposition, a well-documented recombinational phenomenon associated with transposons as stated above.

The recombinant plasmids pUB404 and pUB399 apparently carry two copies of Tn*501* as inverted repeats, demonstrated by intramolecular heteroduplex experiments (3). Double insertions of Tn*A* and Tn*801* have also been reported, with the two copies inverted with respect to one another (3), perhaps necessary for molecular stability avoiding intramolecular recombination. Tn*501* has been shown to exist integrated into the chromosome of *Escherichia coli* strain UB5203, appearing to be quite stable with no detectable ($<10^{-9}$) auxotrophic mutations (96).

The terminal nucleotide sequences of the mercury resistance transposon Tn*501* have recently been determined with pUB781 (ColE1::Tn*501* hybrid) which was previously described by Bennett and co-workers (3). The terminal sequences were found to be inverted repeat sequences of 38 nucleotide base pairs (bp) at either end. The transposon was also found to be flanked by a 5-bp direct repeat, presumably generated in recipient DNA upon insertion (87). The inverted repeats of Tn*501* are similar to those found in Tn*3* and others, suggesting a functional similarity in the transpositional process. They may have a common evolutionary origin, as the inverted repeated sequences are required for transposition, presumably to act as recognition sites for specific nucleases.

A *Pseudomonas putida* mercury resistance determinant has been shown to be a 5.2-Mdal transposon, designated Tn*1861* (27). Tn*1861* is described as a discrete element that can transpose from the chromosome of *Pseudomonas putida* to plasmids, then to the *Escherichia coli* chromosome, and back again to other plasmids as deduced from various transformation studies with RP4 and RSF1010 plasmids (27). These investigators failed to isolate plasmid DNA from a mercury-resistant *Pseudomonas putida* strain, even after long-term cultivation in the presence of 160 μg of Hg^{2+} ml^{-1}. The level of mercury used was much higher than the 12- to 15-μg ml^{-1} levels routinely used by others. Transfer of the Mer^r phenotype was not detectable ($<10^{-9}$ $cell^{-1}$) in these plasmidless Mer^r *Pseudomonas putida* strains, whereas additional mutations causing phenotypes such as Ade^-, Trp^-, and Thr^- were detected at a frequency of 10^{-2} to 10^{-3} $cell^{-1}$ $generation^{-1}$. Spontaneous reversion of Ade^- to Ade^+ phenotype in Ade^- mutants of *Pseudomonas putida* strain AC77 (AC1000, Met^-/Ade^-) was about 10^{-8} (27).

Transposition of Mer resistance from chromosome to broad-host-range plasmids RP4 and RSF1010, and thereby

transmission to other strains, was studied by Friello and Chakrabarty (27). Broad-host-range plasmids RP4 and RSF1010 were introduced into *Pseudomonas putida* strain AC100, subsequently isolated, and transformed into *Escherichia coli* and other *Pseudomonas putida* strains. With RP4, 15 to 20% of the transformants were both Cb^r (an RP4 marker) and Hg^r, i.e., a transposition frequency of 1.5×10^{-1} to 2.0×10^{-1}, exhibiting a 1 to 2% loss of Hg^r when grown in the absence of Hg^{2+}. In contrast, the acquisition of mercury resistance by *Escherichia coli* and *Pseudomonas putida* transformants, using RSF1010, was 2 to 5%, i.e., a transposition frequency of 2×10^{-2} to 5.0×10^{-2} and a 50 to 70% loss of Hg^r when grown in the absence of Hg^{2+} for 20 generations. This may indicate there is some site preference for Tn*1861* in RP4 or instability in RSF1010. Tn*1861* transposition and mutation induction were found to be independent of host recombination functions, as indicated by performing experiments in RecA$^-$ hosts. Analysis of RP4::Tn*1861* hybrids by restriction endonuclease digestion and electron microscopic examination showed Tn*1861* to be 5.5 (± 0.2) Mdal in 90% of the molecules examined. The remaining 10% of the hybrids showed a 11.5-Mdal increase, indicating the acquisition of two Tn*1861* molecules (24). Tn*1861* and Tn*501* are similar in several respects. Both were originally isolated from the nonconjugative plasmid pVS1 in *Pseudomonas aeruginosa*. They are similar in molecular weight (5.2×10^6 versus 5.5×10^6), and both have demonstrated the ability to transpose from one plasmid to another or to the host chromosome independent of host recombination systems (3, 27, 96). In addition, they appear to be immunologically similar. A crude enzyme preparation of the mercuric reductase from *Pseudomonas putida* AC77 was immunologically similar to a large number of mercuric reductases, including those specified by *Escherichia coli* plasmid R100, *Pseudomonas fluorescens* B69, and from Tn*501* (92). However, whereas both transposons have three *Eco*RI restriction sites and a single *Hin*dIII site, Tn*501* has a unique *Sal*GI site and both appear as multiple copies on hybrid plasmids studied. The most striking dissimilarity between the two transposons is the genetic instability of both *Escherichia coli* and *Pseudomonas putida* host strains harboring Tn*1861*, resulting in multiple auxotrophic mutants which have not been observed with Tn*501*. However, Friello and Chakrabarty (27) did not specify background auxotrophic mutation frequencies in strains not harboring Tn*1861*, nor did they investigate the possibility of the role of other transposons in such mutational events.

The nucleotide sequences at the ends of Tn*1861* have not been determined. Further work in this area is warranted to determine the extent of similarity between Tn*1861* and Tn*501* which has been sequenced (8). Tn*1861* may possess a modified transposase or resolvase gene that may account for the observed differences between it and Tn*501*, including the increased frequency of mutations.

A number of other transposons have been shown to carry genes for mercury resistance (Hg^{2+}). Some well-characterized examples are presented in Table 1, and these have been described in detail previously (13, 37, 107). In studies of deletion mutants, Tn*21* has been shown to possess genes designated *tnpA* and *tnpR* which encode a transposase and resolvase, respectively (13). These genes are necessary for transposition to occur via cointegrate formation (*tnpA*) and resolution of the cointegrates (*tnpR*). The products of the *tnpA* genes of Tn*21* and Tn*501* have been shown to compliment one another as indicated by transposition complementation assays with *tnpA* mutants (13). The terminal inverted repeat sequences of Tn*21* and Tn*501* are homologous in 30 of 38 bp, as expected, because the product of the *tnpA* gene is known to act on the ends of these transposable elements. The resolvase activity of both Tn*501* and Tn*21* is well below that of Tn*3*, making secondary rearrangements a greater possibility, such as an increased mutation frequency or incorporation of new genes.

The transposition of *mer* genes from the IncH plasmid R826 to IncN plasmid R45 in an *Escherichia coli recA* background has been investigated (105). With a *recA*$^-$ donor and a *recA*$^-$ (R$^-$) recipient, at temperatures restrictive to the transfer of R826, 50% of the transconjugants had all of the markers associated with R45 plus mercury resistance. Four of these Mer^r transconjugants were studied further. Only two (RAS305 and RAS324) were stable with regard to the mercury phenotype; the other two were considerably less stable and lost mercury resistance if Hg^{2+} was not present for selection. One of the stable derivatives, RAS324, had lost Tc^r, suggesting that insertion of *mer* genes had inactivated this gene. Inactivation of insertion was also discovered with Tn*501* and Tn*1861*. Although a *recA*$^-$-independent transposition of sequences, including the *mer* resistance genes, appears to occur, a consistent amount of DNA was not transferred in all cases. Sequences ranging from 8.5 to 11.2 Mdal in size were found in the Mer^r transconjugants, as determined by restriction digests. These acquired sequences were also found to be larger than those of several well-characterized mercury resistance transposons or operons (e.g., 6.2 Mdal in R100 and 5.2 Mdal for Tn*501*). In addition, no inverted repeat sequences were observed in heteroduplex studies of the Mer^r transconjugants, whereas there was some evidence for tandem repeats of *mer* genes in one transconjugant. These observations are problematic and argue against the existence of a unique *mer* transposon in the strains studied.

Examples of transposons in the gram-positive organism *Staphylococcus aureus* have been demonstrated, such as Tn*551*, a 5.2-kilobase transposon carrying erythromycin resistance genes (76). No mercury resistance transposon has been demonstrated in any of the broad-spectrum Mer^r strains of *Staphylococccus aureus*.

The existence of transposons encoding mercury resistance genes has a number of implications with regard to the evolution and dissemination of mercury resistance in both clinical and natural environments. Further studies involving more stringent selections for transposition of mercury resistance genes and heteroduplex analysis between R45 derivatives and the cloned segments of other *mer* transposons are warranted. In a recent study, the molecular interrelationships were examined among a family of transposons encoding Hg resistance and resistance to multiple antibiotics (108). Physical and functional mapping indicated that the multiple-antibiotic-resistant transposons Tn*21*, Tn*2603*, Tn*2607*, and Tn*4* have evolved from an ancestral mercury transposon.

A new Hg resistance transposon designated Tn*26131* was detected on the naturally occurring plasmid pCS229 and is believed to be this ancestral transposon (108). Tn*2613* was similar to Tn*501* except for a difference in the right-hand end. Tn*2613* appears to have acquired streptomycin and sulfonamide resistance genes, resulting in Tn*2608*, with the structural features of Tn*2613* conserved. This suggests a close evolutionary relationship between Tn*2613* and Tn*2608*. The other multiple-antibiotic-resistance transposons Tn*21*, Tn*2603*, Tn*2607*, and Tn*4* were shown to have sequences similar to those of Tn*2613* and Tn*2608*. It is conceivable that the transposons have evolved by the sequential acquisition

of antibiotic resistance genes and additional sequences from Tn*2613*.

THE *mer* OPERON

The nature and organization of the genes encoding mercury resistance have been an area of intense study. Physical and genetic mapping of the narrow-spectrum resistance plasmid R100 from *Escherichia coli* and several broad-spectrum plasmids of *Staphylococcus aureus*, as well as biochemical analysis of the gene products of the R100 plasmid, have generated a clearer picture of the organization and relatedness of *mer* operons in both systems. Based on restriction patterns, deletion mapping, and transpositional mutagenesis techniques, resistance to mercury has been shown to be inducible and governed by a group of at least four genes: *merA*, which encodes the mercuric reductase enzyme; *merB*, encoding the organomercurial lyase enzyme in broad-spectrum resistant strains; *merT*, the gene believed to govern the Hg^{2+} uptake function; and *merR*, which codes for the regulatory protein responsible for the inducibility of the system (14, 16, 22, 23, 45, 64, 98, 106). A model (Fig. 2) of the *mer* operon of the R100 Hg^r plasmid has been proposed by Silver and Kinscherf (92).

The *merB* gene is not presented on this map because it is not present on R100. However, on *Staphylococcus aureus* broad-spectrum Hg^r plasmids such as pI258, pI524, and pI6187, the *merB* gene has been shown to be downstream or distal to the *merA* gene (76, 92). This may also be true with the broad-spectrum Hg^r plasmids of *Escherichia coli* and *Pseudomonas* spp. which have not been mapped; thus, further study is warranted. Evidence from studies with mutants of R100 suggests that the system is under positive control (22, 98). Recently two new *mer* genes, designated as *merC* and *merD*, have been tentatively identified, using *mer*::Tn*5* insertions with the R100 *mer* genes cloned into pBR322 (74). The functions of *merC* and *merD* genes are presently unknown. The work of these investigators (74) confirmed the order of *merRTA* and indicated, based on restriction map analyses, that the region including *merC* and *merD* had the gene order *merRTCAD*. Regulation of the *mer* genes was also investigated by using *mer-lac* fusions, which suggested that *merR* may act as both inducer and repressor of the *mer* operon as previously described. The evidence for the *mer* operon model and its regulation are presented below.

Mapping the *mer* Operon

Elucidation of the genetic and physical structure of the mercury resistance genes of the IncFII plasmid R100, considered a prototype of narrow-spectrum Hg^r resistance plasmids, has been accomplished through the use of deletion mapping, restriction endonuclease mapping, transposition mutagenesis with Tn*801*, and lambda::R100 cointegrates (14–16, 22, 23, 64, 106). The drug and mercury resistance genes of R100 were found to be on the r-determinant, a region bounded by IS1a and IS1b as determined by heteroduplex DNA/DNA analysis (40). A physical map of R100 has been constructed by analyzing restriction patterns and partial digests, using several restriction endonucleases as well as lambda transducing phages derived from R100::lambda cointegrates containing mercury resistance genes. Since lambda insertions are frequently strongly polar they can be used to identify operons. In addition, deletion mutants were selected, using λ *c*I857 survivors at high temperatures (15, 16, 106). Both methods produced the same order of resistance genes on R100.

The locations of the antibiotic and *mer* resistance genes of R100 were more precisely determined in cloning experiments, in which *Eco*RI restriction endonuclease fragments were cloned into the plasmid vector RSF2124 (61, 64). An Hg^r recombinant designated pRR130 contained RSF2124 and both H and I *Eco*RI fragments of R100. The expression of mercury resistance was shown to require both *Eco*RI-I and *Eco*RI-H fragments of R100, suggesting that an *Eco*RI

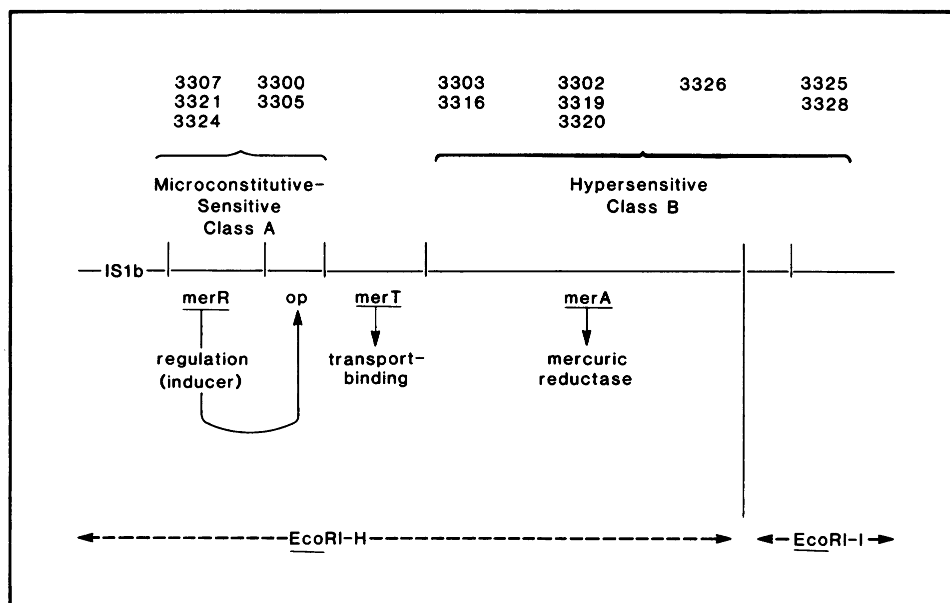

FIG. 2. Genetic map of *mer* operon of the narrow-spectrum resistance plasmid R100. Two additional genes, *merC* and *merD*, have been identified with the gene order *merRTCAD* (74), but the functions of *merC* and *merD* are presently not known.

cleavage site lies within the *mer* operon. This was determined by examining Hg^r transformants containing *Eco*RI fragment H or I. The transformants containing H or I only did not confer mercury resistance, regardless of their orientation. Additional studies were performed to confirm that both fragments were required to confer the Hg^r phenotype and to rule out the possibility that the orientation of either fragment alone could confer resistance. Plasmid DNA from pRR132 (containing PSF2124 plus fragment I) and pRR134 (containing RSF2124 plus fragment H), as well as a mixture of both, was treated with *Eco*RI, ligated, and used in transformations to select for Hg^r (64). Results indicated that only a mixture of the DNA from both plasmids yielded Hg^r transformants. Similar conclusions were reached with R100.1 and R6-5 plasmids by other investigators (10, 61).

The organization of the genes on the mercury resistance region of the *mer* operon of R100 was determined primarily by examining point and deletion mutants constructed by transpositional mutagenesis and subsequent complementation and recombinant analysis (22, 23, 71, 106). Preliminary studies indicated that Tn*801* insertions into R100 resulted in mercuric ion sensitivity at a relatively high frequency, thus leading to its use as a mutagenic probe for biochemical and genetic analyses of the *mer* operon (23). A series of 23 transposon Tn*801*-induced Hg^s mutants of pDU202 (a tetracycline-sensitive derivative of R100.0 which is a mutant of R100 depressed for conjugal transfer) were studied. Basically two classes of mutants were obtained upon testing for sensitivity to $HgCl_2$, merbromin, and FMA: (i) sensitive mutants which were shown to be as sensitive to the mercury compounds as were the plasmidless strains; and (ii) hypersensitive mutants which were four- to sevenfold more sensitive than the plasmidless strains. The hypersensitive mutants were also hypersensitive to the organomercurials merbromin and FMA, and they were shown to be inducible such that uninduced cells were as sensitive as the plasmidless strains and upon induction became hypersensitive. These mutants were assayed for mercuric reductase enzyme, using both whole cells and cell-free extracts. In 18 sensitive and hypersensitive mutants of the 23 examined, there was no detectable enzyme at the whole-cell level (at a resolution limit of <0.1% of the wild-type activity). The other five sensitive strains, designated pDU3300, pDU3305, pDU3307, pDU3321, and pDU3324, exhibited a low level of activity that was expressed constitutively. No in vitro enzyme activity was found in the sensitive or hypersensitive mutants, whereas the microconstitutive-sensitive mutants had low levels of Hg(II) reductase, consistent with their phenotype. Thus, no cryptic mutants were observed, i.e., low-activity mutants that had high cell-free levels of Hg(II) reductase enzyme (23). In studies with the recombinants of RSF2124 containing R100 *Eco*RI-H and -I fragments, it has been demonstrated that pRR130 (RSF2124 plus both *Eco*RI fragments) exhibited wild-type resistance levels, whereas strains containing pRR132 (RSF2124 plus the *Eco*RI-I fragment) were as sensitive to Hg^{2+}, merbromin, and FMA as the plasmidless strains. However, the strains containing pRR134 (RSF2124 plus *Eco*RI-H fragment) were hypersensitive to Hg^{2+} and the organomercurials with the phenotype indistinguishable from that of the hypersensitive Tn*801* mutants (71).

The organization and the nature of the genes of the R100 *mer* operon were determined by using the Tn*801* insertion mutants described above. Complementation tests revealed that the majority of the mutants fell into two groups, designated A and B, based on their ability to form mercury-resistant colonies at high frequencies with other mutants. A third class of mutants did not complement mutants in either class A or B and were shown to have extensive deletions. The mutants were divided into four major groups as follows: (i) a group of microconstitutive mutants belonging to class A which are able to complement class B mutants to full Hg^{2+} resistance; (ii) 2 microconstitutive-sensitive mutants in class B and therefore capable of complementing class A mutants to full Hg^{2+} resistance; (iii) 13 hypersensitive mutants which produced no Hg(II) reductase under any circumstances and were capable of complementing class A mutants; and (iv) a group of sensitive mutants which produced no Hg(II) reductase enzyme and were unable to complement either group A or B mutants (21). The properties of these mutants are summarized in Table 5.

Tn*801* insertions were assigned to either *Eco*RI-I or *Eco*RI-H fragments based on the mutants and pRR132 or pRR134 recombinant plasmid. The data from the complementation and recombination tests (combined with restriction digest analysis using *Eco*RI and *Bam*HI; a *Bam*HI site is located asymmetrically in Tn*801*) provided evidence needed to order the genes on the operon. The Tn*801* insertion sites and the order of the *mer* genes are presented in Fig. 2. Seven sensitive and hypersensitive mutants failed to recombine with either *Eco*RI-I or *Eco*RI-H fragments, as they carried deletions that covered both fragments. The hypersensitive

TABLE 5. pDU202::Tn*801* mutants (23)

Group	Mutant	Complementation group	Phenotype	Enzyme activity	Recombination with: pRR132	Recombination with: pRR134
1	3307 3321 3324	A	Sensitive	Microconstitutive	−	+
2	3300 3305	B	Sensitive	Microconstitutive	−	+
3	3303 3302 3312 3319 3320 3326 3325 3328	B	Hypersensitive	None	+[a]	+
4		Noncomplementing	Sensitive	None	−	−

[a] Some mutants recombined with pRR132, some with pRR134, and some with both.

deletion mutants are not believed to extend into the *merT* gene or the O/P regions, as otherwise they would exhibit a sensitive or microconstitutive phenotype. All microconstitutive mutants were found to have Tn*501* inserted into the *Eco*RI-H fragment, indicating that *merT*, *merR*, and at least part of *merA* reside on this fragment. The hypersensitive mutants were shown to have Tn*801* inserted in both *Eco*RI-I and *Eco*RI-H fragments, indicating that the mercuric reductase gene spans both fragments.

The ability of the class B hypersensitive and sensitive mutants as well as that of the cloned *Eco*RI-H fragment to complement class A mutants suggests the existence of a transacting regulatory element encoded by the *Eco*RI-H fragment that is nonfunctional in the class A mutants. The failure of the two class B sensitive-microconstitutive mutants to be complemented by the *Eco*RI-H cloned fragment or other class B hypersensitive mutants suggests the existence of a *cis*-acting regulatory site (23). That the mercuric reductase function can be altered without altering the *merT* transport function has led to the hypothesis that the order of genes on the *mer* operon is O/P-*merT*-*merA* and that transcription proceeds from the *Eco*RI-H to the *Eco*RI-I fragment. This proposed order is corroborated by the studies conducted on the products of the *mer* operon (15, 45).

To overcome the incompatibility problem associated with using mutants of the same Inc group, Tn*1*-generated mutants of Inc plasmid R702 originally isolated from *Proteus mirabilis* was used in crosses with the Tn*801*::pDU202 mutants discussed above, with crosses being done in both directions to detect any host effects. The Tn*1* insertion mutants of R702 were found to be hypersensitive, have no detectable Hg(II) reductase, and exhibit inducible uptake of Hg^{2+}, indicating that the insert is in the *merA* gene (98). This was confirmed by complementation and recombination tests with the known point and deletion mutants of pDU202 described above. The R702 Tn*1* insertion mutants were unable to complement *merA* pDU202 mutants, but they were able to complement class A mutants. These results suggest that the R702 Hg^s mutants have a functional *merR* gene that can act on the O/P region of the pDU202 IncFII plasmid. Host cell background had no apparent effect.

In a recent study, a 260-bp fragment of the *Eco*RI-H fragment of R100, believed to be the promoter of the *mer* operon, was cloned into the promoter-cloning vehicle pBRH4 (7). This vehicle expresses tetracycline resistance only when the deleted *tet* promoter is replaced by a sequence that can serve as a promoter. Recombinant plasmid pFB4 containing the 260 bp of R100 was shown to direct expression of tetracycline resistance only when there is a second plasmid in the strain that carries the wild-type *merR* gene, encoding the regulatory protein, and tetracycline resistance was directly proportional to the concentration of Hg^{2+} present. When microconstitutive *merR* mutants were used instead of the wild-type Hg^r plasmid, low levels of tetracycline were observed (7). These observations show that this 260-bp cloned fragment responds to *merR* and *merA* mutants in the same manner as the *mer* operon does. Further studies are warranted in the area of promoter identification.

Hypersensitivity and Hyperbinding Activity

Hypersensitivity to mercurials is believed to be the result of a functional mercury uptake system, encoded by the *merT* gene located on the *Eco*RI-H fragment in the absence of a functional mercuric reductase coded by both *Eco*RI-H and -I fragments (22, 23). The uptake of binding of $^{203}Hg^{2+}$ by induced and uninduced hypersensitive and sensitive Tn*801* insertion mutants and strains containing the cloned *Eco*RI-H and -I fragments was examined to test this model (23, 71).

Binding activity was measured in Hg^{2+}-induced and uninduced cells, which were grown in the presence of $^{203}Hg^{2+}$ at levels that distinguished between sensitive and hypersensitive strains. Samples were periodically removed, filtered on membrane filters, washed, and counted by liquid scintillation spectroscopy. By this method, the Tn*801*-generated hypersensitive mutants were shown to bind three to five times more $^{203}Hg^{2+}$ when induced compared with induced sensitive mutants or sensitive plasmidless strains. In contrast, the microconstitutive mutants bound no more $^{203}Hg^{2+}$ when induced than when they were not, the same behavior exhibited by wild-type Hg^r strains (23). This indicates that the hyperbinding activity expressed by the hypersensitive mutants is inducible in nature, and thus it is governed by an inducible transport system. In the case of the wild-type and microconstitutive strains it is presumed that the transport system and mercuric reductase are intact so that mercury is reduced and volatilized as fast as it is taken up. Induction of hyperbinding activity in hypersensitive cells carrying pRR134 was found to be approximately coordinate with the induction of mercury volatilization activity of the wild-type resistant cells carrying pRR130 (71).

Similar experiments were conducted with $^{203}Hg^{2+}$-induced and uninduced strains carrying pRR130, pRR132, and pRR134 recombinant plasmids. At low levels (2 μM) of Hg^{2+}, the induced hypersensitive strains (pRR134) exhibited greater binding of mercury than the induced sensitive strain containing pRR132, with the total $^{203}Hg^{2+}$ content constant. The resistant strain harboring pRR130 was able to volatilize added Hg^{2+} and it bound less mercury than the sensitive strain (71). However, when the Hg^{2+} concentration was increased to 10 to 20 μM, a level that caused rapid inhibition of metabolism in both sensitive and hypersensitive strains, the sensitive strains bound increasingly more $^{203}Hg^{2+}$, with both strains showing saturation at approximately the same level. The hypersensitive cells bound the $^{203}Hg^{2+}$ rapidly, reaching a maximum of 15 μmol g of cells^{-1} within 2 min, after which the binding leveled off, whereas the sensitive cells reached a maximum of 5 μmol g of cells^{-1} only after 60 min. This suggests a very specific binding mechanism.

Factors affecting Hg^{2+} binding have also been investigated. Hypersensitive strains such as pRR134 confer hypersensitivity to merbromin and FMA. Although it has been demonstrated that neither one binds to the cells to any appreciable extent, they inhibit binding of Hg^{2+} by induced hypersensitive cells at 700 and 25 μM, respectively (71). In addition, thiol reagents such as cysteine and β-mercaptoethanol (100 μM each) have been shown to have essentially no effect on Hg^{2+} binding, whereas dithiothreitol has a somewhat inhibitory effect. The reversibility and energy dependence of Hg^{2+} binding by sensitive and hypersensitive cells have also been tested. Binding of $^{203}Hg^{2+}$ by both sensitive (pRR132) and hypersensitive (pRR134) strains was inhibited by incubation at 40°C, and whereas carbonyl cyanide *m*-chlorophenylhydrazone (100 to 200 μM) inhibited initial binding by induced hypersensitive cells, NaCN (1 mM) had no effect (71). Cells treated with agents such as toluene or sodium deoxycholate that increase permeability were found to bind more $^{203}Hg^{2+}$.

The bound $^{203}Hg^{2+}$ was found to be precipitated by cold trichloroacetic acid, but under conditions that dissolve cellular protein the bound $^{203}Hg^{2+}$ was solubilized (71). The distribution of the bound $^{203}Hg^{2+}$ was determined by analyzing French press-disrupted cells subjected to high-speed

centrifugation. The distribution was found to be the same for both sensitive and hypersensitive cells, with most of the $^{203}Hg^{2+}$ (140,000 × *g*) in the pellet containing both membrane material and ribosomes.

Pseudomonas fluorescens B69 contains a 34-Mdal plasmid and is resistant to mercury. Curing of this plasmid resulted in a hypersensitive phenotype, B69A, from which a sensitive derivative, B69AR1, could be isolated by using Hg^{2+} as the selective agent (1). Neither phenotype B69A nor B69AR1 was able to volatilize Hg^{2+}. Transconjugants of the hypersensitive and sensitive strains carrying the 34-Mdal plasmid were able to volatilize Hg^{2+}, indicating that the 34-Mdal plasmid encoded the Hg(II) reductase in these strains. However, the transconjugant of the sensitive strain exhibited only 25% of the activity that was detected in B69 and in the transconjugant of the hypersensitive B69A. The sensitive strain and its transconjugant contained more mercury in the cell material than did the transconjugant of the hypersensitive strain. Such differences may reflect alterations in cell wall permeability and the mercury transport system and are yet to be explained in further work.

Regulation of the *mer* Operon

The *mer* operon described above is believed to be under positive regulation. This hypothesis rests on several lines of evidence such as: (i) isolation of temperature-sensitive induction mutants; and (ii) the isolation of microconstitutive sensitive mutants (71, 92). The system may be under simple positive regulation such that *merR* of the microconstitutive mutants is altered in such a way that it acts as an activator (albeit a poor one) regardless of the presence of Hg^{2+}. Under simple positive regulation the presence of the wild-type *merR* gene would not affect the phenotype. However, if the system is under positive/negative regulation such as that seen in the arabinose operon, the microconstitutive mutants would have to be modified in both the repressor and activator functions. In this instance, the presence of the wild-type *merR* would repress the system in the absence of Hg^{2+}. This hypothesis was examined by forming stable heteroplasmid strains, using R702 *merA* mutants and pDU202 microconstitutive *merR* mutants, and assaying the effect of wild-type *merR* (98). Strains carrying both the wild type and the microconstitutive-sensitive mutant had the same uninduced Hg-volatilizing activity as that of a strain harboring the microconstitutive mutant plasmid alone. Therefore, the microconstitutive mutant plasmid appears not to be switched off in the presence of wild-type *merA* allele, suggesting that the operon is under simple positive regulation.

Recent studies have shown that the *mer* operon is subject to a 2.5-fold decrease in expression when glucose is added with the inducer (99). Two types of experiments were conducted: (i) short-term or "transient repression" experiments using a strain with wild-type *crp* and *cya* alleles; and (ii) experiments using *cya* and *crp* mutants. During transient repression experiments, the addition of glucose and $HgCl_2$ resulted in a 2.0- to 2.7-fold repression in Hg(II) reductase activity depending on growth stage at the time of induction. The addition of cyclic AMP at the same time as the glucose caused a significant increase in activity. Permanent catabolite repression of the operon was observed in mutants lacking either the *crp* or *cya* protein, resulting in a 1.6- to 1.9-fold decrease in expression. The *cya* mutation could be overcome by the addition of cyclic AMP, but had no effect on the *crp* mutant (99). Similar effects were observed with cell-free extracts of Hg(II) reductase from wild type, *crp*, and *cya* mutants, indicating that the catabolite repression effect seen in intact cells is not simply due to an alteration in Hg^{2+} uptake.

Under conditions of transient catabolite repression, glucose did not appear to have a negative effect; indeed, it had a positive one on the induction of Hg^{2+} uptake in a hypersensitive mutant strain. Whereas cyclic AMP had a slight negative effect, the combination of cyclic AMP and glucose was the same as that of glucose alone. These results were confirmed in *crp* and *cya* mutants and are essentially the opposite of those demonstrated for the induction of the Hg(II) reductase activity. Since Hg(II) reductase is a flavin adenine dinucleotide (FAD)-containing enzyme and appeared to be under catabolite repression while the Hg^{2+} uptake system was not, it seems possible that a decrease in cellular FAD levels could account for the observed results. However, flavin levels (per milligram of cell protein) remained constant under both conditions.

Gene Copy Number Effects

The gene copy number effects in the *mer* operon of plasmid R100(NR1) have recently been examined. Three R100 variants were analyzed in an *Escherichia coli* K-12 strain: (i) wild-type R100; (ii) pRR12, a round of replication mutant containing three to four times as many plasmid copies as wild-type R100; and (iii) pRR130, a high-copy-number plasmid containing five to eight times as many copies per cell as the wild-type R100. *Proteus mirabilis* strain ϕS38 harboring R100 wild-type plasmid and *Proteus mirabilis* ϕS38(R11) "transitioned" to a high r-determinant gene copy number of exposure to chloramphenicol were also examined. Resistance levels to Hg^{2+} and the Hg^{2+} volatilization rates from intact cells and cell-free extracts were determined.

There were no detectable differences in the levels of resistance to Hg^{2+} among the *Escherichia coli* copy number variants. The *Proteus mirabilis* ϕS38 cells were more sensitive than the *Escherichia coli* cells, but when transitioned, resistance levels increased to those observed for *Escherichia coli*. The volatilization rates of mercury from Hg^{2+} by washed intact cells were found to parallel the levels of Hg^{2+} resistance by all strains; however, high levels of Hg^{2+} resulted in substrate inhibition. Substrate inhibition was not observed for the cell-free extracts of these strains up to 50 μM Hg^{2+} (70).

Cell-free preparations demonstrated typical Michaelis-Menten kinetics with a K_m value of 8 to 10 μM Hg^{2+} and V_{max} values that increased with increasing copy number. Extracts from both low- and high-copy-number strains of *Proteus mirabilis* had lower activity compared with intact cells of this bacterium or extracts of *Escherichia coli*. In contrast, cell-free extracts of *Escherichia coli* copy number variants exhibited the effect of increasing copy number, whereas the intact cell did not.

The results suggest that the *Proteus mirabilis* cell uptake function is to concentrate the Hg^{2+}, thereby resulting in higher whole-cell volatilization rates; i.e., the uptake protein/mercuric reductase ratio is low. In the *Escherichia coli* R100 wild-type strain the whole-cell and cell-free volatilization rates were essentially equivalent, whereas the rates of pRR12 and pRR130 variants exhibited higher cell-free volatilization rates. These results suggest that the increased enzyme levels in the latter cells are effectively cryptic and the uptake is the limiting step.

This represents additional support for the existence of a transport protein and demonstrates the existence of host cell

effects on the uptake and volatilization functions expressed by the *mer* operon.

Products of the *mer* Operon

Early efforts to identify the gene products of the *mer* operon were made by examining polypeptides synthesized after the infection of UV-irradiated cells with specialized lambda transducing phages carrying *mer* DNA from IncFII plasmid R100 (12, 14). More recently, a different approach has been taken by analyzing $HgCl_2$-induced polypeptides synthesized by *Escherichia coli* minicells harboring either natural or recombinant Hg^r plasmids (45, 46).

Phage-directed protein synthesis in UV-irradiated lambda *ind* lysogens showed Hg^{2+}-induced synthesis of at least six proteins (14). Three of the Hg^{2+}-induced polypeptides (67.6, 11.7, and 8.0 kilodaltons [kdal]) were heavily labeled, whereas the other three were only moderately labeled (52.4, 32.6, and 13.5 kdal). The region encoding the *mer* genes is 5.6 kilobases, which could direct the synthesis of proteins of a total molecular weight of 205,300 (assuming a weight of 110 for an average amino acid residue). The three heavily and moderately labeled Hg^{2+}-inducible polypeptides total 186,000. However, there were consistently four and occasionally six additional faintly labeled Hg^{2+}-induced polypeptides, bringing the total to 330,000. In this case the total size of the proteins exceeds the coding capacity by a factor of 1.5. It is possible that there are overlapping genes or that the lightly labeled proteins are being transcribed from the irradiated chromosomes or phage DNA. The lightly labeled polypeptides are seen in $HgCl_2$-induced bacteriophage that did not contain *mer* DNA, which would support the latter explanation (14).

In the *Escherichia coli* minicell system $HgCl_2$-inducible polypeptides from a number of different Hg^r plasmids were examined by labeling with [^{35}S]methionine and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) techniques (45). The plasmids examined and the Hg^{2+}-induced polypeptides each one expressed are listed in Table 6. The Hg^{2+}-induced polypeptides fall into five classes based on SDS-PAGE analysis. All plasmids examined exhibited a sharp, intensely labeled band in the 69- to 71-kdal range, whereas all but R831 (IncH) and R702 (IncP) also encoded a peptide in the 64- to 66-kdal range. Thus, it would appear that the Hg(II) reductases encoded by Tn*501*, R100, and R538-1 are more closely related.

The remaining Hg^{2+}-inducible peptides present in all strains included two polypeptides in the 14- to 17-kdal range, which varied in size and intensity among the strains, and a 12-kdal polypeptide. In cells labeled with ^{14}C-amino acids and [^{35}S]cysteine, it has been shown that there were no additional Hg^{2+}-induced polypeptides and that the 12-kdal peptide was more heavily labeled in the presence of ^{14}C-labeled amino acids and showed no incorporation of [^{35}S]cysteine (45, 92). The strain carrying the broad-spectrum plasmid R831 produced two additional polypeptides with molecular weights of 22,000 and 24,000. The latter are similar to the subunit molecular weights of 17,000 and 22,000 reported for the partially purified organomercurial lyase enzyme produced by this strain (90). The sum of the molecular mass of the Hg^{2+}-inducible polypeptides observed in this system, using pRR130 (which is to date the most stringent example of the *mer* operon), is 110 kdal, not including the 66-kdal protein.

The 69- to 71-kdal polypeptide is believed to be the monomer of the Hg(II) reductase enzyme. This assignment is based on several lines of evidence: (i) it is present as a strongly labeled inducible band in all strains tested; (ii) the molecular weight corresponds to those observed for the purified Hg(II) reductase from several strains including R831; and (iii) this protein strongly reacts with antibody to purified Tn*501* Hg(II) reductase. The 66-kdal protein also reacts with the purified antiserum to Tn*501* Hg(II) reductase, which in fact was itself only 66 kdal. These findings supported the idea that the 66-kdal polypeptide is actually a proteolytically modified form of the 69-kdal polypeptide (47).

Pulse-labeling of the Hg^{2+}-induced minicells carrying pRR130 with [^{35}S]methionine followed by the addition of chloramphenicol and an excess of nonradioactive methionine during a chase period provided evidence to support this hypothesis. During the pulse period the 69-kdal peptide was heavily labeled, whereas the 66-kdal peptide was not observed until 5 min after the addition of the chloramphenicol. The evidence shows that the 66-kdal protein is produced under conditions that inhibit protein synthesis and its pro-

TABLE 6. Hg^{2+}-induced polypeptides expressed in *Escherichia coli* minicells (45)

Plasmid	Resistance spectrum	$HgCl_2$-induced polypeptide mol wt ($\times 10^3$)					Antigenic relationship [reaction with Ab to Tn*502* Hg(II) reductase]
		(1) 69–71	(2) 64–66	(3) 25	(4) 14–17	(5) 12	
R538-1 (IncFII)	Narrow	69	64–66	None	15.1 14	12	ND[a]
NRI(R100) (IncFII)	Narrow	69	64–66	None	15.1 14	12	ND
R702 (IncP)	Narrow	69	None	None	15.1 14	12	ND
ColE1:Tn*501* (from IncFII)	Narrow	69	None	None	16.5 14	12	ND
pRR130 (*Eco*RI-H+I from R100)	Narrow	69	None	None	15.1 14	12	Both 66- and 69-kdal peptides inactivated by antisera
pRR132 (*Eco*RI-I from R100)	Narrow	None	None	None	15	None	ND
pRR134 (*Eco*RI-H from R100)	Narrow	40, amino terminus	None	None	15.1 14	12	40-kdal peptide reacts strongly
R831 (IncH)	Broad	71	None	22 24	15.1 14	12	ND

[a] ND, Not determined.

duction is accompanied by a concomitant decrease in the 69-kdal peptide, whereas the sum of the 69- and 66-kdal proteins is produced from the 69-kdal protein by in vivo modification and simply due to nonspecific proteolysis (42). Degraded forms of the Hg(II) reductase of Tn*501* and plasmid R831 have been described (24, 90). Similar studies have indicated that the 12-kdal peptide is a modified form of a 13-kdal peptide formed during the pulse period.

The polypeptides encoded by the *Eco*RI-I and *Eco*RI-H cloned fragments were examined (Table 6). Plasmid pRR134 carrying the *Eco*RI-H fragment was shown to encode the three smallest peptides (15.1, 14, and 12 kdal) as well as a 40-kdal protein believed to be the amino terminus of the Hg(II) reductase because it reacted strongly with the antiserum produced against purified Hg(II) reductase of Tn*501*. In contrast, pRR132 produced a single *mer*-specific protein of 15 kdal which did not react with the Tn*501* Hg(II) reductase antiserum. An 81-kdal fusion protein was observed which was produced constitutively and did not react with the antiserum but is believed to contain the 29-kdal carboxy terminus of Hg(II) reductase (45). The lack of antigenic cross-reactivity of the three smallest peptides with the antiserum to purified Hg(II) reductase and their association with the *Eco*RI-H fragment indicated that they may have some uptake or regulatory role.

The sum of the molecular weights of the Hg^{2+}-induced polypeptides in the minicell system is 110×10^3, excluding the 66×10^3-molecular-weight peptide, thus requiring a minimum of 3,000 bp of DNA (45). The *Eco*RI-H fragment from the IS*1b* element to the *Eco*RI-I junction is 2,700 bp (23). If the 40-kdal amino terminus of the Hg(II) reductase is encoded by the *Eco*RI-I fragment, this leaves 1,610 bp to encode the remaining 41.1 kdal of the polypeptides observed in the minicell system. This leaves 490 bp unaccounted for, which could possibly encode a polypeptide that has not yet been detected, such as a regulatory protein that may be present in very small quantities.

The minicell Hg^{2+}-induced polypeptides correspond to some extent to those observed with the lambda-*mer* system. The heavily labeled 68-kdal protein of the lambda-*mer* system is believed to correspond to the 69- to 71-kdal proteins observed in the minicell system, whereas either of the smaller lambda-*mer* peptides (11.5 or 8.5 kdal) may correspond to the 12-kdal polypeptide from the minicell system. Only the lightly labeled 13.5-kdal peptide of the lambda-*mer* system corresponds to either the 15.1- or the 14-kdal peptide observed in the *Escherichia coli* minicell system (12, 42). Alternatively, it may be that the 8.5-kdal peptide corresponds to the 12-kdal minicell protein, whereas the 11.5- and 13-kdal proteins represent the 14- and 15.1-kdal minicell proteins, owing to poor resolution in the lower-molecular-weight range.

In studies of labeling Hg^{2+}-induced minicells in the presence of ethanol, which has been shown to inhibit the processing of outer membrane and periplasmic proteins in *Escherichia coli*, it was found that only the 66- and 12-kdal Mer proteins disappeared, whereas the 69-, 15.1-, and 14-kdal Mer polypeptides were not affected (46). The cellular location of the Hg^{2+}-induced polypeptides in *Escherichia coli* minicells was determined by SDS-PAGE analysis of soluble and cell membrane fractions as prepared by French press disruption of the cells and sucrose density centrifugation techniques (44). The membrane fraction was found to contain both the 15.1- and 14-kdal Mer proteins, whereas the 69-, 66-, and 12-kdal proteins were in the soluble fraction. During sucrose density centrifugation the 15.1- and 14-kdal proteins were found in the light and intermediate density fractions, along with the 36-kdal *tet*-induced inner membrane protein, indicating that they are located in the inner membrane. These findings were confirmed by the solubilization of both the 15.1- and 14-kdal proteins by Sarcosyl, which specifically solubilizes the inner membranes of *Escherichia coli*. Whereas the 66-kdal protein is predominantly in the soluble fraction, a small but consistent amount of the protein was found to be associated with the inner membrane fraction of the sucrose density gradients. As neither the 69- nor 12-kdal protein was associated with this fraction, the 66-kdal protein is not believed to be due to contamination of the envelope fraction by soluble proteins (46).

Thus, it appears that the 15.1- and 14-kdal peptides are components of the inner membranes, but unlike many membrane and periplasmic enzymes they do not appear to be derived from larger peptides.The 12-kdal protein, associated predominantly with the soluble fraction, appears to be processed from a 13-kdal *mer*-specific peptide which is inhibited in the presence of ethanol (46). These results are consistent with the hypothesis that the 12-kdal protein is located in the periplasmic space rather than in the cytosol. A model based on the data presented above is shown in Fig. 3. It may be that the 15.1- and 14-kdal proteins participate in Hg^{2+} uptake, whereas the 12-kdal proteins may act as a Hg^{2+} binding protein, bringing the Hg^{2+} in contact with the Hg(II) reductase enzyme. It is likely then that all of the 15.1- and the 14-kdal proteins are products of the *merT* gene, as may also be the case for the 12-kdal protein. Although a membrane-bound Hg(II) reductase has never been clearly demonstrated, the loose association of the 66-kdal degradation products with the membrane fraction may serve a purpose in allowing closer contact with the Hg^{2+} as it is brought into the cell. Such a closely coordinated system would have obvious advantages in terms of efficiency and reduced toxicity to the cell. The regulatory protein which would be expected to be present at very low levels may not yet have been detected and warrants further investigation.

Analysis of the *mer* Operon in *Staphylococcus aureus*

All mercury-resistant strains of *Staphylococcus aureus* have been shown to express broad-spectrum resistance (117). The *Staphylococcus aureus* Hg^r plasmids pI258, pII147, and pI524 have been studied most thoroughly, and all are believed to have the genes for resistance to both Hg^{2+} and PMA in close proximity (66, 76). The mapping of these plasmids was accomplished by using a combination of deletion mapping, restriction analysis, heteroduplex DNA/DNA mapping, cloning of restriction fragments, and transposon insertion techniques in the same manner as for *Escherichia coli* plasmid R100.

Restriction digests of pI524 with *Eco*RI generated six fragments and revealed that both fragments C and E are required to confer resistance to both Hg^{2+} and PMA. The *Eco*RI cleavage appears to be in the *merB* gene encoding the organomercurial lyase enzyme such that resistance to PMA and volatilization of mercury from PMA required both fragments cloned together (61). Transpositional mutagenesis with plasmid pI258 revealed that the regulatory genes and the Hg(II) reductase gene are encoded by the proximal fragment, whereas the *merB* gene is either cleaved by *Eco*RI or exists totally on the distal fragment. The Tn inserts of pI258 inactivated either Hg^{2+} and PMA resistance both or PMA resistance alone (76). In another study, Hg^s mutants of *Staphylococcus aureus* were able to lose either reductase or lyase alone, indicating separate genetic loci for the *merA* and

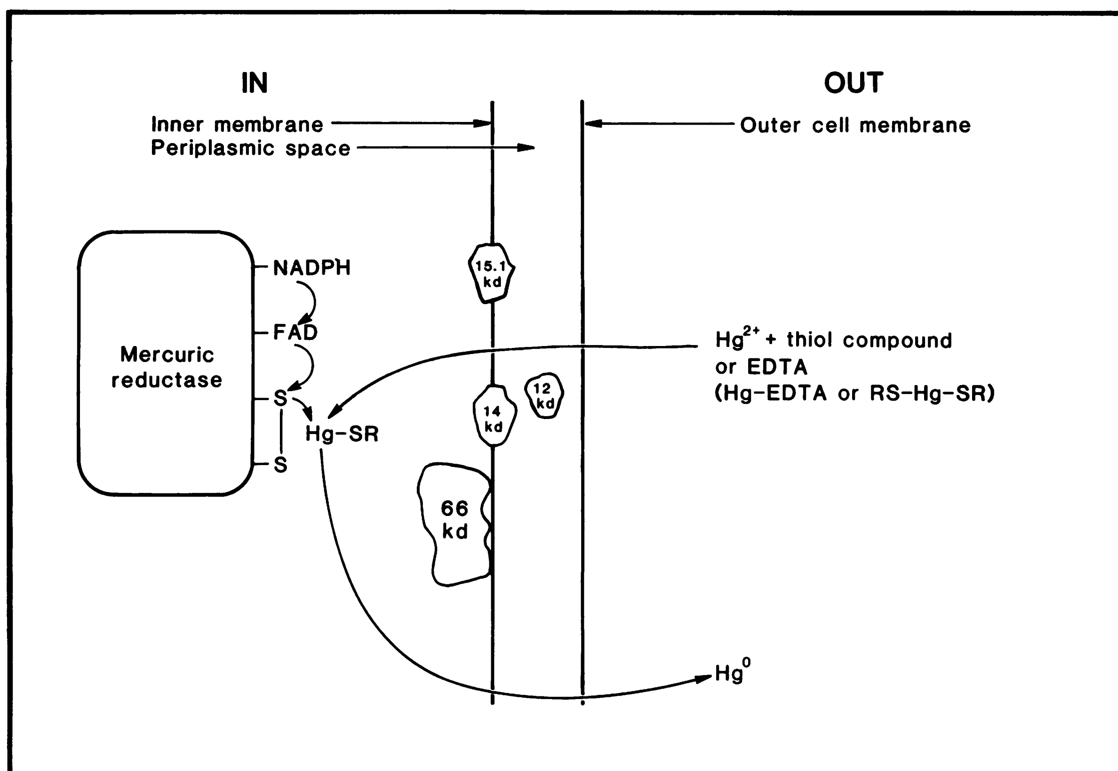

FIG. 3. *mer* polypeptides ($HgCl_2$-inducible polypeptides) encoded by the *mer* operon on plasmid R100. kd, Kilodaltons.

merB genes (92, 117). The demonstration of coordinate induction of both activities in a resistant strain, and noncoordinate induction in a mutant, suggests that the two genes may be transcribed from different promoters but are regulated in the same manner (117). Other mutants have been isolated that abolish the inducibility of the system, suggesting that *merT* or *merR* is abolished. More detailed studies are required to determine whether the *merA* and *merB* genes are indeed being transcribed from different promoters and whether they are under separate regulation.

Although the mercuric reductase enzymes of *Staphylococcus aureus* and *Escherichia coli* J53-1(R831) appear to be similar, chimeric plasmids of pSC101 and the *Staphylococcus aureus* Hg^r plasmid pI258 do not result in expression of the *mer* genes in *Escherichia coli*. These observations indicate that host cell effects may be present.

ENZYMOLOGY OF THE MERCURY AND ORGANOMERCURIAL DETOXIFICATION SYSTEMS

Mercuric Reductase Enzyme

The mercuric reductase enzyme is a soluble flavoprotein located in the cytoplasm which catalyzes the NADPH-dependent reduction of mercuric ions to metallic mercury, and it is believed to play a key role in the mercury detoxification system of many bacteria (92, 97, 104). It has been shown to require an excess of exogenous thiols for activity and to be mechanistically similar to enzymes in the class of FAD-containing disulfide reductases such as glutathione reductase and lipoamide dehydrogenase (25, 87). The overall reaction catalyzed by the Hg(II) reductase enzyme is as follows (24): $NADPH + RS\text{-}Hg\text{-}SR + H^+ \rightarrow NADP^+ + Hg^0 + 2RSH$. The enzyme has been purified from both narrow- and broad-spectrum Hg^r strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* spp., and *T. ferrooxidans* (7a, 27, 29, 30, 87, 90, 109, 110, 117).

Location of Mercuric Reductase Enzyme

The cytoplasmic location of the Hg(II) reductase has been demonstrated with both whole cells and spheroplasts. In all cases tested, cell-free extracts of the enzyme can be detected in the high-speed supernatant fraction, suggesting that it is located in the cytoplasmic fraction (29, 44, 45, 56, 90, 104). It is considered to be truly cytoplasmic and not periplasmic as determined by using the periplasmic enzyme alkaline phosphatase as a marker (45, 104).

Although there is no evidence that a membrane-bound Hg(II) reductase exists, the 66-kdal polypeptide expressed in *Escherichia coli* is associated with the inner membrane of the minicells. A cytoplasmic location may be advantageous for an enzyme that requires cofactors such as NADPH, whereas association with the membrane has the advantage of placing the enzyme in closer contact with the toxic substrate. It may be that two forms of the enzyme have a biological role in increasing the efficiency of the system.

Properties of Mercuric Reductase Enzyme

The variations in molecular weight among the purified Hg(II) reductase enzymes appear to be the most significant difference. The molecular weights determined by using the *Escherichia coli* minicell system are presented in Table 6. The subunit and native enzyme molecular weights for various purified enzymes are presented in Table 7.

The Hg(II) reductase expressed by the broad-spectrum Hg^r plasmid R831 in *Escherichia coli* J53-1 has been well characterized. This enzyme has a native molecular weight of

TABLE 7. Comparison of Hg(II) reductase enzymes isolated from various bacterial genera

Strain(plasmid)	Mol wt of Hg(II) reductase		pH optimum	Bound FAD as coenzyme	Hg^{2+}-dependent volatilization		Reference(s)
	Native enzyme	Subunit			K_m	V_{max} (min^{-1} mg^{-1})	
Escherichia coli K-12 J531(R831)	180,000[a,b] 175,000	63,000 ± 2,000 (mean) (trimer)	7.5 in phosphate buffer	3.12/mol of enzyme (1/subunit)[c]	13 μM $HgCl_2$[d,e,f] 16 μM $HgCl_2$	1,050 nmol	90
E. coli W2252 (R factor)	ND[g]	ND	7.7 in Tris-hydrochloride	Component of purified enzyme; no ratio determined	20 μM $HgCl_2$[e]	ND	44
E. coli KP245 (pRR130) Cloned *Eco*RI-H and -I fragments	110,000[b]	56,000 (dimer)	ND	FAD bound to enzyme; no ratio determined	0.46 μM Hg(II)-EDTA[f,h] 70 μM RS-Hg-SR 14 μM Hg(II) + EDTA + SH	1,038 nmol [Hg(II)-EDTA] 1,044 nmol	87
Pseudomonas aeruginosa PAO 9501 (pVS1/Tn*502*)	123,000 ± 5,000[b]	56,000 62,000 (dimer)	ND	1 FAD/subunit	12 μM $HgCl_2$[d,e,h]	12 μmol	24
Pseudomonas sp. K62 (no plasmid isolated)	67,000[b]	ND	ND	FAD bound to enzyme; no ratio determined	ND	14 μmol	29, 30
Staphylococcus aureus	170,000[b]	ND	ND	ND	10 μM $HgCl_2$[f]	16 μmol	117
Thiobacillus ferrooxidans BA-4 (no plasmid identified)	ND	ND	8.1	ND	15 μM $HgCl_2$[f]	ND 10% of level in *E. coli*	80
T. ferrooxidans TFI-29	130,000	62,000 54,000	7.5	ND	8.9 μM $HgCl_2$[f]	ND	7a

[a] Determined by disc gel electrophoresis.
[b] Determined by gel filtration.
[c] Determined by thin-layer chromatography.
[d] Measured as Hg^0 volatilization.
[e] Measured as NADPH oxidation.
[f] Demonstrates Michaelis-Menten kinetics.
[g] ND, Not determined.
[h] Biphasic kinetics observed.

180,000 as determined by disc gel electrophoresis. However, when subjected to PAGE, two major protein bands were resolved. Both bands were shown to have activity. A second enzyme preparation which was reported to require a much longer preparation time and a lower yield did not exhibit two bands under these conditions (90). When the enzyme preparation was resolved by the SDS-PAGE method, two major bands accounted for 80% of the protein on the gel and migrated corresponding to 64 and 55 kdal. In addition, when the two bands seen on the polyacrylamide gel were run separately on SDS-polyacrylamide gels, only one protein band was seen on each gel, with relative migrations corresponding to 64 and 56 kdal. The low-yield enzyme preparation had a single band of 56 kdal when run under these conditions. These values are in close agreement with those observed when the purified Hg(II) reductase preparation was analyzed directly by SDS-PAGE methods. A molecular weight of 71,000 was reported for the Hg(II) reductase monomer of R831 in the *Escherichia coli* minicell system (45).

Schottel (90) attributes the presence of two bands to modification of the enzyme, perhaps a degradation product formed during its isolation and purification. Although modified forms of the Hg(II) reductase monomers were observed in *Escherichia coli* minicells, none were reported for R831. The discrepancy noted between these two systems may be due to some type of modification unique to the system or simply due to differences in the purification method. Based on the native enzyme molecular weight of 180,000 and a mean subunit molecular weight of 63,000, Schottel (90) believes the active enzyme is composed of three subunits of identical molecular weight. Further support of a trimeric enzyme involves the finding that 3 mol of bound FAD are present mol of enzyme^{-1} as determined by thin-layer chro-

matography, using the supernatant of boiled purified enzyme preparation (86). This represents the only trimeric Hg(II) reductase. The Hg(II) reductase from a *Staphylococcus aureus* strain containing a penicillinase plasmid has a reported molecular weight of 180,000, like R831, but a subunit configuration is not reported (117).

The purified Hg(II) reductase from *Escherichia coli* KP245 containing the cloned mercury resistance genes from plasmid R100(NR1) is a dimer with a molecular weight of 110,000 ± 10,000 and subunits of 56 kdal as determined by gel filtration on a Sephadex-200 column and SDS-gel electrophoresis (81). This is in contrast to the 69-kdal monomer and a 54-kdal peptide seen occasionally in the *Escherichia coli* minicell system (42). It is also of interest to note that, again, a modified 66-kdal protein was present in the minicell preparations, whereas J. W. Williams (personal communication) has only detected the 56-kdal monomer. This contradiction was recently resolved (Williams, personal communication) by the demonstration that the 66-kdal protein in the minicell system was the same as the 56-kdal protein when tested by the same method (45).

Recently, the Hg(II) reductase from Tn*501* has been shown to be a dimer with a molecular weight of 123,000 ± 5,000, with subunits of 56,000 and 62,000 as determined by gel filtration on a Sephadex G-150 column (24). The two bands of 56 and 62 kdal seen on SDS-polyacrylamide gels comprised 95% of the protein on the gel. At the time of isolation, 80 to 85% of the protein was in the higher-molecular-weight form, whereas upon storage up to 90% was found in the lower-molecular-weight band. There was no significant change in the activity of the preparation despite the shift in molecular weight. Although an in vivo modified form of the Tn*501* Hg(II) reductase was detected in the *Escherichia coli* minicells, it is unclear whether this is the case here.

The 67,000 molecular weight observed for *Pseudomonas* sp. K62 Hg(II) reductase is much lower than those discussed above. It may be that this represents the monomer molecular weight, in which case it agrees closely with the other enzymes listed in Table 7. For the *T. ferrooxidans* Hg(II) reductase a molecular weight of 130,000 has been determined (7a); this protein has been separated to 62- and 54-kdal subunits by SDS-PAGE.

Antigenic Relationships Among Purified Mercuric Reductase Enzymes

A recent comparative study was conducted with antiserum prepared to both Tn*501*- and R831-purified Hg(II) reductase enzymes. All gram-negative bacterial Hg(II) reductases tested were inactivated by the antiserum prepared against the R831 enzymes (92). These enzymes included those expressed by various enteric organisms and those from *Pseudomonas* and *Proteus* spp. (91). In contrast, antisera to purified Tn*501* Hg(II) reductase separated the gram-negative bacterial enzymes into three serotypes based on spur formation in double-diffusion precipitation tests. Neither one of the antisera cross-reacted with the Hg(II) reductase of *Staphylococcus aureus*. These results indicate that the gram-negative and gram-positive Hg(II) reductase enzymes examined thus far are immunologically distinct. Interestingly, the Hg(II) reductase purified from *T. ferrooxidans* was not inactivated by either of the antisera, but was nonspecifically inhibited by both (92).

The thermostability of the purified Hg(II) reductase enzymes reinforces this immunological distinction between gram-negative and gram-positive sources. All of the Hg(II) reductase enzymes purified from gram-negative bacteria have been found to retain activity upon incubation at 80°C for 10 min. In contrast, the Hg(II) reductase of *Staphylococcus aureus* is completely inactivated at 55°C (92). The Hg(II) reductase enzyme purified from *T. ferrooxidans* is reported to be at least as heat resistant as that of *Escherichia coli* Hg^r plasmid R100, retaining activity after 10 min at 80°C (80).

Cofactor Requirements of Mercuric Reductase

All Hg(II) reductase enzymes have been shown to contain bound FAD as coenzyme (24, 29, 30, 43, 87, 90). In most instances this was determined by examining the supernatant of the boiled, purified enzyme, using thin-layer chromatography. The number of moles of FAD per subunit of the enzyme was determined fluorometrically, with an extinction coefficient of FAD in mercuric reductase of 11.3 $mM\ cm^{-1}$ at 458 nm (24). Values of 1 FAD per subunit of the Hg(II) enzyme have been reported (Table 8) (24, 87, 90). The absorption spectra of the purified enzymes of pRR130 and Tn*501* have maxima at both 370 and 460 nm, identifying them as flavoproteins (24, 87). A listing of all cofactor requirements is presented in Table 8.

A requirement for NADP(H) as a cofactor in the reduction of Hg^{2+} to Hg^0 has been demonstrated for all purified Hg(II) reductase enzymes studied to date (11, 24, 29, 30, 43, 55, 87, 90, 104). NADPH serves as the electron donor in the reduction of Hg^{2+}. Some studies have demonstrated that NADH can substitute for NADPH as the electron donor in various *Escherichia coli* and *Pseudomonas* sp. strains as well as in *T. ferrooxidans* and *Yersinia enterocolitica*, but it was much less effective than NADPH (6, 29, 30, 43, 80).

The stoichiometry of the NADPH-dependent reduction of Hg^{2+} has been shown to be 1:1 (NADPH:Hg^{2+}) for all except *Escherichia coli* W2252, which was found to be 2:1 (Table 8) (44). The degree of coupling of the system has been examined by measuring NADPH oxidation and Hg^{2+} reduction in parallel. The ratio was 1.3 mol of NADPH consumed 1.0 mol of Hg^{2+} reduced^{-1}, with <0.3% of the total electron flow going to O_2 as measured with an O_2 electrode (24).

An additional cofactor requirement shared by all Hg(II) reductase enzymes is that of thiol reagents for activity. However, the nature of the thiol as well as the levels required for maximum activity appear to vary among the enzymes examined (Table 8). One consistent finding is the need for an excess of the preferred thiol reagent. This is presumably to insure the reduced state of the enzyme active site and also that Hg be present as RS-Hg^{2+} or RS-Hg-SR (92, 97). Although an excess of thiol reagent is required for activity, inhibition may occur. For example, the Hg(II) reductase purified from *T. ferrooxidans* exhibited optimal activity at a 250:1 ratio of β-mercaptoethanol, whereas at 2,500:1 there was a 50% inhibition (80).

The role of EDTA has proven to be less clear. In many cases EDTA has not been included in the reaction mixture (see Table 8). EDTA was found to be required for maximum enzyme activity of the Hg(II) reductase partially purified from *T. ferrooxidans*. In the absence of EDTA, there was a 95% reduction in activity from that observed at the optimal concentration of 5 mM EDTA (13). The same optimal level of EDTA was observed for *Escherichia coli* C600(pDU1003) when tested under the same conditions. A highly purified Hg(II) reductase from *Escherichia coli* Kp245(pRR130) was shown to require EDTA or thiol reagents. No activity was observed if both were omitted, and the kinetics of the reaction were dependent on which reagents were present (87).

TABLE 8. Characteristics of mercuric reductase enzymes

Strain(plasmid)	Mercuric reductase cofactors: EDTA	Mercuric reductase cofactors: Sulfhydryl	Mercuric reductase cofactors: NADPH/NADH (ratio of NADPH oxidized/Hg^{2+} reduced)	Maximum absorption (nm)	Inhibitors	Reference(s)
Escherichia coli K-12 J53-1, (R831)	(+)	(+) L-Cysteine optimal	NADPH dependent NADH not effective (1:1)	ND[a]	ND	90
E. coli W2252 (R factor)	ND	(+) β-Mercaptoethanol optimal	NADPH dependent Some activity with NADH (2:1)	268 370 450	Ag^{+}, Cu^{2+}, Cd^{2-}	44
E. coli KP245 (pR130)	(+) (Requires either EDTA or sulfhydryl)	(+)	NADPH dependent NADH not tested	370 450	Cd^{2+}, Ag^{+}, Cu^{2+}, Au^{3+}	87
Pseudomonas aeruginosa PAO9501 [pVS1 (Tn*501*)]	ND	(+)	NADPH dependent	270 370 450	ND	24
Pseudomonas sp. K62 (no plasmid identified)	ND	(+) β-Mercaptoethanol	NADPH or NADH dependent	270 365 460	Cd^{2+}, Cu^{2+}	29, 30
Staphylococcus aureus (penicillinase plasmid)	(+) (Removal had no effect as long as sulfhydryl present)	(+) L-Cysteine optimal	NADPH dependent No activity with NADH	ND	ND	117
Thiobacillus ferrooxidans BA-4 (no plasmid identified)	(+) (Without EDTA activity reduced 90%)	(+) β-Mercaptoethanol optimal	NADPH dependent Some activity with NADH	ND	ND	80
T. ferrooxidans TFI-29	(+)	(+)	NADPH dependent 25% activity with NADH	455	Cu^{2+}, Ag^{+}, Co^{2+}, Fe^{3+}	7a

[a] ND, Not determined.

Mechanism of Mercuric Reductase

The nature of the enzymatic mechanism involved in the NADPH-dependent reduction of Hg^{2+} to Hg^0 has not been entirely elucidated, but recent studies have introduced some important findings (24, 87). The kinetic constants of the various Hg(II) reductase enzymes are presented in Table 7. Assays for Hg(II) reductase activity involve (i) monitoring the loss or volatilization of $^{203}Hg^0$ from the reaction mixture or (ii) following the oxidation of NADPH spectrophotometrically at 340 nm. The K_m values for $HgCl_2$ vary from 0.5 to 20 μM, whereas the V_{max} values suggest that the affinity of the enzymes for the substrate is of the same order, but that the rate of catalysis or release of Hg^0 is quite variable among the different enzymes. NADPH-dependent Hg^{2+} volatilization kinetics have also been examined.

NADPH-dependent Hg^{2+} volatilization does not exhibit Michaelis-Menten kinetics, but rather sigmoidal or biphasic kinetics. This was demonstrated for purified Hg(II) reductase enzymes of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Table 8). (24, 90, 110). When assayed under standard assay conditions (EDTA and thiol compounds present), the reduction of Hg^{2+} shows Michaelis-Menten saturation kinetics for the purified Hg(II) reductase enzymes of *Escherichia coli* strains J53-1(R831) and KP245(pRR130), *Staphylococcus aureus*, and *T. ferrooxidans* (Table 7). The purified Hg(II) reductase enzyme of Tn*501* and pRR130 has been well characterized, and the details of the kinetic and mechanistic studies have provided important information (24, 87).

The oxidation of NADPH by the pRR130 Hg(II) reductase is biphasic under standard assay conditions (EDTA and thiol reagent) and shows a rapid initial phase followed by a slower, linear phase (87). Biphasic kinetics are also observed in the absence of EDTA and a constant concentration of thiol reagent (β-mercaptoethanol). If β-mercaptoethanol is removed, the slower, linear phase is eliminated and the reaction rate declines to zero. When both EDTA and β-mercaptoethanol are omitted there is no detectable activity.

By varying the Hg^{2+} concentration while holding the

EDTA levels constant in the absence of β-mercaptoethanol, there is a time-dependent decrease in the reaction rate which varies as a function of the Hg^{2+} concentration. At high levels of Hg^{2+}, the reaction ceases after only a few minutes. This suggests the formation of an enzyme-inhibitory complex. The same results were obtained when the enzyme was preincubated with Hg^{2+} and EDTA and the reaction was initiated with NADPH. Substantial substrate inhibition by Hg^{2+} in the presence of EDTA is observed. No detectable reaction rate is observed until the ratio of EDTA/Hg^{2+} reaches 1, after which it rises rapidly, remaining unchanged over a 10-fold increase in the ratio (87). Thus it appears that it is the EDTA-Hg^{2+} complex that acts as the substrate in the absence of thiol compounds.

Thiol compounds exert a markedly different effect. In the absence of EDTA and a constant β-mercaptoethanol concentration, the reaction is biphasic. When preincubated with β-mercaptoethanol, the initial phase is eliminated, indicating that the enzyme exists in two separate forms which are converted from one to the other by the thiol compound. A mechanism which accounts for the observed behavior has been proposed by Rinderle et al. (87) (Fig. 4). The K_m values for the first and second phases are 70 and 53 μM $HgCl_2$, respectively, with V_{max} values of 1,044 and 152 μmol min^{-1} mg of $protein^{-1}$. It appears that, although the affinity of the enzyme for the substrate is not altered greatly in the second phase, the rate of catalysis or release of product is much slower. The V_{max} values are similar for assays run with either β-mercaptoethanol/Hg^{2+} or EDTA/Hg^{2+} but the K_m values indicate that the Hg(II)-EDTA complex has more affinity for the enzyme than the Hg(II)–β-mercaptoethanol complex. However, incubation with thiols reactivates the inhibited EDTA-Hg(II) enzyme complex, presumably by competing as ligands for the enzyme-bound Hg^{2+}.

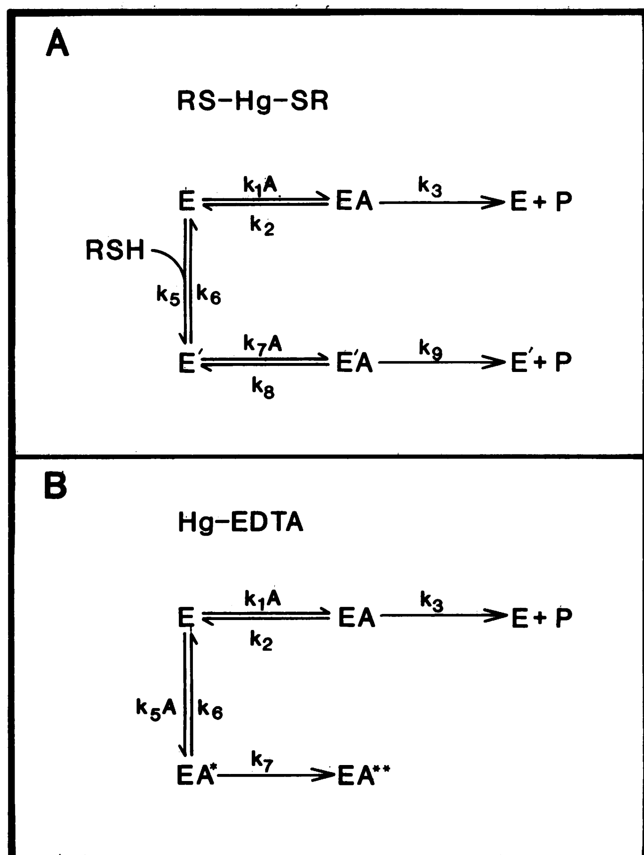

FIG. 4. Kinetic mechanism of mercuric reductase from *Escherichia coli* (87).

Relationship of Mercuric Reductase to Other Flavoenzymes

Spectroscopic and thiol titration data identify the Tn*501* and pRR130 Hg(II) reductase enzymes as belonging to the pyridine-nucleotide disulfide reductase class of proteins. Upon addition of reducing equivalents (NADPH or dithionite) the spectrum of Hg(II) reductase is similar to the two-electron-reduced (EH_2) form of glutathione reductase and lipoamide dehydrogenase (24, 87). When glutathione reductase and lipoamide dehydrogenase are reduced by two electrons, the EH_2 species contains oxidized FAD and a reduced disulfide, but when reduced by four electrons EH_4 is formed, which contains reduced flavin and reduced disulfide as indicated by spectra and oxidation-reduction potential couplings (24, 87).

When the Hg(II) reductase enzyme was reduced by two electron equivalents per FAD, a species was formed with absorbance at 540 nm, and two more electrons were required for complete flavin reduction. This indicates that four electrons are required to reduce FAD bound to the enzyme, suggesting a second two-electron acceptor on the active site. Thiol titrations with 5,5′-dithiobis(2-nitrobenzoate) indicate the presence of an oxidation-reduction active disulfide on both enzymes (24, 87). The species characterized by long-wavelength absorbance generated upon the addition of reducing equivalents bears a strong resemblance to the charge transfer complex between reduced thiols and FAD on both glutathione reductase and lipoamide dehydrogenase. These enzymes are known to possess an additional electron acceptor, an oxidation-reduction active disulfide (a cysteine residue) (121). In addition, the oxidation-reduction potentials of E/EH_2 and EH_2/EH_4 complexes of Hg(II) reductase and lipoamide dehydrogenase are very similar (24). No substrate cross-reactivity between Tn*501* Hg(II) reductase and pig heart lipoamide dehydrogenase, yeast glutathione reductase, or thioredoxin reductase has been reported (24). However, 5,5′-dithiobis(2-nitrobenzoate) is reportedly a substrate for the pRR130 Hg(II) reductase, with a K_m of 3 mM and a V_{max} of about 1% of that observed for Hg^{2+} (81).

These results have evolutionary implications. The amino acid sequence of the active site of the pRR130 Hg(II) reductase has been determined and found to be very similar to that of lipoamide dehydrogenase (25). Homologous amino acid sequences have also been found in Tn*501* mercuric reductase and human erythrocyte glutathione reductase (9). It is interesting to note that a mercury-detoxifying enzyme encoded on a plasmid may be similar to enzymes encoded by the chromosome that are essential to the cell. It is not too difficult to imagine that selective pressure, i.e., the presence of toxic mercurials, could result in a slightly modified enzyme capable of reducing Hg^{2+} to elemental mercury which is effectively removed from the microenvironment of the cell.

Further studies are required to determine whether the enzyme cycles between EH/EH_2, with FAD acting as mediator in the electron flow from NADPH to disulfide, or from EH_2/EH_4 with electrons flowing to FAD creating $FADH_2$, which is the actual reductant, and then on to Hg^{2+}. In the latter case the disulfide-dithiol site may act as a substrate-binding site, accessible only after it has been reduced.

Sequencing the active sites of the Hg(II) reductase enzymes should be pursued to determine the relationships among the various enzymes as well as to the three enzymes of the class of FAD-containing disulfide reductase enzymes. As it seems unlikely that an excess of thiol compounds would be present in excess under environmental conditions, the validity of such a mechanism may be in question.

Organomercurial Lyase Enzyme

The organomercurial lyase enzymes, responsible for the detoxification of organomercurials, have not been as fully characterized as the Hg(II) reductase enzymes described above. The organomercurial lyase enzyme is responsible for catalyzing the cleavage of the carbon-mercury linkages of organomercurial compounds (Fig. 2), and it has been characterized in the broad-spectrum mercury-resistant *Pseudomonas* sp. K62 and *Escherichia coli* J53-1(R831) strain (90, 109). A crude enzyme preparation of the broad-spectrum, mercury-resistant, plasmid-bearing *Staphylococccus aureus* strain has also been shown to possess activity towards PMA, but has not been sufficiently resolved from the Hg(II) reductase enzyme to allow further study (117). Both organomercurial lyase and mercuric reductase enzymes are required for detoxification of organomercurials.

For *Pseudomonas* sp. K62 it was first reported that a metallic mercury-releasing enzyme present in cell-free extracts was responsible for catalyzing the reduction of Hg^{2+} and organomercurials to elemental mercury (29, 30). The enzyme was first shown to require NADPH equivalents and a cytochrome c_1 fraction for activity. In subsequent work, however, it has been shown that the original preparation contained the mercuric reductase enzyme and that two separate organomercurial lyase enzymes (designated S-1 and S-2) were present in the cell-free extracts (109, 110). The S-1 and S-2 enzymes were purified from cell-free extracts by treatment with streptomycin, precipitation with ammonium sulfate, and successive chromatography on Sephadex G-150, DEAE-Sephadex, and DEAE-cellulose. It was discovered that the cytochrome c_1 fraction was not involved in the decomposition of organomercurials. This misconception was due to the inability to separate the S-1 and S-2 enzymes from the cytochrome c_1 fraction.

The S-1 enzyme catalyzes the splitting of carbon-mercury bonds of both aryl- and alkylmercury compounds. This enzyme is capable of catalyzing the decomposition of MMC, EMC, PMA, and pCMB (Table 9) (109). The purified enzyme preparation showed a single band on polyacrylamide gels with a molecular weight of 19,000 estimated by gel filtration on Sephadex G-75 (Table 9). The pH and temperature optima were determined to be 7.0 and 40°C, respectively. The enzyme is stable up to 50°C, but at 60°C almost 100% loss of activity occurred rapidly. Thiol compounds were required for activity. L-Cysteine is the most effective in promoting decomposition, compared with reduced glutathione, DL-homocysteine, thiomalate, and thioglycolate. β-Mercaptoethanol and dithiothreitol were much less effective, with only 3% of the activity observed for L-cysteine, and L-cysteine had no detectable activity (109). As with the mercuric reductase enzyme, the actual substrate is believed to be the mercaptide form rather than the organomercurial itself.

The splitting of carbon-mercury linkages were determined for MMC, EMC, PMA, and pCMB in assay mixtures containing thioglycolate and the mercuric reductase enzyme. When these compounds were incubated with the S-1 enzyme in the presence of an excess of thioglycolate, methane,

TABLE 9. Comparison of organomercurial lyase enzymes isolated from several bacterial genera[a]

Strain(plasmid)	Mol wt	Substrate	Kinetic constants: K_m (μM)	Kinetic constants: V_{max} (min^{-1} mg^{-1})	Cofactor requirements	Reference(s)
Pseudomonas sp. K62 (no plasmid identified)	a. S-1, 19,000 (pH 7.0 optimal, 50°C optimal)	pCMB PMA MMC EMC	78 45 20 ND	0.26 μmol 0.68 μmol 5.4 nmol ND	1. NADPH 2. Sulfhydryl (L-cysteine most effective)	105, 109
	b. S-2, 22,000 (pH 5.0 optimal, 40°C optimal)	pCMB PMA MMC	180 250 NA	3.1 μmol 20 μmol NA		
Escherichia coli J53-1(R831)	a. Native enzyme 43,000 ± 4,000 (disc gel electrophoresis) 25,000 (gel filtration)	PMA MMC EMC (Substrate inhibition observed with EMC, >2 μM; PMA, >200 μM)	a. 0.24–0.3 b. >200 6.7–7.7 0.56–0.61	26 nmol 2,800 nmol 13 nmol 16 nmol	1. NADPH 2. EDTA 3. Sulfhydryl (L-cysteine most effective)	90
	b. Subunit (SDS-PAGE) 22,000 16,500					
Staphylococcus aureus (penicillinase plasmid-bearing strain)	ND	PMA No activity towards MM or EM	25	8.4 nmol	1. NADPH 2. Sulfhydryl (L-cysteine most effective)	117

[a] ND, Not determined; NA, not applicable; MM, methylmercury; EM, ethylmercury.

ethane, benzene, and benzoic acid were produced, respectively (109). Benzoic acid was detected by thin-layer chromatography, whereas the other products were identified by gas-liquid chromatography methods. Although 100% of PMA was decomposed in 2 h, <50% EMC and about 25% MMC were decomposed during 3 h of incubation. The substrates and their kinetic constants are presented in Table 9. Kinetic data indicate that the enzyme has greater affinity for MMC than for PMA, but a slower rate of decomposition. No activity was observed in the absence of the mercuric reductase enzyme.

The molecular weight of the S-2 enzyme is 20,000 as determined by gel filtration on a Sephadex G-75 column, and the enzyme was found to migrate as a single band with a molecular weight of 17,000 on a 10% SDS-polyacrylamide gel. The enzyme was found to have pH and temperature optima of 5.0 and 40°C (completely inactivated at 55°C), respectively (Table 9), indicating that the enzyme is more acidic and more heat labile than the S-1 enzyme (110).

Both PMA and pCMB can serve as substrates for this enzyme, whereas MMC and EMC cannot, suggesting that the S-2 enzyme is involved in the splitting of carbon-mercury linkages of arylmercury compounds. In the presence of an excess of thioglycolate, benzene and benzoic acid were produced from PMA and pCMB, respectively. When ^{14}C-labeled PMA was used as the substrate, [^{14}C]benzene was produced and decomposition could be followed by the loss of radioactivity from the reaction mixture (110). Michaelis-Menten kinetics were observed with PMA and pCMB as substrates. The K_m and V_{max} values are listed in Table 9. The S-2 enzyme appears to have higher affinity for both substrates than does S-1, but lower rates of decomposition.

The S-2 enzyme also requires an excess of thiols for maximum activity and is inactivated by reagents which react with sulfhydryl groups such as monoiodoacetic acid, monoiodoacetamide, PMA, pCMB, MMC, and $HgCl_2$ but not by EDTA, cyanide, and azide (110). The lack of inhibition by EDTA, cyanide, and azide suggests that EDTA does not form an S-2–EDTA complex, as it does with Hg^{2+}, and is not coupled to energy transduction and respiration.

Another organomercurial lyase enzyme has been purified from the broad-spectrum plasmid-bearing *Escherichia coli* J53-1(R831) (24). This enzyme was purified in a manner similar to the S-1 and S-2 enzymes and was monitored for the lyase activity by using MMC and PMA throughout the purification to detect the presence of more than one lyase. Enzyme activity is dependent on the presence of EDTA and a sulfhydryl compound. L-Cysteine is the most effective sulfhydryl compound whereas dithiothreitol is the least effective. Enzyme activities of 50 and 25% were obtained with thioglycolate and β-mercaptoethanol, respectively. The same order of effectiveness of sulfhydryl compounds is not seen with the Hg(II) reductase from the same strain (90).

Lyase activity was measured by following the loss of Hg^{2+} from a reaction mixture containing phosphate buffer, EDTA, Mg-acetate, L-cysteine, bovine serum albumin, and ^{203}Hg-labeled substrate. NADPH and either purified Hg(II) reductase or $SnCl_2$ were added to reduce Hg^{2+} formed by lyase action. A need for high concentrations of protein was suggested by a 65 to 80% loss of activity when bovine serum albumin is eliminated. This was not observed with either S-1 or S-2 lyase. In addition, when Hg(II) reductase or NADPH was omitted from the reaction mixture containing $SnCl_2$, the activity decreased by approximately 85%. This suggests that Hg(II) reductase stimulates the lyase activity and that the lyase reaction is the rate-limiting step (90).

The molecular weight of the R8331 lyase is 25,000 ± 1,000 as determined by gel filtration on a Sephadex G-200 column and 39,000 and 47,000 in other experiments using disc gel electrophoresis (90). This discrepancy was also observed for the Hg(II) reductase of the same strain and may simply represent a poor correlation between the two techniques. Two protein bands in equimolar amounts were seen on SDS-polyacrylamide gels, with molecular weights of 22,000 and 16,500. The enzyme may be a monomer of 25 kdal with a 16-kdal degradation product. It may also be a dimer based on a molecular weight of 39 × 10^3 with dissimilar subunits of 22 × 10^3 and 16 × 10^3. Further characterization is needed to resolve this discrepancy. In *Escherichia coli* minicells containing R831, two polypeptides of 22 and 24 kdal were produced (45). These peptides were present only in R831, which was the only broad-spectrum Hg^r plasmid studied, and are similar in molecular weight to those reported for the subunits of the partially purified lyase from R831 described above. The activity of these polypeptides towards organomercurials was not determined.

The substrates and kinetic constants for the R831 lyase enzyme are listed in Table 9. The organomercurial lyase enzyme of R831 can decompose both aryl- and alkylmercury compounds. Although Schottel (90) was not able to detect or separate two organomercurial lyases from this strain, kinetic analysis showed two K_m and two V_{max} values for PMA, suggesting the possibility of two enzymes. Further characterization of this enzyme is required to determine whether two separate enzymes do indeed exist, or perhaps there are two active sites, or a modulation or modification of the enzymes may result in differential activity.

The differences noted in the kinetic properties of the lyase enzymes of *Escherichia coli* and *Pseudomonas* spp. may be due in part to host strain effects. These effects could be determined by using a common host strain and by comparison of the products in the *Escherichia coli* minicell system described previously. Since the Hg(II) reductase of *Staphylococcus aureus* appears to be quite different from the gram-negative Hg(II) reductase enzymes, it would be of interest to determine the relationship of its lyase enzyme to those from gram-negative sources. More detailed mechanistic studies are warranted. The location of the lyase enzyme has not yet been determined. Although it appears to be in the soluble fraction, it may be periplasmic or have a limited association with the membrane.

CONCLUSIONS

The relationship among the *mer* operons of different bacterial species needs to be examined in even greater detail. Characteristic patterns of resistance to mercury compounds among different genera have emerged. Only A-C, L, and H incompatibility groups of enteric plasmids specify broad-spectrum resistance capabilities, whereas all *Staphylococcus aureus* plasmids studied to date fall into this class. These patterns may suggest a limitation in transfer among strains, maintenance instability, or additional host cell background effects. Examples of host cell effects that have been documented include: (i) the lack of expression of *Staphylococcus aureus mer* genes in *Escherichia coli* cells, using chimeric plasmids of pSC101 and the *Staphylococcus aureus mer* region; (ii) the inability of *Escherichia coli* cells to express resistance to pHMB from a broad-spectrum resistance plasmid from *Pseudomonas aeruginosa* that expresses resistance to this compound; and (iii) the variations in expression of *mer* operon of R100 in *Escherichia coli* and *Proteus mirabilis* in studies on gene copy number effects. Variations

in resistance range and levels may be attributed to the host background effects in a number of instances, suggesting that further efforts in identifying such effects on regulation and expression of *mer* operons in these systems are warranted.

The mercuric reductase enzymes from gram-negative and gram-positive bacteria differ in a number of aspects as presented above. Examination of the *Staphylococcus aureus* mercury-induced polypeptides and their location within the cell, using the *Escherichia coli* minicell system for direct comparison with gram-negative strains, would provide valuable information on the extent of such differences. It is especially interesting to note that in gram-negative systems there is a polypeptide believed to be located in the periplasmic space that may act as the mercury-binding protein. The existence and location of an analogous protein in *Staphylococcus aureus* cells would be useful in understanding the mechanism of mercury uptake and how it may differ among mercury-resistant isolates. Sequencing of the *mer* region of *Staphylococcus aureus* should also be undertaken to establish the homology with *mer* genes from other sources.

The relationship of the mercuric reductase enzymes encoded by Tn*501* and pRR130 to the glutathione reductase and lipoamide dehydrogenase enzymes might provide a link in the evolution of mercury resistance systems. The use of X-ray crystallography in addition to amino acid and DNA sequencing would provide useful information on the degree of homology among these enzymes.

The specificity of thiol requirements and the effects of these compounds on the mercuric reductase enzymes also need clarification. The differences may reflect heterogeneity among the enzymes or differences in the affinity of the compound for the substrate, and its ability to exchange onto the active site, thus promoting its reduction.

Identification of the *merT* uptake protein is of major importance. The 12-kdal protein shown in the *Escherichia coli* minicells to be in the periplasmic space has no cysteine residues which may be necessary for a protein that binds mercury to prevent its inactivation. This may also explain the preference for L-cysteine as sulfhydryl compound, as it would allow for the exchange of mercury from the carrier protein to the active site on the enzyme. The isolation of *merT* mutants and examination of their products in the minicell system would be one approach to identifying the product(s) of this gene. The uptake function appears to be a highly specific and high-affinity process as indicated by binding studies. This may be a rate-limiting step in the detoxification of both mercuric ions and organomercury compounds, as observed with the high-copy-number variants. Studying the effects of various ionophores may be useful in establishing the energetics of the uptake system.

The location of the organomercurial lyase enzymes has not yet been established. Difficulties in purifying and resolving this enzyme have slowed down research in this area. The isolation of two different lyase enzymes from the *Pseudomonas* sp. K62 strain and a single lyase enzyme from *Escherichia coli* with what appears to have different substrate specificities needs clarification. There is some evidence that the organomercurial lyase enzyme is transcribed from a different promoter than the mercuric reductase enzyme but is under control of the *mer* operon. This is indicated by the lack of coordinate induction, preferential induction by merbromin, and isolation of mutants defective in mercuric reductase activity that retain the lyase activity. Establishing the number and position of promoters on the *mer* operon region of broad-spectum plasmids will aid in resolving this point. Detailed studies of coordinate induction of the mercuric reductase and lyase activities of different plasmids in a common host strain would indicate whether this is a common occurrence.

One of the most important questions that remains is the mechanism of resistance to organomercurials such as merbromin and FMA, which does not include degradation. It is not readily apparent why the carbon-mercury bond in these compounds would not be catalyzed by the lyase enzyme while the others are. This may be explained simply as the result of the lower toxicity of these compounds or that of a modified uptake system. However, these compounds are known to inhibit the hyperbinding of mercuric ions by hypersensitive mutants but are not themselves bound to the cell. It may be that these compounds actually bind the mercuric ions, making them unavailable, or they may bind loosely to the uptake protein specified by the *mer* operon and will result in induction but not uptake.

The methylation of mercury by microorganisms may represent a detoxification mechanism which may or may not be enzymatic in nature, a question that must be clarified. Studying methylation in *Escherichia coli*, which does not produce methylcobalamin, and mutants in other strains that do produce methylcobalamin may provide answers to this question. The balance of methylation and demethylation of mercury should be explored. It may be that under conditions in which methylation is optimum (pH 4.5 and no thiol requirement) fewer organisms that degrade methylmercury (pH 7.0 and presence of thiol compounds) or a reduced rate may be observed. Bacteria that possess well-characterized mercury reduction and organomercurial degradation systems should be assayed for the ability to methylate mercury under conditions known to be optimum for methylation. The differences in optimal conditions for these two activities may make them mutually exclusive if they exist in the same organism or may result in microenvironments in situ in which one activity predominates.

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LITERATURE CITED

1. **Barkay, T., and R. R. Colwell.** 1983. Altered mercury transport in a mutant of *Pseudomonas fluorescens* B69. J. Gen. Microbiol. **129**:2945–2950.
2. **Beneš, P., E. T. Gjessing, and E. Steinnes.** 1976. Interactions between humus and trace elements in fresh water. Water Res. **10**:711–716.
3. **Bennett, P. M., J. Grinsted, C. L. Choi, and M. H. Richmond.** 1978. Characterization of Tn501, a transposon determining resistance to mercuric ions. Mol. Gen. Genet. **159**:101–106.
4. **Berlin, M.** 1979. Mercury, p. 503–530. *In* L. Friberg, G. F. Nordberg, and V. B. Vouk (ed.), Handbook on the toxicology of metals. Elsevier/Holland Biomedical Press, Amsterdam.
5. **Bertilsson, L., and H. Y. Neujahr.** 1971. Methylation of mercury compounds by methylcobalamin. Biochemistry **10**:2805–2808.
6. **Blaghen, M., M.-C. Lett, and D. J.-M. Vidon.** 1983. Mercuric reductase activity in a mercury-resistant strain of *Yersinia enterocolitica*. FEMS Microbiol. Lett. **19**:93–96.
7. **Bohlander, F. A., A. O. Summers, and R. B. Meagher.** 1981. Cloning a promoter that puts the expression of tetracycline resistance under the control of the regulatory elements of the *mer* operon. Gene **15**:395–403.

7a. **Booth, J. E., and J. W. Williams.** 1984. The isolation of a mercuric ion-reducing flavoprotein from *Thiobacillus ferrooxidans*. J. Gen. Microbiol. **130**:725–730.

8. **Brown, N. L., C.-L. Choi, J. Grinsted, M. H. Richmond, and**

P. R. Whitehead. 1980. Nucleotide sequences at the ends of the mercury resistance transposon, Tn501. Nucleic Acids Res. **8:**1933–1945.

9. **Brown, N. L., S. J. Ford, R. D. Pridmore, and D. C. Fritzinger.** 1983. Nucleotide sequence of a gene from the *Pseudomonas* transposon Tn501 encoding mercuric reductase. Biochemistry **22:**4089–4095.
10. **Cabello, F., K. Timmis, and S. N. Cohen.** 1978. Cloning of *Hin*dIII and *Eco*RI fragments of R6-5 plasmid by insertional inactivation, p. 42–44. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
11. **Clark, D. L., A. A. Weiss, and S. Silver.** 1977. Mercury and organomercurial resistances determined by plasmids in *Pseudomonas*. J. Bacteriol. **132:**186–196.
12. **Craig, P. J., and P. D. Bartlett.** 1978. The role of hydrogen sulphide in environmental transport of mercury. Nature (London) **275:**635–637.
13. **De La Cruz, F., and J. Grinsted.** 1982. Genetic and molecular characterization of Tn*21*, a multiple resistance transposon from R100.1. J. Bacteriol. **151:**222–228.
14. **Dempsey, W. B., and S. A. McIntire.** 1979. Lambda transducing phages derived from a FinO$^-$ R100::λ cointegrate plasmid: proteins encoded by the R100 replication/incompatibility region and the antibiotic resistance determinant. Mol. Gen. Genet. **176:**319–334.
15. **Dempsey, W. B., S. A. McIntire, N. Willetts, J. Schottel, T. G. Kinsherf, S. Silver, and W. A. Shannon, Jr.** 1978. Properties of lambda transducing bacteriophages carrying R100 plasmid DNA: mercury resistance genes. J. Bacteriol. **136:**1084–1093.
16. **Dempsey, W. B., and N. S. Willetts.** 1976. Plasmid co-integrates of prophage lambda and R factor R100. J. Bacteriol. **126:**166–176.
17. **DeSimone, R. E., M. W. Penley, L. Charbonneau, S. G. Smith, J. M. Wood, H. A. O. Hill, J. M. Pratt, S. Ridsdale, and R. J. P. Williams.** 1973. The kinetics and mechanism of cobalamin-dependent methyl and ethyl transfer to mercuric ion. Biochim. Biophys. Acta **304:**851–863.
18. **D'Itri, F. M.** 1972. The environmental mercury problem. CRC Press, Inc., Boca Raton, Fla.
19. **Fagerström, T., and A. Jernelöv.** 1971. Formation of methyl mercury from pure mercuric sulphide in aerobic organic sediment. Water Res. **5:**121–122.
20. **Fagerström, T., and A Jernelöv.** 1972. Some aspects of the quantitative ecology of mercury. Water Res. **6:**1193–1202.
21. **Faust, S. D., and O. M. Aly.** 1981. Chemistry of natural waters. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.
22. **Foster, T. J., and H. Nakahara.** 1979. Deletions in the r-determinant *mer* region of plasmid R100-1 selected for loss of mercury hypersensitivity. J. Bacteriol. **140:**301–305.
23. **Foster, T. J., H. Nakahara, A. A. Weiss, and S. Silver.** 1979. Transposon *A*-generated mutations in the mercuric resistance genes of plasmid R100-1. J. Bacteriol. **140:**167–181.
24. **Fox, B., and C. T. Walsh.** 1982. Mercuric reductase: purification and characterization of a transposon-encoded flavoprotein containing an oxidation-reduction-active disulfide. J. Biol. Chem. **257:**2498–2503.
25. **Fox, B. S., and C. T. Walsh.** 1983. Mercuric reductase: homology to glutathione reductase and lipoamide dehydrogenase. Iodoacetamide alkylation and sequence of the active site peptide. Biochemistry **22:**4082–4088.
26. **Friberg, L., and J. Vostal (ed.).** 1972. Mercury in the environment. CRC Press, Inc., Boca Raton, Fla.
27. **Friello, D. A., and A. M. Chakrabarty.** 1980. Transposable mercury resistance in *Pseudomonas putida*, p. 249–260. *In* C. Stuttard and K. R. Rozee (ed.), Plasmids and transposons: environmental effects and maintenance mechanisms. Academic Press, Inc., New York.
28. **Furukawa, K., T. Suzuki, and K. Tonomura.** 1969. Decomposition of organic mercurial compounds by mercury-resistant bacteria. Agric. Biol. Chem. **33:**128–130.
29. **Furukawa, K., and K. Tonomura.** 1972. Metallic mercury-releasing enzyme in mercury-resistant *Pseudomonas*. Agric. Biol. Chem. **36:**217–226.
30. **Furukawa, K., and K. Tonomura.** 1972. Induction of metallic mercury-releasing enzyme in mercury-resistant *Pseudomonas*. Agric. Biol. Chem. **36:**2441–2448.
31. **Gadd, G. M., and A. J. Griffiths.** 1978. Microorganisms and heavy metal toxicity. Microb. Ecol. **4:**303–317.
32. **Gavis, J., and J. F. Ferguson.** 1972. The cycling of mercury through the environment. Water Res. **6:**989–1008.
33. **Grinsted, J., P. M. Bennett, S. Higginson, and M. H. Richmond.** 1978. Regional preference of insertion of Tn501 and Tn802 into RP1 and its derivatives. Mol. Gen. Genet. **166:**313–320.
34. **Groves, P. J., H. Short, A. J. Thewaini, and F. E. Young.** 1975. Epidemiology of antibiotic and heavy metal resistance in bacteria: resistance patterns in staphylococci isolated from populations in Iraq exposed and not exposed to heavy metals or antibiotics. Antimicrob. Agents Chemother. **7:**622–628.
35. **Groves, D. J., and F. E. Young.** 1975. Epidemiology of antibiotic and heavy metal resistance in bacteria: resistance patterns in staphylococci isolated from populations not known to be exposed to heavy metals. Antimicrob. Agents Chemother. **7:**614–621.
36. **Hamdy, M. K., and O. R. Noyes.** 1975. Formation of methyl mercury by bacteria. Appl. Microbiol. **30:**424–432.
37. **Hänni, C., J. Meyer, S. Iida, and W. Arber.** 1982. Occurrence and properties of composite transposon Tn*2672*: evolution of multiple drug resistance transposons. J. Bacteriol. **150:**1266–1273.
38. **Hatch, W. R., and W. L. Ott.** 1968. Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Anal. Chem. **40:**2085–2087.
39. **Holm, H. W., and M. F. Cox.** 1975. Transformation of elemental mercury by bacteria. Appl. Microbiol. **29:**491–494.
40. **Hu, S., E. Ohtsubo, N. Davidson, and H. Saedler.** 1975. Electron microscope heteroduplex studies of sequence relations among bacterial plasmids: identification and mapping of the insertion sequences IS1 and IS2 in F and R plasmids. J. Bacteriol. **122:**764–775.
41. **Iida, S., C. Hänni, C. Echarti, and W. Arber.** 1981. Is the IS1-flanked r-determinant of the R plasmid NR1 a transposon? J. Gen. Microbiol. **126:**413–425.
42. **Imura, N., E. Sukegawa, S. Pan, K. Nagao, J. Kim, T. Kwan, and T. Ukita.** 1971. Chemical methylation of inorganic mercury and methylcobalamin, a vitamin B_{12} analog. Science **172:**1248–1249.
43. **Izaki, K.** 1977. Enzymatic reduction of mercurous ions in *Escherichia coli* bearing R factor. J. Bacteriol. **131:**696–698.
44. **Izaki, K., Y. Tashiro, and T. Funaba.** 1974. Mechanism of mercuric chloride resistance in microorganisms. III. Purification and properties of a mercuric ion reducing enzyme from *Escherichia coli* bearing R factor. J. Biochem. (Tokyo) **75:**591–599.
45. **Jackson, W. J., and A. O. Summers.** 1982. Polypeptides encoded by the *mer* operon. J. Bacteriol. **149:**479–487.
46. **Jackson, W. J., and A. O. Summers.** 1982. Biochemical characterization of $HgCl_2$-induced polypeptides encoded by the *mer* operon of plasmid R100. J. Bacteriol. **151:**962–970.
47. **Jensen, S., and A. Jernelöv.** 1969. Biological methylation of mercury in aquatic organisms. Nature (London) **223:**753–754.
48. **Jernelöv, A., and A. L. Martin.** 1975. Ecological implications of metal metabolism by microorganisms. Annu. Rev. Microbiol. **29:**61–77.
49. **Jernelöv, A., and T. Wallin.** 1973. Air-borne mercury fall-out on snow around five Swedish chlor-alkali plants. Atmos. Environ. **7:**209–214.
50. **Joensuu, O. I.** 1971. Fossil fuels as a source of mercury pollution. Science **172:**1027–1028.
51. **Johnson, D. L., and R. S. Braman.** 1974. Distribution of atmospheric mercury species near ground. Environ. Sci. Technol. **8:**1003–1009.
52. **Kamps, L. R., R. Carr, and H. Miller.** 1972. Total mercury-monomethylmercury content of several species of fish. Bull. Environ. Contam. Toxicol. **8:**273–279.

53. **Kleckner, N.** 1981. Transposable elements in prokaryotes. Annu. Rev. Genet. **15**:341–404.
54. **Kleckner, N.** 1977. Translocatable elements in procaryotes. Cell **11**:11–23.
55. **Kōmura, I., and K. Izaki.** 1971. Mechanism of mercuric chloride resistance in microorganisms. I. Vaporization of a mercury compound from mercuric chloride by multiple drug resistance strains of *Escherichia coli*. J. Biochem. (Tokyo) **70**:885–893.
56. **Kōmura, 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.
57. **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.
58. **Kopecko, D. J., J. Brevet, and S. N. Cohen.** 1976. Involvement of multiple translocating DNA segments and recombinational hotspots in the structural evolution of bacterial plasmids. J. Mol. Biol. **108**:333–360.
59. **Kothny, E. L. (ed.).** 1973. Trace elements in the environment. American Chemical Society, Washington, D.C.
60. **Landner, L.** 1971. Biochemical model for the biological methylation of mercury suggested from methylation studies *in vivo* with *Neurospora crassa*. Nature (London) **230**:452–454.
61. **Lane, D., and M. Chandler.** 1977. Mapping of the drug resistance genes carried by the r-determinant of the R100.1 plasmid. Mol. Gen. Genet. **157**:17–23.
62. **Matsumura, F., Y. Gotoh, and G. M. Boush.** 1971. Phenylmercuric acetate: metabolic conversion by microorganisms. Science **173**:49–51.
63. **Meissner, P. S., and J. O. Falkinham III.** 1984. Plasmid-encoded mercuric reductase in *Mycobacterium scrofulaceum*. J. Bacteriol. **157**:669–672.
64. **Miki, T., A. M. Easton, and R. H. Rownd.** 1978. Mapping of the resistance genes of the R plasmid *NR1*. Mol. Gen. Genet. **158**:217–224.
65. **Moser, H. C., and A. F. Voight.** 1957. Dismutation of the mercurous dimer in dilute solutions. J. Am. Chem. Soc. **79**:1837–1839.
66. **Murphy, E., and R. P. Novick.** 1979. Physical mapping of *Staphylococcus aureus* penicillinase plasmid pI524: characterization of an invertible region. Mol. Gen. Genet. **175**:19–30.
67. **Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo, H. Kozukue, and S. Mitsuhashi.** 1977. Mercury resistance and R plasmids in *Escherichia coli* isolated from clinical lesions in Japan. Antimicrob. Agents Chemother. **11**:999–1003.
68. **Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo, H. Kozukue, and S. Silver.** 1977. Linkage of mercury, cadmium, and arsenate and drug resistance in clinical isolates of *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **33**:975–976.
69. **Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo, and S. Mitsuhashi.** 1977. Frequency of heavy-metal resistance in bacteria from inpatients in Japan. Nature (London) **266**:165–167.
70. **Nakahara, H., T. G. Kinscherf, S. Silver, T. Miki, A. M. Easton, and R. H. Rownd.** 1979. Gene copy number effects in the *mer* operon of plasmid NR1. J. Bacteriol. **138**:284–287.
71. **Nakahara, H., S. Silver, T. Miki, and R. H. Rownd.** 1979. Hypersensitivity to Hg^{2+} and hyperbinding activity associated with cloned fragments of the mercurial resistance operon of plasmid NR1. J. Bacteriol. **140**:161–166.
72. **Neet, K. E., and G. R. Ainslie, Jr.** 1980. Hysteretic enzymes. Methods Enzymol. **64**:192–226.
73. **Nelson, J. D., W. Blair, F. E. Brinkman, R. R. Colwell, and W. O. Iverson.** 1973. Biodegradation of phenylmercuric acetate by mercury-resistant bacteria. Appl. Microbiol. **26**:321–326.
74. **Ni' Bhriain, N. N., S. Silver, and T. J. Foster.** 1983. Tn*5* insertion mutations in the mercuric ion resistance genes derived from plasmid R100. J. Bacteriol. **155**:690–703.
75. **Nishimura, H., and M. Kumagai.** 1983. Mercury pollution of fishes in Minamata Bay and surrounding water: analysis of pathway of mercury. Water Air Soil Pollut. **20**:401–411.
76. **Novick, R. P., I. Edelman, M. D. Schwesinger, A. D. Gruss, E. C. Swanson, and P. A. Pattee.** 1979. Genetic translocation in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U.S.A. **76**:400–404.
77. **Novick, R. P., and C. Roth.** 1968. Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. J. Bacteriol. **95**:1335–1342.
78. **Olson, B. H., and R. C. Cooper.** 1976. Comparison of aerobic and anaerobic methylation of mercuric chloride by San Francisco Bay sediments. Water Res. **10**:113–116.
79. **Olson, G. J., W. P. Iverson, and F. E. Brinkman.** 1981. Volatilization of mercury by *Thiobacillus ferrooxidans*. Curr. Microbiol. **5**:115–118.
80. **Olson, G. J., F. D. Porter, J. Rubinstein, and S. Silver.** 1982. Mercuric reductase enzyme from a mercury-volatilizing strain of *Thiobacillus ferrooxidans*. J. Bacteriol. **151**:1230–1236.
81. **Pickup, R. W., R. J. Lewis, and P. A. Williams.** 1983. *Pseudomonas* sp. MT14, a soil isolate which contains two large catabolic plasmids, one a TOL plasmid and one coding for phenylacetate catabolism and mercury resistance. J. Gen. Microbiol. **129**:153–158.
82. **Pinney, R. J.** 1978. Survival of plasmid-containing strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in phenylmercuric nitrate and thiomersal. J. Pharm. Pharmacol. **30**:228–232.
83. **Porter, F. D., S. Silver, C. Ong, and H. Nakahara.** 1982. Selection for mercurial resistance in hospital settings. Antimicrob. Agents Chemother. **22**:852–858.
84. **Ramamoorthy, S., T. C. Cheng, and D. J. Kushner.** 1982. Effect of microbial life stages on the fate of methylmercury in natural waters. Bull. Environ. Contam. Toxicol. **29**:167–173.
85. **Ramamoorthy, S., and D. J. Kushner.** 1975. Binding of mercuric and other heavy metals ions by microbial growth media. Microb. Ecol. **2**:162–176.
86. **Ridley, W. P., L. J. Dizikes, and J. W. Wood.** 1977. Biomethylation of toxic elements in the environment. Science **197**:329–332.
87. **Rinderle, S. J., J. E. Booth, and J. W. Williams.** 1982. Mercuric reductase from R-plasmid NR1: characterization and mechanistic study. Biochemistry **22**:869–876.
88. **Rowland, I. R., M. J. Davies, and P. Grasso.** 1977. Volatilization of methylmercuric chloride by hydrogen sulphide. Nature (London) **265**:718–719.
89. **Rowland, I. R., P. Grasso, and M. J. Davies.** 1975. The methylation of mercuric chloride by human intestinal bacteria. Experientia **31**:1064–1065.
90. **Schottel, J. L.** 1978. The mercuric and organomercurial detoxifying enzymes from a plasmid-bearing strain of *Escherichia coli*. J. Biol. Chem. **253**:4341–4349.
91. **Schottel, J., A. Mandal, D. Clark, S. Silver, and R. W. Hedges.** 1974. Volatilisation of mercury and organomercurials determined by inducible R-factor systems in enteric bacteria. Nature (London) **251**:335–337.
92. **Silver, S., and T. G. Kinscherf.** 1982. Genetic and biochemical basis for microbial transformations and detoxification of mercury and mercurial compounds, p. 85–103. *In* A. M. Chakrabarty (ed.), Biodegradation and detoxification of environmental pollutants. CRC Press, Inc., Boca Raton, Fla.
93. **Soldano, B. A., P. Bien, and P. Kwan.** 1975. Air-borne organomercury and elemental mercury emissions with emphasis on central sewage facilities. Atmos. Environ. **9**:941–944.
94. **Spangler, W. J., J. L. Spigarelli, J. M. Rose, R. S. Flippin, and H. H. Miller.** 1973. Degradation of methylmercury by bacteria isolated from environmental samples. Appl. Microbiol. **25**:488–493.
95. **Spangler, W. J., J. L. Spigarelli, J. M. Rose, and H. H. Miller.** 1973. Methylmercury: bacterial degradation in lake sediments. Science **180**:192–193.
96. **Stanisich, V. A., P. M. Bennett, and M. H. Richmond.** 1977. Characterization of a translocation unit encoding resistance to mercuric ions that occurs on a nonconjugative plasmid in *Pseudomonas aeruginosa*. J. Bacteriol. **129**:1227–1233.

97. **Summers, A. O., G. A. Jacoby, M. N. Swartz, G. McHugh, and L. Sutton.** 1978. Metal cation and oxyanion resistances in plasmids of gram-negative bacteria, p. 128–131. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
98. **Summers, A. O., and L. Kight-Olliff.** 1980. Tnl generated mutants in the mercuric ion reductase of the Inc P plasmid, R702. Mol. Gen. Genet. **180**:91–97.
99. **Summers, A. O., L. Kight-Olliff, and C. Slater.** 1982. Effect of catabolite repression on the *mer* operon. J. Bacteriol. **149**:191–197.
100. **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.
101. **Summers, A. O., J. Schottel, D. Clark, and S. Silver.** 1974. Plasmid-borne Hg(II) and organomercurial resistance, p. 219–226. *In* D. Schlessinger (ed.), Microbiology—1974. American Society for Microbiology, Washington, D.C.
102. **Summers, A. O., and S. Silver.** 1972. Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. J. Bacteriol. **112**:1228–1236.
103. **Summers, A. O., and S. Silver.** 1978. Microbial transformations of metals. Annu. Rev. Microbiol. **32**:637–672.
104. **Summers, A. O., and L. I. Sugarman.** 1974. Cell-free mercury(II)-reducing activity in a plasmid-bearing strain of *Escherichia coli*. J. Bacteriol. **119**:242–249.
105. **Summers, A. O., R. B. Weiss, and G. A. Jacoby.** 1980. Transposition of mercury resistance from a transferable R plasmid of *Escherichia coli*. Plasmid **3**:35–47.
106. **Tanaka, M., J. H. Cramer, and R. H. Rownd.** 1976. *Eco*RI restriction endonuclease map of the composite R plasmid NR1. J. Bacteriol. **127**:619–636.
107. **Tanaka, M., H. Harafuji, and T. Yamamoto.** 1982. A gene and its product required for transposition of resistance transposon Tn*2603*. J. Bacteriol. **151**:723–728.
108. **Tanaka, M., T. Yamamoto, and T. Sawai.** 1983. Evolution of complex resistance transposons from an ancestral mercury transposon. J. Bacteriol. **153**:1432–1438.
109. **Tezuka, T., and K. Tonomura.** 1976. Purification and properties of an enzyme catalyzing the splitting of carbon-mercury linkages from mercury-resistant *Pseudomonas* K-62 strain. J. Biochem. (Tokyo) **80**:79–87.
110. **Tezuka, T., and K. Tonomura.** 1978. Purification and properties of a second enzyme catalyzing the splitting of carbon-mercury linkages from mercury-resistant *Pseudomonas* K-62. J. Bacteriol. **135**:138–143.
111. **Timoney, J. F., J. Port, J. Giles, and J. Spanier.** 1978. Heavy-metal and antibiotic resistance in the bacterial flora of sediments of New York Bight. Appl. Environ. Microbiol. **36**:465–472.
112. **Tonomura, K., and F. Kanzaki.** 1969. The reductive decomposition of organic mercurials by cell-free extracts of a mercury-resistant pseudomonad. Biochim. Biophys. Acta **184**:227–229.
113. **Tonomura, K., T. Nakagami, F. Futai, and K. Maeda.** 1968. Studies on the action of mercury-resistant microorganisms on mercurials. I. The isolation of mercury-resistant bacterium and the binding of mercurials to the cells. J. Ferment. Technol. **46**:506–512.
114. **Vonk, J. W., and A. K. Sijpesteijn.** 1973. Studies on the methylation of mercuric chloride by pure cultures of bacteria and fungi. Antonie van Leeuwenhoek J. Microbiol. Serol. **39**:505–513.
115. **Vouk, V. B.** 1979. General chemistry of metals, p. 15–31. *In* L. Friberg, G. F. Nordberg, and V. B. Vouk (ed.), Handbook on the toxicology of metals. Elsevier/North-Holland Biomedical Press, Amsterdam.
116. **Walker, J. D., and R. R. Colwell.** 1974. Mercury-resistant bacteria and petroleum degradation. Appl. Microbiol. **27**:285–287.
117. **Weiss, A. A., S. D. Murphy, and S. Silver.** 1977. Mercury and organomercurial resistances determined by plasmids in *Staphylococcus aureus*. J. Bacteriol. **132**:197–208.
118. **Weiss, A. A., J. L. Schottel, D. L. Clark, R. G. Beller, and S. Silver.** 1978. Mercury and organomercurial resistance with enteric, staphylococcal, and pseudomonad plasmids, p. 121–124. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
119. **Weiss, H. V., M. Koide, and E. D. Goldberg.** 1971. Mercury in a Greenland ice sheet: evidence of recent input by man. Science **174**:692–694.
120. **Westöö, B.** 1973. Methylmercury as percentage of total mercury in flesh and viscera of salmon and sea trout of various ages. Science **181**:567–568.
121. **Williams, J. R., and J. F. Morrison.** 1979. The kinetics of reversible tight-binding inhibition. Methods Enzymol. **63**:437–467.
122. **Wood, J. M.** 1974. Biological cycles for toxic elements in the environment. Science **183**:1049–1052.
123. **Wood, J. M., A. Cheh, L. J. Dizikes, W. P. Ridley, S. Rakow, and J. R. Lakowicz.** 1978. Mechanisms for the biomethylation of metals and metalloids. Fed. Proc. **37**:16–21.
124. **Wood, J. M., F. S. Kennedy, and C. G. Rosen.** 1968. Synthesis of methyl-mercury compounds by extract of a methanogenic bacterium. Nature (London) **220**:173–174.
125. **Yamada, M., and K. Tonomura.** 1972. Formations of methylmercury compounds from inorganic mercury by *Clostridium cochlearium*. J. Ferment. Technol. **50**:159–166.
126. **Yamada, M., and K. Tonomura.** 1972. Further study of formation of methylmercury from inorganic mercury by *Clostridium cochlearium* T-2. J. Ferment. Technol. **50**:893–900.
127. **Yamada, M., and K. Tonomura.** 1972. Microbial methylation of mercury in hydrogen sulfide-evolving environments. J. Ferment. Technol. **50**:901–909.