The Three Modern Faces of Mercury

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The three modern "faces" of mercury are our perceptions of risk from the exposure of billions of people to methyl mercury in fish, mercury vapor from amalgam tooth fillings, and ethyl mercury in the form of thimerosal added as an antiseptic to widely used vaccines. In this article I review human exposure to and the toxicology of each of these three species of mercury. Mechanisms of action are discussed where possible. Key gaps in our current knowledge are identified from the points of view both of risk assessment and of mechanisms of action. ***Key words:*** **amalgam, ethyl mercury, mercury, methyl mercury, thimerosal.** ***Environ Health Perspect*** **110(suppl 1):11–23 (2002).**
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Several reviews of the toxicology of mercury have appeared recently (*1–4*). In this present review I do not repeat all the material presented in these extensive reviews. Instead I focus on three chemical species of mercury that are currently the source of intense public health interest.

Public health concerns about methyl mercury in edible tissue of fish suddenly erupted in 1969 when fish from Lake St. Clair bordering Michigan were found to have high levels. This and other findings discussed in this review have maintained public health concerns over this form of mercury. In 1997, the U.S. Environmental Protection Agency (U.S. EPA) reduced recommended safe intakes of methyl mercury by a factor of about five (*1*), which brought public apprehension to new heights.

The U.S. EPA-recommended safe intake level is referred to as the reference dose and is defined as that dose that can be absorbed daily for a lifetime without a significant risk of adverse effects. The new reference dose was estimated in 1997 to be 0.1 μg methyl mercury/kg body weight/day. This dose implies that the amount of methyl mercury ingested in just one 7-oz can of tuna fish per week would equal or even slightly exceed the new limit, depending on the consumer's body weight. Other federal regulatory agency guidelines allow higher levels (*4*): The U.S. Food and Drug Administration (FDA) guideline is equivalent to 0.5 μg Hg/kg/day, and that of the Agency for Toxic Substances and Disease Registry (ATSDR) is 0.3 μg Hg/kg/day.

Mercury amalgam tooth fillings have been used since the early nineteenth century. Periodically, debates have arisen about the potential danger from mercury. These debates are sometimes referred to as the "amalgam wars." The most recent began with an observation in the 1970s that mercury vapor was released from amalgam, especially during the process of chewing, and that this vapor could be inhaled. Concentrations of mercury vapor measured in the air of the oral cavity approached and even exceeded occupational health limits. The debate has now reached new heights (or lows, depending on the side of the argument) with claims that chronic degenerative diseases of the nervous system such as Parkinson's disease and Alzheimer's disease are caused or exacerbated by mercury released from amalgam.

In the late summer of 1999, concern was expressed by the American Academy of Pediatrics and by the U.S. Public Health Service about the safety of a mercury preservative used in many vaccine preparations routinely administered to infants (*5*). Within about 18 months, the mercury preservative was removed by the manufacturers from all vaccines destined for use in the United States.

The mercury preservative has the molecular formula $CH_3CH_2–Hg–S–C_6H_4–COOH$. This preservative was introduced into vaccines in the early 1930s and has been used ever since (*6*). It was given a clean bill of health by the FDA in 1976 (*7*). However, the U.S. EPA later lowered its allowable safe long-term daily intake for mercury, as discussed above. As a result, a more recent review of thimerosal by the FDA raised questions about possible health risks.

My objective in this review, therefore, is to give the toxicologic background for these three species of mercury and the public health issues surrounding them. In each case, I address human exposure, disposition in the body, and adverse effects. Where possible, I discuss the underlying mechanisms. Emphasis is on the human target. I also discuss ecologic aspects only inasmuch as they may play a role in human exposure.

Quantitative estimates of human health risks are not made in this review. Such a task is left to "expert committees" covering a range of disciplines that cannot be mastered by one individual. Nevertheless, the toxicologic background presented here should give at least a qualitative idea of the type of health risks we face from these forms of mercurys.

Methyl Mercury in Fish

History of Human Exposure

The first methyl mercury compounds were synthesized in a chemical laboratory in London in the 1860s (*8*). Two of the laboratory technicians died of methyl mercury poisoning. This so shocked the chemical community that methyl mercury compounds were given a wide berth for the rest of the century. However, early in the twentieth century the potent antifungal properties of the short-chain alkyl mercury compounds were discovered, leading to application to seed grains, especially for cereal crops. The widespread global use of these mercury compounds was found to be highly protective of seed grain from what otherwise would be devastating fungal infections and the loss of the grain harvest.

Despite this widespread use, few cases of poisoning were reported for the first half of the twentieth century. However, in the late 1950s and early 1960s serious outbreaks of alkyl mercury poisoning erupted in several developing countries (*9*). The largest, most recent outbreak occurred in rural Iraq in the winter of 1971–1972 (*10*). Some 6,000 cases were admitted to hospitals. An epidemiologic follow-up suggested that as many as 40,000 individuals may have been poisoned.

These outbreaks were caused by preparing homemade bread directly from the treated seed grain. Several factors contributed to these mass health disasters. The warning labels were not written in the local language. Well-known symbols for poisons in the Western world, such as the skull and crossbones, have no meaning to rural Arabs unfamiliar with stories of "pirates on the Spanish Main." Typically, a red dye is added to the treated grain to indicate the presence of a fungicide. This was counterproductive, as the victims washed away the dye, thinking they had also removed the poison. The insidious properties of methyl mercury were another important factor, as there is a long

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latent period between ingestion and the first appearance of symptoms (*10*).

Also in the late 1950s, evidence emerged of environmental damage from treated grain (*11*). It was observed in Sweden that predatory birds were developing neurologic disorders. These birds were at the top of a food chain that began with small mammals consuming the treated grain freshly planted in the fields. Analysis of feathers from museum-preserved birds indicated a sharp rise in mercury levels at the time when mercurial compounds were introduced as agricultural fungicides. Because some of these birds were migratory, it was possible to show that elevated mercury levels were found only in those feathers that grew when the birds were in Sweden.

As a control measure, the Swedish investigators decided to check mercury levels in the feathers of fish-eating birds, where mercury levels were assumed to be low. To their astonishment, mercury levels were elevated despite that these birds had no dietary connection with the treated grain. Eventually this finding led to a landmark discovery on the environmental fate of mercury, namely that microorganisms in the aquatic environment are capable of converting inorganic mercury to methyl mercury. This is the first step in the aquatic food chains, where methyl mercury bioaccumulates in higher organisms to plankton, herbivorous, and finally in the top fish predators such as sharks and fish-eating marine mammals. A similar food chain exists in bodies of freshwater, with such species as pike and bass having some of the highest levels of methyl mercury.

The potential for bioaccumulation in aquatic food chains was demonstrated dramatically in two outbreaks of human poisoning in Japan at about this time. The Japanese health authorities in Minamata had been aware for some time that fishermen and their families were suffering from a neurologic disease, exhibiting signs of incoordination, constricted visual fields, and numbness in the extremities. The cause was elusive until a visiting physician from Scotland recognized the neurologic signs and symptoms from cases of occupational methyl mercury poisoning he had seen in England in 1939 (*8*). Eventually the source in Japan was traced to a factory manufacturing acetaldehyde, where inorganic compounds of mercury were used as a catalyst. The producers were unaware that the synthetic process converted some of the mercury to methyl mercury, which was discharged into Minamata Bay. It was difficult to believe that methyl mercury released into a large ocean bay could be bioaccumulated to such an extent that the fish carried levels of methyl mercury that would prove lethal when consumed by humans.

Global Cycling of Mercury

The twin discoveries of biomethylation and bioaccumulation aroused intense interest in the environmental fate of mercury and in pathways to human exposure. Methyl mercury was soon detected in all species of fish and in fish-consuming animals. The source appeared to be inorganic mercury biomethylated by microorganisms in sediments of both fresh and ocean water.

Many anthropogenic sources were identified. Chloralkali plants discharged inorganic mercury as waste into rivers, lakes, and ocean bays. Paper pulp factories likewise discharged a variety of mercury compounds used a slimicides. These practices now have been eliminated, but contamination of aquatic sediments now occurs worldwide because of extensive goldmining operations, for example, in the Amazon basin (*12*).

Large quantities of liquid mercury are used to extract the sedimentary gold found in river beds. Pure gold is recovered when the mercury is evaporated from the amalgam by heating. It has been estimated that over 130 tons of mercury have been released each year into the Amazon basin alone (*13*).

The global cycling of mercury begins with the evaporation of mercury vapor from land and sea surfaces. Volcanoes can be an important natural source (*14*). The burning of fossil fuel, especially coal and municipal waste incineration, is a major anthropogenic source to the atmosphere. Mercury vapor is a chemically stable monatomic gas. Its residence time in the general atmosphere is estimated to be about 1 year. Thus, mercury vapor is globally distributed even from point sources. By processes not yet fully understood, the vapor is oxidized in the upper atmosphere to a water-soluble ionic mercury, which is returned to the earth's surface in rainwater. This global cycling of mercury results in the distribution of mercury to the most remote regions of the planet. For example, environmental mercury levels even in the arctic water may not differ greatly from levels in more southern latitudes.

The global cycling of mercury, along with the processes of biomethylation and bioaccumulation, implies that humans must have consumed methyl mercury in fish dating back to times before *Homo sapiens* evolved (*15*). It could be argued that environmental levels of mercury vapor were much higher in an earlier period of the earth's history when oxygen had not yet appeared in the atmosphere. As levels of oxygen began to rise, increasing amounts of the vapor would be converted to the ionic form. Life forms at those Archean times had to protect themselves not only from this new toxic gas, oxygen, but also from ionic mercury pouring down in rainwater. Perhaps it is no coincidence that those proteins and antioxidant molecules present in today's cellular machinery to protect against oxygen also are our main line of defense against mercury.

Disposition in the Body

The U.S. EPA (*1,2*) and ATSDR (*3*) in recent reviews give extensive details on the disposition of methyl mercury in the body. A brief review and update are provided here.

About 95% of methyl mercury ingested in fish is absorbed in the gastrointestinal tract, although the exact site of absorption is not known. It is distributed to all tissues in a process completed in about 30 hr. About 5% is found in the blood compartment and about 10% in brain. The concentration in red blood cells is about 20 times the concentration in plasma. Methyl mercury crosses the placental barrier. Levels in cord blood are proportional to but slightly higher than levels in maternal blood. Levels in the fetal brain are about 5–7 times that in maternal blood (*16*). Brain-to-blood ratios in adult humans and other primates are approximately in the same range.

Methyl mercury avidly accumulates in growing scalp hair. Concentrations in hair are proportional to simultaneous concentrations in blood but are about 250 times higher. They are also proportional to concentrations in the target tissue, the brain (*16*). Longitudinal analysis of strands of scalp hair can recapitulate past blood and brain levels (*17*). Hair and blood are used as biologic indicator media for methyl mercury in both the adult and fetal brain (in the latter case, maternal hair or cord blood).

Methyl mercury is slowly metabolized to inorganic mercury mainly by microflora in the intestines, probably at a rate of about 1% of the body burden per day. Some demethylation also occurs in phagocytic cells The biochemical mechanism is unknown. Although methyl mercury is the predominant form of mercury during exposure, inorganic mercury slowly accumulates and resides for long periods in the central nervous system. It is believed to be in an inert form, probably insoluble mercury selenide (*18*).

Urinary excretion is negligible, of the order of 10% or less of total elimination from the body. Methyl mercury undergoes extensive enterohepatic cycling. It is secreted into bile and partly reabsorbed into the portal circulation and thereby returned to the liver. A fraction of the biliary mercury is converted by microflora to inorganic mercury. The latter is reabsorbed only to a small extent. Thus, most of the methyl mercury is eliminated from the body by demethylation and excretion of the inorganic form in the feces. The processes of biliary secretion and demethylation by

microflora do not occur in suckling animals. The role of these two processes in suckling human infants is unknown.

The high mobility of methyl mercury in the body is not due to lipid solubility, as claimed in some textbooks. Methyl mercury is present in the body as water-soluble complexes mainly if not exclusively attached to the sulfur atom of thiol ligands. It enters the endothelial cells of the blood–brain barrier as a complex with L-cysteine. The process is so specific that the complex with the optical isomer D-cysteine is not transported. Structurally, the L-complex is similar to the large neutral amino acid L-methionine and is carried across the cell membrane on the large neutral amino acid carrier (*19*).

Methyl mercury is pumped out of mammalian cells as a complex with reduced glutathione. For example, it is secreted into bile as a glutathione complex. The glutathione moiety is degraded in the bile duct and gall bladder to a dipeptide and finally to the L-cysteine complex. Presumably, in this form it is reabsorbed into the bloodstream to be returned to the liver, thereby completing the enterohepatic cycle (*20–22*).

The elimination of methyl mercury from the body approximates first-order kinetics. Half-times vary from one tissue to another but generally fall in the range of 45–70 days. Thus, individuals with long-term regular exposure to methyl mercury attain a steady-state body burden in about 1 year (five half-times).

Several thiol-containing complexing agents have been successfully used to remove methyl mercury from the body [e.g., in the Iraq outbreak; see Clarkson et al. (*23*)]. An interesting example is a thiol-containing resin that, when given by mouth, traps the methyl mercury secreted in bile and carries it into the feces. Perhaps the most promising complexing agent is *N*-acetylcysteine (*24*). It enhances methyl mercury excretion when given orally, has a low toxicity, and is widely available in the clinical setting.

Adverse Effects

The major toxic effects of methyl mercury are on the central nervous system. Its toxic action on the developing brain differs in both mechanism and outcome from its action on the mature organ, so the two actions are treated separately here [for detailed reviews, see U.S. EPA and ATSDR (*2,3*)]. However, recent reports have raised the possibility that methyl mercury may have adverse effects on other targets in the body.

The mature central nervous system. The action of methyl mercury on adults is characterized by a latent period between exposure and onset of symptoms. The period can be several weeks or even months, depending of the dose and exposure period. Perhaps the most dramatic example of latency was in the case of severe, ultimately fatal poisoning of a chemistry professor from exposure to dimethyl mercury (*25*). A single exposure from a spill of liquid dimethyl mercury took place in August. The professor continued her normal professional work without any apparent ill effects. In November she presented a paper at an overseas conference. It was not until late December that the first symptoms appeared. Within a few weeks the full syndrome of severe methyl mercury poisoning became manifest. Despite many decades of research on methyl mercury toxicology, the mechanism underlying this long latent period is still unknown.

Paresthesia, a numbness or a "pins and needles" sensation, is the first symptom to appear at the lowest dose (*10*). This may progress to cerebellar ataxia, dysarthria, constriction of the visual fields, and loss of hearing. These signs and symptoms are caused by the loss of neuronal cells in specific anatomical regions of the brain. For example, ataxia results from the loss of the granule cells in the cerebellum. The neighboring Purkinje cells are relatively unaffected.

The mechanism underlying the focal damage to the adult brain is still not established with any certainty. Syversen (*26*) examined the effect on protein synthesis in various areas of brain of rats poisoned with methyl mercury. Protein synthesis was inhibited in all three areas studied—the granule and Purkinje cells of the cerebellum, and the cells from the cortical areas of the brain. Protein synthesis recovered in two types of neurons but not in the granule cells. These data suggest that the focal damage to the brain is not due to the initial insult but depends on the capacity of neuronal cells for repair, as suggested by Jacobs et al. (*27*). Apparently the small granule cells lack the repair systems present in the other larger cells. Sarafian et al. (*28*) have suggested that the selective vulnerability of cells in the nervous system may arise from a "critical absence of inherent protective mechanisms."

Cellular defenses may be decisive in determining the toxic outcome and deserve further study. If we understand the defense mechanism, we may be able to predict which individuals are most susceptible. Thiol compounds probably play a key role (*29*). Resistant cells have higher levels of the thiol-containing peptide glutathione (*30*). Glutathione also plays a key role in the excretion of methyl mercury [for further discussion, see Sarafian et al. (*28*)].

Selenium is a dietary component that may affect the disposition and toxicity of methyl mercury. Ganther et al. (*31*) were the first to observe that selenium compounds could delay the onset of toxic effects in animals fed methyl mercury in tuna. This gave rise to a series of studies by his group and others. However, despite promising indications from animal studies, no definite studies have yet been carried out on human populations co-exposed to methyl mercury and selenium [for a recent review, see National Research Council (*4*)].

Methyl mercury is converted to inorganic mercury in the brain. It is possible that the inorganic ion is the proximate toxic agent responsible for the brain damage. However, experiments on rats comparing methyl and ethyl mercury compounds suggest that the intact methyl mercury radical is the toxic agent (*32*). Ethyl mercury converts to inorganic mercury more rapidly than methyl mercury, but the latter produces more severe brain damage.

Autopsy samples taken years after exposure to methyl mercury reveal that inorganic species account for most if not all of the remaining mercury in the brain (*33*). It has been suggested that the long residence time is due to inorganic mercury forming an insoluble complex with selenium (*18*). However, Charleston et al. (*34*) have challenged this view, suggesting that inorganic mercury released in brain tissue from methyl mercury may be the proximate toxic agent. The toxicologic role of inorganic mercury remains a matter of debate.

Other adverse effects in adults. Most epidemiologic studies and clinical reports on adults [for review, see WHO (*18*)] have identified neurologic signs and symptoms of poisoning associated mainly with the central nervous system. An important exception is an extensive study on the relationship between fish consumption, levels of mercury in urine and scalp hair, and risk of cardiovascular disease in adult male residents living in eastern Finland (*35*). A statistically significant correlation was found between mercury levels and cardiovascular disease even after correction for numerous cardiovascular risk factors. A subsequent study by the same group found a correlation between mercury accumulation and accelerated progression of carotid atherosclerosis (*36*).

However, it is difficult to draw firm conclusions. Stress, believed to be a major risk factor (*37*), was not directly measured. The highest recorded hair level of 15.7 ppm was more than six standard deviations from the mean. A histogram of hair levels was not presented, but these statistics imply that a small percentage of the study group had high mercury levels. Outlying and "influential points" may play a major role in studies of this type [e.g., Myers et al. (*38*)]. It would have been of interest to see if these

correlations persisted when the very high mercury levels were excluded.

Given the serious health implications, a repeat of this study in another population is needed. If these findings are confirmed, two long-held dogmas may have to be abandoned, namely, that methyl mercury primarily affects the central nervous system and that the prenatal period (see below) is the most susceptible part of the life cycle.

Effects on the developing brain. The first indication of the special susceptibility of the developing brain to prenatal exposure to methyl mercury came from anecdotal reports from Minamata that mothers with mild symptoms gave birth to offspring with severe brain damage. The Iraq outbreak confirmed that severe brain damage can occur from high prenatal exposure. A milder syndrome was also identified in the Iraq outbreak (*39*). Children apparently normal nevertheless had a history of delayed achievement of developmental milestones and, on examination, exhibited neurologic abnormalities such as brisk tendon reflexes. When the prenatal exposure was determined from mercury levels in maternal hair samples, it was possible to construct a dose–response relationship between peak hair mercury levels in pregnancy versus number of abnormal offspring showing developmental delays and abnormal neurologic findings (*39,40*).

This study was of interest for two reasons. First, a dose–response relationship has been established for prenatal exposures to a toxicant; that is, a dose to the mother predicts the probability of effects in her offspring. This discovery laid the groundwork for further quantitative estimate of prenatal risks from methyl mercury. No doubt this relationship was made possible by the parallel between levels of mercury in maternal and fetal tissues. Indeed, it was later demonstrated in another study that maternal hair levels of mercury were proportional to levels in autopsy samples of brain tissue from infants who died shortly after birth (*16*). Ernhart et al. (*41*), at virtually the same time, published a dose–response curve for prenatal exposures to ethanol. Probably as with methyl mercury, the high mobility of ethanol ensures that maternal levels predict those in the fetus.

A second unique aspect of the Iraqi study (*40*) was the application of continuous single-strand hair analysis to determine peak levels during pregnancy. By the use of X-ray fluorescence analysis, it was possible to measure the concentration of mercury in contiguous 2-mm segments of a single strand of maternal hair, thus giving a complete picture of mercury levels in pregnancy. Moreover, because exposure in Iraq took place over a single period of time, it was possible to fit the hair data with a single compartment model covering both the rising levels during intake and the exponential fall afterward. This allowed the true peak value to be calculated from the curve by fitting all the data points, as opposed to taking the single highest value, which would be more prone to error. It is unfortunate that this method of analysis was not used in subsequent studies of prenatal exposure.

The studies of the Iraq outbreak confirmed what had been suspected from the outbreak in Japan, that the fetal brain was more sensitive than the mature organ. A Swedish expert group (*11*) had estimated a threshold level for neurologic effects in adults at about 50 ppm in hair, an estimate confirmed by the findings in Iraq (*10*). This level may be compared with an estimated threshold as low as 10 ppm for prenatal effects (milestones of development and neurologic change) in Iraq (*40*). As these studies were being conducted and early findings presented at scientific meetings, concern arose that methyl mercury in fish normally consumed in our diet might present risks of prenatal damage. Several large epidemiologic studies were conducted in people consuming freshwater fish (*42*) and ocean water fish (*43*), and large-scale studies are continuing even to this day focusing on neuropsychologic development [e.g., (*44,45*)]. These studies have not yet provided a consistent picture of the lowest prenatal levels that offer a measurable risk of damage to the developing brain. However, at this time it can be said that these studies on fish-eating populations taken as a whole are consistent with the original findings in Iraq that effects can be detected in the range of 10 ppm in maternal hair. Indeed, a U.S. EPA reference dose published recently (*2*) is identical to the previous estimate from the Iraq data (*1*).

Mechanism of prenatal damage. Several studies have given some insight into the mechanism underlying prenatal brain damage. Autopsy brain samples from the Minamata outbreak indicated widespread damage to all areas of the fetal brain, as opposed to the focal lesions seen in adult tissue. Microcephaly was also observed (*18*). Autopsy tissue from Iraq also gave invaluable clues to the nature of prenatal brain damage (*46*). The normally ordered parallel arrays of neuronal cells in the cortex were found to be disrupted, which is indicative of a general disturbance in the developmental growth of the brain. Moreover, neurons were present such as Purkinje cells that had failed to migrate to the cerebellum. These findings from both Japan and Iraq indicated that the most basic processes in brain development were affected, namely, neuronal cell division and migration.

Experimental work in animals and *in vitro* has provided a mechanism explaining why methyl mercury inhibits both cell division and migration (*47–49*). These studies show that the cytoarchitecture first affected at the lowest levels of methyl mercury is the microtubular system. Intact microtubules are required for both cell division and migration. Microtubules are formed by a treadmilling process whereby assembly from α-and β-tubulin monomers occurs at one end and disassembly at the other. Apparently, methyl mercury binds to thiol ligands (–SH) groups on the tubulin monomers and blocks the assembly process. The disassembly continues unchanged, thus leading to the complete loss of the tubule.

Other adverse effects of prenatal exposures. Studies in 7-year-old children revealed an elevation in both systolic and diastolic blood pressure that correlated with prenatal exposure to methyl mercury (*50*). The study was conducted in the Faroe Islands on a large cohort of children whose mothers had ingested methyl mercury mainly from whale meat but also from fish. This effect is seen only at the lower range of blood levels from about 1 to 10 μg Hg/L. Above this range no further increase is seen, even at blood levels in the mother ranging as high as 250 μg Hg/L.

As elevated blood pressure in children may be indicative of later cardiovascular problems, this finding is of public health concern. Further work is needed to confirm this finding and to understand its mechanism.

Thimerosal in Vaccines

Mercury in the thimerosal molecule is in the form of ethyl mercury (CH_3CH_2-Hg^+), for which there is limited toxicologic information. Thus, estimates of health risks from thimerosal in vaccines (*7*) were based on the assumption that ethyl mercury is toxicologically similar to its close chemical relative, methyl mercury (CH_3–Hg^+), about which much is known. However, as discussed below, there are reasons to believe that this assumption is not necessarily correct for all aspects of the disposition and toxicity of ethyl mercury compounds, including thimerosal.

History of Human Exposure

Ethyl mercury compounds were first synthesized in the nineteenth century in a chemical laboratory in London (*8*). In the late 1880s diethyl mercury was first used in the treatment of syphilis, a practice soon abandoned because of the toxic properties of this agent. However, early in the twentieth century, the fungicidal properties of the short-chain alkyl mercury compounds led to commercial applications in agriculture. For

example, they are especially effective in a plant root disease in wheat caused by *Telletia triticia*. In fact, many different organic mercury compounds were being used to prevent seed-borne diseases of cereal by 1914 (*51,52*).

Generally speaking, the ethyl mercury fungicides were used effectively and safely. However, a number of outbreaks of poisoning occurred in some developing countries (*8*). For example, two outbreaks occurred in rural Iraq in 1956 and 1960 from the misuse use of the fungicide ethyl mercury toluene sulfonilamide (*51*). The farmers' families prepared homemade bread directly from the treated grain instead of planting it. Hundreds of cases of severe poisoning occurred, many of which had a fatal outcome. Cases of ethyl mercury poisoning have occurred in China as recently as the 1970s. The exposure pathway was the same as in Iraq: The farmers consumed rice treated with ethyl mercury chloride (*53*).

Ethyl mercury in the form of thimerosal has found wide application in medicine as a disinfectant. Axton (*54*) reported case histories of four children and two adults severely poisoned by accidental exposure. Five of the six cases died. Rohyans et al. (*55*) reported a case of severe poisoning from treatment of an infected ear. Pfab et al. (*56*) reported on an attempted suicide from drinking a solution of thimerosal, resulting in severe poisoning. Treatment of infants with omphaloceles resulted in high levels of mercury in autopsy tissues (*57*). Cases of human poisoning have also occurred from infusion of large volumes of plasma containing thimerosal as a preservative (*58,59*).

Disposition in the Body

If, after injection of the vaccine, the thimerosal molecules were to remain intact for a period sufficient to allow diffusion to the bloodstream and thence to the kidneys, rapid excretion might take place. The carboxyl group of thiosalicylic acid might allow thimerosal to be a substrate for the system responsible for the tubular secretion of weak acids. Rapid urinary excretion of thimerosal would then be possible.

This possibility seems unlikely. Thimerosal contains the ethyl mercury radical attached to the sulfur atom of the thiol group of salicylic acid. Generally, mercuric ions bind tightly but reversibly to thiol ligands (*60*). It is likely, therefore, that the ethyl mercury cation will dissociate from the thiosalicylic acid moiety immediately after injection to bind to the surrounding thiol ligands present in great excess in tissue proteins.

Thimerosal is used as a thiol titration reagent in numerous experimental studies [e.g., Elferink (*61*)]. This application would be possible only if rapid dissociation of ethyl mercury took place in the presence of endogenous thiol groups in the cells and tissues under study. *In vitro*, the thiosalicylate moiety is degraded by oxidation to dithiosalicylic acid followed by further oxidation to 2-sulfinobenzoic acid (*62*).

Ulfvarson (*63*) demonstrated that the type of anion attached to the alkyl mercury radical made little difference to the ultimate disposition in the body. These include such anions as hydroxyl, cyanide, and even the thiol-containing propane diolmercaptide. These findings suggest that the mercury radical rapidly dissociates from the anion in the parent compound to attach to ligands in tissues.

Therefore, it is assumed that administration of thimerosal results in the immediate release of the ethyl mercury to the surrounding tissues. Toxicologically, ethyl mercury in thimerosal is assumed to follow the same pathways of disposition as ethyl mercury absorbed into the body from other ethyl mercury compounds.

Patterns of tissue disposition and excretion. Little is known about mercury levels in human tissue after administration of thimerosal. Suzuki et al. (*58*) reported levels of total and inorganic mercury in the tissues of a 13-year-old boy who had died 5 days after receiving infusion of artificial human plasma containing thimerosal as a preservative. The infusion of plasma had taken place over a period of 6 months, with a total estimated dose of 284–450 mg Hg. The levels of total mercury from high to low were in the following order: liver, kidneys, skin, brain, spleen, and lowest in plasma. The red cell levels were at least 10-fold higher than plasma. The distribution pattern is generally similar to that seen for methyl mercury. These findings are supported by studies in primates dosed with thimerosal (*64*).

It is interesting that hair levels were high. The section proximal to the scalp had a level of 187 μg Hg/g, whereas the level in blood was approximately 7 μg Hg/mL, giving a hair-to-blood ratio of 27:1. This is lower than the commonly assumed ratio for methyl mercury of 250:1, but possible redistribution of mercury in autopsy blood samples and uncertainty in the length and exact position of the proximal segment make the estimates of the hair-to-blood ratio uncertain. However, it does indicate that ethyl mercury, like methyl mercury, is accumulated in scalp hair.

Matheson et al. (*59*) reported on blood and urine levels in one patient exposed to thimerosal in long-term injections of gamma globulin. Specifically, they reported on levels of total and inorganic mercury before and after one injection of gamma globulin. The data allow a rough calculation of how similar the observed increase in blood level is to that expected from methyl mercury. The injected dose was 0.6 mL/kg containing 50.3 μg Hg/mL, to give a total mercury dose of 30 μg Hg/kg. The disposition parameters for methyl mercury in adult humans (*18*) predict that 5% of the dose—1.5 μg Hg—is deposited in the blood compartment. The volume of the latter is 70 mL, assuming the blood compartment is 7% of the body weight. Thus, 1.5 μg Hg would be deposited in 70 mL of blood to give an increase on concentration of 1500/70 μg Hg/L = 21 μg Hg/L. The observed increase was 18 μg Hg/L. This calculation suggests that the disposition of mercury after thimerosal is not very different from that expected from methyl mercury.

The pattern of urinary excretion also indicates similarities to that with methyl mercury. Matheson et al. (*59*) do not quote a specific figure for the change in urinary excretion after injection of thimerosal, but the graph published in their article indicates little change. They state that 90% of the total mercury in urine was in the inorganic form. Adult humans exposed to methyl mercury excrete little mercury in urine and all in the inorganic form (*10*).

Conversion to inorganic mercury. There is, however, one important difference from methyl mercury illustrated in the report from Matheson et al. (*59*). Inorganic mercury accounted for about 50% of the total mercury in blood samples collected from this patient. This is in marked distinction from methyl mercury, where inorganic mercury accounts for only about 10% of total mercury in blood (*10*).

Similar findings were made in the case described by Suzuki et al. (*58*). A significant fraction of the total mercury in both gray and white matter of the brain was in the form of inorganic mercury of the order of 30–40%. The kidney cortex had the highest percentage. These findings are confirmed by studies on experimental animals (*32*). Blood and tissue levels, including the brain, were higher in animals dosed with ethyl mercury compared with an equivalent dose of a methyl mercury compound. The high tissue levels of inorganic mercury seen in both humans and animals indicate that ethyl mercury breaks down to inorganic mercury more rapidly than methyl mercury.

Blood levels from thimerosal in vaccines. Stajich et al. (*65*) are the first and only investigators to measure disposition of mercury before and after administration of vaccines containing thimerosal. They reported on blood levels of mercury before and 48–72 hr after administration of a single dose of hepatitis vaccine in the first week after birth. Seven

were preterm infants (average birth weight, 748 g) and five were term infants (average birth weight, 3,588 g). Prevaccination blood levels were 0.04–0.5 μg Hg/L. The preterm infant levels rose to an average value of 7.4 μg Hg/L, whereas the levels in term infants were 2.2 μg Hg/L.

These levels are similar to those expected from methyl mercury. The dose was the same for all infants, 12.5 μg Hg. Five percent, or 0.625 μg Hg, should be deposited in the blood compartment, which is assumed to be 8% of the infant's body weight. Thus, for the preterm infants, 0.625 μg Hg would be deposited in a blood volume of 0.08 × 748 = 60 mL to give a predicted concentration of 0.625 × 1,000/60 = 10.4 μg Hg/L. This compares to an observed increase of 6.8 μg Hg/L. The predicted increase for the term infants based on methyl mercury is 0.625 × 1,000/287 = 2.2. The observed increase was identical, 2.2 μg Hg/L.

These estimates also suggest that the disposition of mercury after a dose of thimerosal is similar to that expected from methyl mercury. However, these estimates can be regarded as approximate at best. Individual values for each infant were not reported. The blood levels in samples collected between 48 and 72 hr may not have been the true maximum levels after distribution of the injected dose.

A preliminary report by Pichichero et al. (*66*) indicated blood levels of mercury in infants lower than what would be expected from methyl mercury. These infants, ≤6 months of age, had received some vaccines containing thimerosal. Most blood samples were collected 1 week or more after the last vaccination. The highest recorded level was 4.1 μg Hg/L, and many were below the detection limit of about 0.5 μg Hg/L. When the authors performed calculations similar to those described above, they found that the methyl mercury dispositional parameters predicted significantly higher levels than those observed.

The main difference in design of the Pichichero et al. (*66*) study compared with that of the Stajich et al. (*65*) study is that in the former, samples were collected much later after the last dose of thimerosal. Both studies could be consistent if the half-time for ethyl mercury in blood is shorter than that for methyl mercury. The time after collection of 48–72 hr is too short for a measurable decline in blood levels in the Stajich et al. study. The urine levels in the Pichichero et al. study were low, which is consistent with other observations on thimerosal discussed previously. Significant amounts of mercury were found in fecal samples that might account for the lower blood levels.

In conclusion, both animal and human studies indicated that the pattern of tissue disposition of ethyl mercury was qualitatively similar to that of methyl mercury, with brain levels of the intact mercury being slightly higher for methyl than for ethyl. The conversion in body tissues to inorganic mercury appears to be substantially faster from ethyl than from methyl. We know little about the kinetics of elimination of mercury from the body when dosed with ethyl mercury compounds. The feces is the main pathway of elimination. The residence time in the body is probably shorter for ethyl, but quantitative data are lacking.

Adverse Effects

Ball et al. (*7*) have reviewed the animal literature on ethyl mercury toxicity. Toxicity tests conducted before marketing thimerosal in 1931 in several animal species involved high (≤45 mg Hg/kg) acute doses with only a short follow-up period (*67*). The studies have little relevance to today's concern over risks from low doses from vaccines. Chronic carcinogenicity studies were conducted on rats with twice weekly doses ranging from 30 to 1,000 μg Hg/kg. Weight loss was observed at the highest-dose group. Unfortunately, no brain histopathology was reported, making these studies difficult to extrapolate to current human exposure.

Magos et al. (*32*) compared the target organ toxicity of ethyl and methyl mercury in rats. Five daily doses of 8 mg Hg/kg were given by gavage for 5 consecutive days. Brain and kidney histopathology was examined 3 and 10 days after the last dose. In general, kidney damage was more severe after ethyl mercury and brain damage more severe after methyl mercury. However, when the dose of ethyl mercury was increased by only 20%, the brain damage was similar or slightly more severe than that seen from the lower dose of methyl mercury.

Magos (*68*) has reviewed the published cases of human poisoning resulting from exposure to thimerosal. Severe cases of poisoning can result in the same neurologic signs and symptoms associated with methyl mercury poisoning, for example, constriction of the visual fields. Ethyl mercury poisoning was characterized by a latent period of several weeks between first exposure and onset of the first symptom of poisoning, as has been observed for methyl mercury. In distinction from methyl mercury, signs of renal damage are found in severe cases.

A detailed review of case histories on exposure to ethyl mercury including thimerosal allowed Magos (*68*) to construct a table comparing blood levels at the time of onset of symptoms. To estimate such blood levels from samples collected at a later date, he assumed a half-time in blood of 50 days. Severe intoxication was associated with blood levels in excess of 2,000 μg Hg/L, with milder intoxication at 1,000 μg Hg/L. Five cases with blood levels of 140–650 μg Hg/L had no reported adverse effects. Only 18 cases were involved, with ages ranging from infants to 79 years. Because of the small number of individuals, no statistical evaluation is possible in terms of dose–response relationships. However, the data suggest that ethyl mercury is somewhat less potent in producing neurologic signs and symptoms than methyl mercury, where the threshold for neurologic effects has been estimated at about 200 μg Hg/L (*18*).

Allergic reactions. Allergic response, usually by skin application, is well known to occur from organomercurial compounds, including thimerosal (*69*). Santucci et al. (*70*) have demonstrated that contact allergy to thimerosal is due to the ethyl mercury radical and that it is indistinguishable in its allergic action from methyl mercury. Goncalo et al. (*71*) also noted that allergy to thimerosal was mainly related to the mercurial component, but some allergic reactions may be due to the thiosalicylic acid component.

Allergy to thimerosal and related mercury compounds is a rare event. There is evidence that individuals with certain polymorphisms in glutathione transferase genes may be susceptible to allergic reactions to thimerosal (*72*). Glutathione is necessary for the biliary excretion of methyl and inorganic mercury (*20*), and intracellular glutathione is protective against the toxicity of methyl mercury (*29*).

Other effects. Only one case of acrodynia has been reported from exposure to thimerosal (*59*). This occurred in a 20-year-old man receiving regular gamma globulin infusions containing thimerosal as a preservative. The total dose was estimated as 40–50 mg Hg.

Acrodynia is now a rare disease. It was well known to pediatricians when children were exposed to mercury compounds in teething powders, vermifuge preparations, and diaper disinfectants (*73*). The children characteristically have pink hands and feet (hence the alternative name "pink's disease"). They are photophobic and suffer from joint pains. A typical picture is that of a child with head buried in a pillow and continually crying. The distraught parents invariably take the child for medical attention. Thus, it is unlikely that cases of acrodynia would escape attention. It is interesting that not a single case of acrodynia has been reported from exposure to vaccines despite the propensity of thimerosal to produce this syndrome when given in sufficient amounts.

An important characteristic of the disease is that only 1 child in 500 exposed children develops this disease. The reason for

this susceptibility is not known but presumably has a genetic basis. It has made the identification of mercury as the causal agent very difficult (*74*).

Although it is unlikely that thimerosal in vaccines has ever caused acrodynia, the clinical history of this disease gave rise to the idea that a genetic susceptibility to mercury may underlie other rare childhood diseases. For example, Bernard et al. (*75*) claim that autism is a "novel form of mercury poisoning" that occurs in rare infants who are genetically susceptible.

In attempts to estimate health risks from thimerosal in vaccines, a key gap in our knowledge on the human toxicology of mercury has become apparent. Little is known about the tissue disposition and toxicity of mercury in human infants, or in animals for that matter.

Current health risks from thimerosal in vaccines depend on the assumption that ethyl mercury is equally toxic to the nervous system as methyl mercury.

For example, Ball et al. (*7*) quote the U.S. EPA reference dose as a guide for safe intake of ethyl mercury. However, this reference dose is based on data from prenatal exposures to methyl mercury. In the case of vaccines, we are dealing with postnatal exposures. The prenatal stage is believed to be the window of highest susceptibility to methyl mercury (*18*). Also, evidence reviewed above suggests that the systemic toxicity of thimerosal is less than that of methyl mercury compounds.

Dental Amalgam and Mercury Vapor

History of Human Exposure

Dental amalgam. Dental amalgam was introduced more than 150 years ago as a tooth filling restoration. Today it is still the most popular restorative despite the introduction of new types of fillings. It is an amalgam of several metals, but mercury is the principal component, usually accounting for about 50% by weight. Other metals include silver and copper. Periodically throughout the history of dental amalgam, concern has been expressed about health risks because of the high content of mercury. These recurrent concerns have sometimes been referred to as the "amalgam wars," reflecting the arguments between the proponents and opponents of its use. Today we are in the third amalgam war, which started in the early 1970s and continues today unabated.

This present war was started by reports that amalgams released mercury vapor that could be inhaled. Concentrations of mercury vapor in the air in the oral cavity were shown to exceed occupational health standards. This finding provoked further investigations and a series of reviews of potential health risks from amalgam [e.g., World Health Organization (*76*)]. It was soon realized that comparison with occupational health standards gave misleadingly high estimates of health risks. The concentration of mercury vapor in the oral cavity could indeed reach occupational health danger levels, but the quantity of vapor was small because the volume of the cavity was small. Eventually more meaningful data have been obtained indicating that the retained vapor is much less than that inhaled under conditions of occupational exposures, except for an interesting exception to be discussed below.

Levels of mercury vapor in the ambient atmosphere are so low that intake from this source is negligible. Thus, with the exception of certain occupational exposures, dental amalgam is the main source of human exposure to mercury vapor. As discussed further below, other forms of mercury released from amalgam do not appear to be important. Thus, a consideration of health risks from amalgam depends on our knowledge of the toxicology of inhaled mercury vapor and the quantities released and inhaled from amalgam restorations.

Mercury vapor. An important source book on this topic is Leonard Goldwater's *Mercury: A History of Quicksilver* (*77*). Ramazinni's *Diseases of Workers* (*78*), one of the first books on occupational disease, contains fascinating historical details of occupational exposures to this metal, as does Donald Hunter's masterpiece *Diseases of Occupations*, last printed some 30 years ago (*8*). The most important recent source books are by the World Health Organization (*76*), ATSDR (*3*), and the U.S. EPA (*1,2*).

Mercury vapor is a monatomic gas that evaporates from liquid metallic mercury or is produced by chemical or physical processes from chemical compounds of mercury. The principal ore is cinnabar, a brilliant crimson crystalline form of mercuric sulfide. The largest and oldest mine is located in Almaden, Spain, which has production records dating back many centuries. These records show spurts in production as new uses of mercury were discovered. Perhaps its oldest application was in the form of cinnabar first used by the Chinese to make red ink for official documents many centuries before the modern era. Thus, mercury has the dubious distinction as the founder of bureaucracy.

In its liquid metallic form, mercury has found innumerable applications. Spread as a thin film over a sheet of glass, mercury makes an excellent reflecting surface. An island in the vicinity of Venice, Italy, is famous for its mirror makers dating back to the middle ages. Ramazinni (*78*) describes the "mirror makers of Venice" in these terms:

> At Venice on the Island called Murano where huge mirrors are made, you may see these workers gazing with reluctance and scowling at the reflection of their own sufferings in their mirrors and cursing the trade they have adopted.

Besides mirror making and gilding, it was also used in the extraction of gold and silver. Enormous quantities were shipped for extraction of gold and silver from Almaden, as well as from mines in Peru during the Spanish occupation of Central and South America. Today, mercury continues to be used in the largest gold rush of the twentieth century in the Amazon basin (*13*). Exposure to mercury vapor and contamination of local fish also appears to be occurring at other gold mining operations around the world (*79–81*).

Liquid mercury has found important applications in scientific instruments and measuring devices. It has found its way into many homes in thermostats, barometers, and thermometers. It has been contained in household gas regulators. Recent attempts by power companies to replace such meters has led to spills in the homes because of the careless nature by which the meters were removed. As many as 200,000 homes in the Chicago, Illinois, area may have been contaminated in this way (*82*).

Perhaps the earliest medical application may have been in ancient Egypt, where mercury compounds in ointments were used to treat skin infections. The skin sores from syphilis may have prompted the early application of mercury to combat this disease as it swept across Europe soon after the return of Christopher Columbus. Treatment included not only the application of mercury compounds but also the exposure of the person's skin surfaces to mercury vapor. Paracelsus was one of the first advocates for the mercury treatment, which included skin exposure to the vapor. He soon realized, however, that a little too much mercury might kill the patient, hence his famous dictum "Dose makes the poison." So it may be argued that mercury played a key role in establishing the basic guiding principle in modern toxicology and risk assessment.

Thus, the signs and symptoms of poisoning from inhalation of mercury vapor, at least in its severe form, have been known for centuries if not millennia. Severe damage to the brain, kidneys, and lungs may result, depending on the length and intensity of exposure. As discussed below, today's concerns are with subtle changes in brain and kidney function associated with occupational exposure and possibly with amalgam under certain circumstances. Speculations have been put forward that inhalation of mercury vapor from amalgam may be a causative factor in chronic degenerative diseases of the brain such as Alzheimer's disease.

Disposition in the Body

Mercury vapor. Several recent reviews have discussed in detail the uptake, distribution, excretion, metabolism, and kinetics of inhaled mercury vapor (*1,3,76*). A brief summary is presented here with an update from recent reports.

About 80% of inhaled mercury vapor is retained in the body. However, approximately 7–14% is exhaled within a week after exposure. The half-time of the process is about 2 days. The dissolved vapor accumulates in red blood cells and is carried to all tissues in the body. It crosses the blood–brain and placental barriers. The half-time of distribution to the plasma compartment is approximately 5 hr (*83*). The amount of time to reach a peak value is 9 hr, with a range of 7–24 hr in nine adult subjects. The amount of mercury in plasma at the time of the peak concentration was 4% of the inhaled dose (95% confidence limit, 3–5%).

Approximately 7% is deposited in the cranial region after a single exposure to nontoxic levels of the vapor. The kidney is the main depository.

Once the vapor has entered the cell, it is subject to oxidation to divalent inorganic mercury. The oxidation step is catalyzed specifically by the enzyme catalase, with endogenously produced hydrogen peroxide as the other substrate. The process is inhibited by ethanol. As a result, workers imbibing a moderate amount of an alcoholic drink retain less of the inhaled vapor. The finding that the half-time for exhalation from the lung is about 2 days suggests that the half-time for the oxidation in body tissues is about the same.

Studies with radioactive tracers indicate that the rate of overall excretion of mercury from the body can be described by a single half-time of about 58 days, corresponding to an excretion rate of slightly more than 1% of the body burden per day. Most tissues have the same or shorter half-times.

The decline in plasma levels of mercury consists of at least two components: a short half-time of less than 1 day and a longer one of about 10 days. Blood levels therefore reflect recent exposure.

Excretion takes place via both urine and feces. Urinary mercury originates mainly from mercury in kidney tissue. Urine is the commonly used biologic marker, as it reflects the cumulative dose to one of the main target organs, the kidney. The relationship between urinary excretion and levels in the other target tissue, the nervous system, is not well established. As discussed below, urinary mercury levels have been found to show a rough correlation with signs and symptoms of damage to the nervous system.

Dental amalgam. Several studies over the past 30 years or so have demonstrated that amalgam filling releases mercury vapor into the oral cavity. Mouth breathing carries the vapor to the lung, where it is absorbed and distributed to tissues, as discussed above. Mercury levels in autopsy tissue samples, including the brain, have been shown to correlate with the total number of surfaces of amalgam restorations. The estimate for the rate of release in people with amalgam restoration is 2–17 μg Hg/day (*18*). The most recent estimate based on applying pharmacokinetic parameters to steady-state plasma levels in people with amalgam suggests an average intake between 5 and 9 μg Hg/day (*83*). Kingman et al. (*84*), in a study correlating urinary excretion of mercury with amalgam surfaces, estimated that 10 amalgam surfaces would raise urinary levels by 1 μg Hg/L. As discussed below, these are far below toxic levels. However, excessive chewing, such as occurs when smokers try to stop smoking by using nicotine-containing chewing gum, may lead to urine levels in excess of 20 μg Hg/g creatinine, thereby approaching occupational health safe limits (*85*).

Increased amounts of mercury are excreted in feces in individuals with amalgam fillings. Engqvist et al. (*86*) found that only 25% of the total mercury in fecal samples was in the form of amalgam particles in samples taken from six adults with a moderate load of amalgam fillings. About 80% of an oral dose of amalgam particles or mercuric mercury attached to sulfhydryl groups was excreted in the feces. Interestingly, 60% of an oral dose of vapor dissolved in water was retained. Previously it had been assumed that intake of vapor was due solely to inhalation.

Adverse Effects

Mercury vapor. ACUTE TOXICITY. Cases continue to occur of severe poisoning and even fatalities from acute exposure to high levels of mercury vapor [e.g., see Solis et al. (*87*)]. Severe lung damage can lead to death from hypoxia. The poisoning appears to occur in three phases. The initial phase is characterized by flulike symptoms lasting 1–3 days. The intermediate phase is dominated by signs and symptoms of severe pulmonary toxicity. The victim in the final phase will experience gingivostomatitis, tremor, and erethism (memory loss, emotional lability, depression, insomnia, and shyness).

The signs and symptoms of the final phase are identical to those seen in workers chronically exposed to mercury levels. Generally speaking, such cases are rarely seen, at least in developed countries, where industrial hygiene measures are strictly enforced.

THE NERVOUS SYSTEM. Today, health concerns are directed toward the risk from lower levels of exposures. In general, air concentrations above 50 μg Hg/m^3 in the workplace, corresponding to steady-state urinary excretion rates of 60 μg Hg/g creatinine, are associated with fine tremors in the extremities that frequently are not noticed by the worker (*76*). Slowed nerve conduction velocity is another preclinical effect found at these lower levels.

Studies on dentists have suggested adverse effects at air concentrations lower that 50 μg Hg/m^3 [for review, see Langworth et al. (*88*)]. Average air concentrations as low as 14 μg Hg/m^3 were associated with decreased performance on psychomotor tests. Changes in mood and behavior have also been noted, such as emotional lability, somatosensory irritation, and alterations in mood scores. As noted by Langworth et al. (*88*), such effects may be due to mercury exposure. An alternative explanation for the observed correlations is that "dentists with special personality traits are less careful in the handling of mercury spills etc. and thus are more exposed to mercury vapor." If indeed these effects result from exposure to mercury, one should bear in mind that the average levels reported in these studies could be substantially less than peak values that may occur during installation of the amalgam fillings.

Follow-up studies of workers exposed to high levels of mercury vapor and no longer exposed during 10 or more years before being examined have revealed that adverse effects may persist on the nervous system. Mathiesen et al. (*89*) examined 70 previously exposed workers (time from last exposure, 1 to 35 years, average 12.7 years). The average yearly exposure was 8–584 μg Hg/m^3. Peak exposures during any specific year could have been much higher than these average levels. Decreased performance on a number of neuropsychologic tests was found, compared with a control group of 52 workers. Despite these high exposure levels, no residual effects were observed on general intellectual ability or ability to reason logically.

Workers exposed to high levels of vapor at some time during 1953–1966 in a nuclear weapons facility have been the subject of follow-up studies (*90,91*). Columns of liquid mercury were used in the separation of lithium isotopes. The exposure was expressed as "cumulative average quarterly urine mercury measurements" in units of micrograms of mercury per liter, from which information one cannot determine the actual urinary excretion rate (*90*). However, according to comments in the text of this paper (*90*), mercury workers had urine levels in excess of 600 μg Hg/L. In the more recent study (*91*), 104 of the surviving workers were compared with an unexposed group of 201. Residual adverse effects were found primarily on the peripheral

nervous system. Such long-term adverse effects, as quoted from the authors, "were not observed for a measure of dementia or other measures of cognitive function."

As discussed in the following section on amalgam, a suggestion has been made that inhaled vapor originating from amalgam fillings is a cause or predisposing factor to Alzheimer's disease (*92*). The fact that both these studies (*90,91*) were unable to detect any signs or symptoms remotely related to this disease years after heavy exposure to mercury vapor argues strongly against this suggestion. Many of these workers were exposed for many years to intakes of vapor more than 100-fold higher than that experienced from amalgam fillings.

No new information on adverse effects from prenatal exposure has emerged since previous reviews. However, one study (*93*) reported that female squirrel monkeys exposed during their pregnancy to air concentations of 500–1,000 μg Hg/m^3 had blood levels ranging from 25 to 180 μg Hg/L. No difference was observed between exposed and nonexposed offspring in various schedules of reinforcement in terms of lever pressing and other behavioral measures. The exposed offspring, however, appeared to vary more in the test performance. Given the high levels of prenatal exposure and the minimal effects found in the offspring, these data would suggest that the prenatal period may not be especially sensitive to the effects of vapor inhaled by the mother. This is consistent with what is known of the disposition of inhaled vapor in the maternal–fetal unit. Although vapor passes across the placenta, much less accumulates in the fetal brain than in that of the mother. The fetal liver appears capable of oxidizing the vapor in its first pass through this organ. The product of oxidation, divalent inorganic mercury, passes the blood–brain barrier far more slowly than the vapor.

KIDNEYS. Distinct from the action of inorganic mercuric compounds, exposure to mercury vapor does not produce severe kidney damage. However, low-level chronic exposures at air concentrations above 50 g Hg/m^3 do have adverse effects on the kidney (*94*). Decreased selectivity of the glomerular filter is evidenced by increased excretion of albumin. Tubular reabsorptive function is slightly diminished, leading to increased excretion of low-molecular-weight proteins such as retinol-binding protein. Damage to the brush border of the tubular cells is indicated by increased urinary excretion of brush border antigens. Interstitial effects of mercury result in loss of prostaglandins into the urine. These biochemical markers detect effects of mercury well before kidney function is significantly compromised.

Taylor et al. (*94*) reviewed results from a wide variety of urinary markers. The results suggest that mercury, lead, and cadmium may produce different patterns of changes in these markers. The most sensitive tests for the action of mercury are the tubular and interstitial markers.

MECHANISMS OF TOXICITY. The mechanism of action of inhaled mercury vapor on brain function is not known. It is assumed that the vapor is first oxidized to inorganic divalent mercury that functions as the proximate toxic agent. The latter can attach to thiol groups present in most proteins. Thus, almost any enzyme or structural protein is a potential target.

As discussed previously, it appears that the intact mercurial and not its metabolic product, inorganic mercury, is the proximate toxic agent in the neurotoxic action of methyl mercury. Conversely, we assume divalent inorganic mercury is the proximate toxic agent after exposure to mercury vapor. The underlying reason for this apparent conflict is not known. Most likely it is because of the differences in transport and distribution within the brain. Methyl mercury is transported as a water-soluble complex that is metabolized slowly to inorganic mercury only in phagocytic cells not in neuronal cells. Mercury vapor diffuses to all parts of the brain as a lipid-soluble monatomic gas that is rapidly oxidized to inorganic mercury by the catalase–hydrogen peroxide pathway present in all cells.

Pendergrass et al. (*92*) have presented evidence that inhaled vapor may damage the microtubular system in brain cells in a manner somewhat similar to that seen for methyl mercury. They reported that inhaled vapor can inhibit the binding of guanosine triphosphate (GTP) to a β subunit of the tubulin dimer. The microtubules of neuronal and other cells are formed from the polymerization of tubulin protein subunits in a treadmilling process such that as one end of the microtubules is formed, the other end is being depolymerized (*95*). GTP binding is essential for the polymerization step. Thus, if the formation step is inhibited, the microtubule will disappear as the depolymerization continues. Microtubules are key cytoskeletal structures involved in axonal transport, cell division, and cell migration. It will be interesting to see if exposure to mercury vapor leads to disappearance of the microtubules, as has been demonstrated for methyl mercury.

Consistent with action on microtubular structures, Leong et al. (*96*) observed that mercuric ions added *in vitro* to cultured neurons inhibited outgrowth and disrupted-membrane structure. Tests with antibodies for tubulin and actin indicated that the microtubular structure had disintegrated.

The inhibitory action of methyl mercury on the assembly of microtubules is well documented. If further investigation shows that the microtubule assembly is a common biochemical target for both forms of mercury, then we face the problem of explaining why the pathology and clinical signs and symptoms differ so much.

Understanding the mechanisms of cellular defenses is just as important as understanding the mechanisms of damage. Thiol-containing molecules probably play a role in defense as well as being targets for toxicity. Glutathione complexes with inorganic mercury in liver cells are secreted in bile and ultimately in the feces. Intracellular levels of glutathione probably divert mercury from sensitive sites. The thiol-rich family of proteins known generically as metallothioneins also plays a protective role. For example, metallothionein has been shown to protect against kidney damage from inorganic mercury (*97*). More recently, it was shown that lung damage was more severe in metallothionein-null mice than in normal mice after exposure to mercury vapor (*98*).

Amalgam. Contact hypersensitivity to mercury is a well-established adverse effect of amalgam fillings [e.g., see Camisa et al. (*99*)]. According to these authors, a complete remission may be expected about 3 months after the last amalgam filling is removed.

The existence of other adverse health effects due to amalgam is presently unknown but is becoming an area of intensive speculation and controversy. This is partly because of the limited amount of research on the safety of amalgam fillings and partly because of the increased visibility of mercury as a health risk and stringent regulatory actions concerning this metal.

Ahlqwist et al. (*100*) reported on the latest findings of a long-standing study of a cohort of 1,462 Swedish women established in 1968–1969. Follow-up studies were conducted in 1974 and 1975, 1980 and 1981, and in 1992 and 1993. Serum mercury levels correlated with the number of amalgam fillings. Different clusters of symptoms were recorded as well as the incidence of diabetes, myocardial infarction, stroke, and cancer. No association could be found between serum mercury levels and disease in this population of middle-aged and older women.

The finding that dental amalgam does not affect mental health is from two well-conducted epidemiologic studies—one on twins in Sweden (*101*) and the other on older women, the so-called Nun Study in the United States (*102*). The Swedish study involved approximately 587 subjects from an on-going Swedish Adoption/Twin Study of Aging (*103*). The twin study allowed control for genetic predisposition to the toxic effects

of mercury when evaluating the role of amalgam fillings. No negative effects on physical or mental health were found. The mean age of the study group was 66 years.

The study on 129 Catholic nuns aged 75–102 years took advantage of a population with homogeneous adult life styles and environment. No effect of amalgam status (determined by the number and surface area of the occlusal surfaces) could be found on eight different tests of cognitive function.

Cederbrant et al. (*104*) attempted to address the possibility that a susceptible immune system might explain why some individuals with amalgam fillings claimed to have psychologic, sensory, or neurologic symptoms from exposure to mercury. They used an *in vitro* lymphocyte proliferation assay to test for immune sensitivity to inorganic mercury on 23 amalgam patients, 30 healthy blood donors with amalgam, 10 healthy subjects without amalgam, and 9 patients with oral lichen planus (OLP) adjacent to the amalgam. In addition to the lymphocyte proliferation assay, a wide range of immune parameters was measured. None of these end points revealed any significant difference between amalgam patients and controls despite the fact the *in vitro* assay was sensitive to the positive control group (OLP).

Because the inhaled mercury vapor is toxic to the central nervous system, researchers are now speculating that vapor from amalgams may be a cause of or an exacerbating factor in some well-known degenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's disease (AD), multiple sclerosis, and Parkinson's disease. Speculation has been most intense concerning AD after a report that mercury levels were higher in autopsy brains of AD patients than in brains of members of a control group (*105*).

However, subsequent reports have presented an equivocal picture concerning correlations between tissue levels of mercury and AD. Fung et al. (*106*) found no difference between blood mercury levels or mercury to selenium ratios in AD patients and controls. All subjects resided in a nursing home, thus ensuring that environmental and dietary exposures were similar. Conversely, Hock et al. (*107*) found that blood levels in AD patients were higher than in controls. In early-onset AD patients, blood levels were 3 times higher than in controls. Blood mercury correlated with concentrations of amyloid β peptide on the cerebrospinal fluid in a subset of these patients. Interestingly, the increases in blood mercury levels were unrelated to the status of dental amalgam. The reason for the difference in the outcome of the two studies is not clear, although the study by Fung et al. (*106*) may have better control over exposure to mercury.

Subsequent studies on brain and related tissues have also discounted a connection between mercury levels and AD. Fung et al. (*108*) found mercury levels were the same in various anatomical regions of the brain in AD patients and matched controls. Cornett et al. (*109*) found elevated brain levels in most regions of the brain that were measured, but no statistical difference could be established from corresponding mercury levels in control subjects. Mercury levels in pituitary glands of AD patients were found to be similar to those of controls (*110*). In a study of 56 AD patients and 21 controls, Saxe et al. (*111*) found no significant association of AD with number, surface area, or history of having dental amalgam restorations. Mercury levels in the brain were the same in AD and control patients.

Overall studies relating tissue levels of mercury to AD have not produced a convincing picture of any kind of correlation with this disease. Even if one were established, the "chicken and the egg" issue would arise: Is mercury the cause of AD or does AD tissue accumulate mercury more than normal tissue?

Nevertheless, biochemical studies of *in vitro* preparations of nerve cells and of AD tissue continue to raise a question at least on a mechanistic basis that the levels of mercury may in some way be connected with AD. The brain pathology of AD is characterized by plaques of amyloid protein and neurofibrillary tangles (*112*). The latter consist of altered microtubules and microtubule-associated proteins, especially tau (*113*) and are needed for assembly of microtubules from the tubulin monomers. Phosphorylation of this protein blocks its ability to promote microtubule assembly. Mercury can interfere with the complex process of the treadmilling of microtubules.

The study by Leong et al. (*96*) on the effect of mercury on neurite growth also noted the appearance of structures resembling neurofibrillary tangles. The study by Pendergrass et al. (*92*), noted that mercury can block the binding of GTP to tubulin, thus interfering with microtubule assembly. Other studies have indicated that mercury can cause hyperphosphorylation of the tau protein (*114*).

Oxidative stress has been invoked as a cause of AD (*115,116*). Mercury is well known to cause biochemical changes in cells is consistent with oxidative stress (*114*). Indeed, it has been argued that the same enzyme system that protects against oxygen attack also protects against mercury (*15*).

Such biochemical observations offer tantalizing possibilities that mercury can be involved in a mechanism of AD. The process of microtubular treadmilling is controlled largely by thiol-containing proteins. Perhaps mercury is simply acting as a thiol reagent and any other thiol-reactive chemical would produce the same effects. For example, the lipid peroxidation product 4-hydroxynonenal inhibits neurite outgrowth, disrupts neuronal microtubules, and modifies cellular tubulin. Is this also acting by oxidizing thiol groups? As yet we do not have a complete plausible biochemical mechanism for the genesis of AD, nor do we know how mercury interferes with this process *in vitro* or whether or not mercury acts *in vivo*.

Conclusions and Research Needs

The three modern faces of mercury—methyl mercury in fish, mercury vapor from amalgam tooth fillings, and ethyl mercury in vaccines—represent our most recent encounter with this ancient metal. Despite thousands of years of history of human exposure and intense research activity in our lifetime, many of its toxic actions remain unexplained. This review reveals key gaps in our knowledge, gaps that highlight important research needs.

The main features of the disposition of methyl mercury in the body are well known. Nevertheless, some key gaps remain both in pharmacokinetics and in the mechanisms of transport and metabolism.

Fecal excretion is the main pathway of excretion in adults. Animal data indicate that this process does not start until the end of the suckling period. However, we have as yet no confirmation in human infants. Thus, we are unable to estimate the cumulative body burden from methyl mercury known to be secreted in breast milk. This gap in our knowledge is especially critical for risk estimates from thimerosal in vaccines.

Demethylation of methyl mercury by microflora in the gut is a key, probably rate-determining, process in the removal of methyl mercury from the body. The microbes involved have not been identified nor have the biochemical mechanisms of cleavage of the carbon–mercury bond. The demethylation process in the gut might well constitute an important site for interaction between diet and methyl mercury accumulation in the body. The fiber content of the diet has already been shown to affect the excretion rate of mercury (*117*). The diet change at the time of weaning may also affect the activity and composition of the microflora. Further studies in this area might shed light on why there is such a broad range of biologic half-times reported for adults exposed to methyl mercury.

Molecular mechanisms of transport of mercury across cell membranes have been identified, indicating that specific thiol complexes of methyl mercury can enter cells via

the large neutral amino acid carrier and exit on carriers for glutathione. However, no studies have reported to date on how methyl mercury gains entry into the hair follicle and then concentrates over a hundredfold compared to its concentration in whole blood. This is an important research priority, as head hair is the most widely used biologic indicator for this form of mercury. If the same entry mechanism operates for hair follicular cells as has been shown for endothelial cells of the blood–brain barrier, then mercury in hair would represent the species of mercury in blood that enters the brain. This would explain why levels in hair have been shown to parallel levels in brain.

The long latent period between the end of exposure and the sudden appearance of symptoms and signs of neurologic damage is both a fascinating and an insidious property of the action of methyl mercury on the mature central nervous system. A slow release of inorganic mercury might explain this property if the inorganic form were the proximate toxic species. However, animal experiments indicate that this role is played by the intact organomercurial moiety. Because the length of the latent period appears to be independent of the dose, it is also intriguing and argues against the accumulation of a toxic metabolite. It we could determine the mechanisms underlying the latent period, we would learn much more about the toxic action of the "element of mystery."

Two studies have indicted the possibility of adverse effects on the cardiovascular system both in adults and in prenatally exposed children. Such effects appear to be occurring at methyl mercury levels in the body comparable to those associated with the lowest levels affecting the central nervous system. There is an urgent need to confirm these findings in other populations, preferably where no co-exposure is occurring to other persistent organic pollutants such as polychlorinated biphenyls.

Generally, a broad research agenda is needed to develop the toxicology of thimerosal, given the paucity of our current information. Studies should be directed to test the assumption that the toxicology of thimerosal is similar to that of methyl mercury, given the fact the current estimates of human health risks, in particular in infants receiving vaccines, are based on this assumption. The immediate tissue disposition of mercury following a dose of thimerosal appears to be both qualitatively and quantitatively similar to that of methyl mercury, as discussed in this review. However, such limited evidence as now exists suggests that the rate of conversion to inorganic and, subsequently, the rate of excretion are more rapid, perhaps substantially so, compared with methyl mercury. Data on the biologic half-time of the ethyl mercury radical in body tissues, especially the brain, are essential for estimates of tissues burdens and health risk from cumulative exposure from repeated doses of thimerosal in vaccines given to infants. Such information needs to be gathered both during and after the suckling period.

Thimerosal also differs from methyl mercury in that it causes kidney damage at about the same doses that damage the nervous system. Experimental evidence indicated that damage to the nervous system is caused by the intact organomercurial radical, whether methyl or ethyl. However, inorganic mercury released from ethyl mercury may be the proximate toxic agent for kidney damage. Indeed, the suspected greater rate of release from ethyl mercury may explain why kidney damage, if any, occurs only at the later stages of intoxication from methyl mercury. Thus, comparative tests of methyl and ethyl mercury should include the renal–cardiovascular system as well as the nervous system in developing animals.

It is almost 30 years ago that mercury vapor was shown to be emitted from dental amalgam fillings. This led to an outpouring of numerous articles attempting to measure the precise amounts of vapor released and of factors affecting the release rate. In general, levels of inorganic mercury in tissue caused by release of vapor from amalgam are well below those associated with overt toxic effects or even with subtler neurobehavioral and renal affects. However, excessive chewing can raise urine levels close to the lowest safety limits for occupational exposure to mercury vapor. Interest is now focused on possible indirect effects of vapor released from amalgam. Despite the fact that several well-conducted epidemiologic studies have indicated no relationship between dental amalgam and Alzheimer's disease, speculation continues that the small amounts of vapor inhaled from amalgam may in some as yet unknown way exacerbate the progress and severity of this disease. Biochemical studies reviewed in this article raise intriguing possibilities.

Mechanisms of cellular resistance toward and defense against these three faces of mercury have received some research attention. It is suggested that the focal lesions produced in the adult brain by methyl mercury are the result not of selective toxic action but of selective resistance. Those cells having inadequate defense mechanisms succumb to the initial insult. It is likely that intracellular glutathione plays a protective role both in deflecting methyl mercury from sensitive sites in the cell and by enhancing its exit from the cell. Other thiol compounds, such as the metallothioneins, may also play a defensive role for both inorganic and organic forms of mercury (*118*). More detailed biochemical information of these defense processes should lead to the identification of genes controlling cellular resistance and thereby give some genetic insight into host susceptibility.

As we gaze at these three modern faces of mercury and reflect upon the extensive research conducted in our lifetime, we must reluctantly agree with the title of a BBC documentary broadcast over 25 years ago that this metal still remains "an element of mystery."

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