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THE PHARMACOLOGY OF MERCURY COMPOUNDS 6545

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DOSE-RESPONSE RELATIONSHIPS

This subject has been covered by several recent reviews, viz., Brown & Kulkarni (1), MAC Committee (2), and Lu et al (3) covering most mercury compounds; Berglund et al (4), Nelson et al (5), and Kurland (6) with reference to methyl mercury compounds; Goldwater (7) with reference to aryl and alkoxyl aryl mercury compounds; and Smith (8) and Friberg & Nordberg (9) with respect to inorganic mercury. Thus, the dose-response relationships in animals and man have not been included in this review.

ABSORPTION

Elemental mercury vapor undergoes virtually complete absorption across the alveolar membranes in experimental animals and in man (29–31). From the theoretical point of view it seems highly probable that mercury crosses the lung membranes in the elemental form. High diffusibility and lipid solubility are characteristics of this form of mercury and should lead to rapid penetration across cell membranes (32). Magos (117) has demonstrated the presence of dissolved elemental mercury in blood exposed to the radioactive vapor in vitro. Oxidation of the element to the divalent ion appears to take place in the red blood cells and other tissues (27, 33, 34).

More mercury is accumulated in the lungs of experimental animals after exposure to vapor as compared to injection of equivalent doses of inorganic salts (28, 31). After cessation of exposure to the radioactive vapor, mercury leaves the pulmonary tissues of rats with a half-time of approximately 5 hours (28). Presumably the mercury attached to lung tissue has been oxidized in situ to inorganic mercury.

Nielsen Kudsk (29) has shown that a blood-alcohol concentration of 0.04%, w/v, produces a maximum decrease of 30% in the pulmonary absorption of mercury in human subjects. Similar levels of alcohol inhibit the in vitro oxidation of vapor by samples of human blood. The mechanism of alcohol inhibition of vapor retention in the lung is not known. Nonspecific effects on lung permeability cannot be excluded, but Nielsen Kudsk noted that orally ingested alcohol was more effective than inhaled vapors of alcohol. If the rate of oxidation of vapor in the red blood cells influences pulmonary absorption, the inhibitory action of alcohol on the oxidation step may be the cause of decreased lung absorption.

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The pulmonary absorption of the various chemical compounds of mercury will depend upon such factors as solubility and particle size (35). No quantitative measurements have been reported for the pulmonary retention of aerosols of the phenyl and alkyl mercurials. The high lipid solubility of several salts of these mercurials makes it probable that the absorption will be rapid and complete. Klein et al (36) have drawn attention to the high vapor pressure of methyl mercury chloride and the potential hazard to laboratory workers using this salt of methyl mercury.

Friberg et al (37) measured the percutaneous resorption of isotopically labeled mercuric chloride and methyl mercury dicyandiamide using guinea pigs. No significant difference in absorption was noted. The percentage absorption was influenced by the concentration of mercury in the solution applied to the skin. A maximum rate occurred at 16 mg Hg/ml. Pretreatment of the skin with 50% acetone and 1% soap solution did not affect percutaneous resorption. Pretreatment with alkylaryl-sulfonate tended to increase absorption.

The mechanism of skin absorption is not known. Passive diffusion cannot be the only process involved, since the absolute absorption rate of mercury increased with increasing concentration up to "plateau value" (38). Skin absorption of mercuric chloride probably occurs transepidermally rather than via the follicular pathway (39).

Cases have been reported of serious and fatal poisonings resulting from the treatment of fungal skin infections with preparations containing 1% or less of alkyl mercurial compounds (17–20). The application of radioactive phenyl mercury salts to the skin of experimental animals indicates that substantial absorption takes place (literature cited in 7).

Elemental mercury probably penetrates the skin for theoretical reasons previously discussed. No quantitative studies have been found in the literature on rates of skin absorption of elemental mercury and the mercury compounds. This gap in our knowledge is unfortunate. For example, under conditions of occupational exposure, it is difficult to determine the proportion of mercury absorbed through the skin and via inhalation.

Concern in recent years with unusually high levels of mercury in certain foodstuff has stimulated interest in the rates and mechanisms of mercury absorption in the gastrointestinal tract. The type of mercury compound present in food greatly influences the rate of gastrointestinal absorption.

A single dose of methyl mercury salt was completely absorbed in the human gastrointestinal tract whether given as an aqueous solution or bound to fish proteins (12, 26). Inorganic mercury given bound to a preparation of liver was absorbed to the extent of only 15% in human volunteers (12). Chronic feeding of mice with food containing added mercuric chloride or methyl mercuric chloride labeled with the radioisotope similarly indicated complete absorption of the alkyl mercury and a much lower absorption of the inorganic form (40, 41). In these studies, the mercury compounds were allowed to bind to protein components of the food by mixing an equal vol-

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ume of the aqueous solution of the mercurial with the powdered food. At least 90% of the mercury became attached to nondiffusible compounds in food. Methyl mercury salts added to fish homogenate or accumulated biologically in fish living in contaminated lakes were equally well absorbed by experimental animals (42).

Phenyl mercury salts added and allowed to combine with animal foodstuffs underwent virtually complete absorption in mice (41). Early studies of Fitzhugh et al (11), in which rats were given diets containing added mercuric and phenyl mercurial salts, indicated that the organomercurial was much more efficiently reabsorbed (11). Ellis & Fang (43) also noted higher tissue levels of mercury from oral doses of phenyl as compared to mercuric chloride.

The efficiency of gastrointestinal absorption of mercuric chloride and other inorganic salts of mercury is probably dose dependent. Thus in discussing the clinical toxicology of acute lethal doses of mercuric chloride, Gleason et al (10) pointed out that alimentary absorption is "so rapid that the course and prognosis are determined largely by events within the first 10–15 minutes, particularly by the intervention of vomiting or therapeutic lavage." The lowest level of inorganic mercury in foodstuff that leads to damage to the gastrointestinal barrier is not known. Levels as high as 250 ppm of mercury as $HgCl_2$ in food have been given to rats for 2 years without serious gastrointestinal injury (11).

Distribution and Deposition of Mercury in Organs and Tissues

A fascinating aspect of the toxicology of mercury is the difference in patterns of deposition in organs and tissues following administration of different compounds of mercury. Moreover, the same compounds may give rise to remarkable differences in deposition patterns in different animal species. The pattern of organ deposition of the metal appears to be related to the toxic effects of various mercurials. However, compartmentalization of mercury within different parts of the organ or in subcellular structures, the binding of mercury to various chemical compounds within the cell, and the metabolic transformation of mercury and its compounds complicate any attempt to correlate organ damage to average organ levels of total mercury.

Studies published in the 1950s and 1960s have established the main patterns of organ deposition of mercury in animals exposed to elemental mercury, salts of inorganic mercury, and the organomercurials, including the mercurial diuretics and fungicides. Excellent summaries of these findings may be found in review articles by Brown & Kulkarni (1), the Committee on Maximum Allowable Concentration (2), Cafruny (44), and Berglund et al (4).

The main patterns will be briefly discussed with respect to organs and tissues principally involved in the toxic effects of mercury. The kidney in almost all situations accumulates the highest concentration of mercury.

Inorganic mercury is characterized by a markedly nonuniform distribu-

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tion in the body. Data published by Swensson & Ulfvarson (45) indicate kidney levels of mercury 300 times greater than blood levels, and brain concentrations approximately 10 times the blood level, following a single dose of mercuric nitrate. The high kidney levels are established within a few hours of a single dose. The kidneys retain mercury longer than other tissues, so that with increasing time after exposure a greater proportion of the body burden is found in this organ. Rothstein & Hayes (46) reported that the kidneys contained over 85% of the body burden of mercury 15 days or more after a single injection of mercuric chloride into rats.

The deposition of mercury shows a similar highly uneven pattern after exposure to aryl and alkoxy-alkyl mercurials (45, 47) and to the mercurial diuretics (13). Exposure to elemental vapor gives a similar pattern with one important exception. Magos (33) has demonstrated in rats that during exposure to the radioactive vapor, mercury penetrates rapidly to all tissues, resulting in a more uniform pattern of distribution. One important consequence is that brain levels are several-fold higher than after injection of a similar dose of mercuric salts (33, 48). With increasing time after exposure to the vapor, mercury steadily accumulates in the kidneys from other tissues, so as to approach very closely the distribution pattern for salts of inorganic mercury (28, 33). However, brain levels continue to be significantly higher than after inorganic mercury.

The short-chain alkyl mercurials, in contrast to all other classes of mercury compounds, present a much more uniform pattern of deposition. Levels in kidney, brain, and blood usually lie within a two- or threefold range of each other (45). The ability to cross the placental barrier and accumulate in fetal tissue is another unique property of this class of mercurials (49, 50, 145). Tejning (51) has reported that levels of methyl mercury compounds in cord blood are approximately 20% higher than in maternal blood. In a brief report, Matsumoto et al (25) presented data indicating much higher levels in fetal than in maternal brain tissue in experimental animals. Thus, the fact that brains of both the adult and fetal animal are the site of the most serious toxic effects appears to be due to the ability of these mercurials to penetrate the blood-brain and placental barriers.

The distribution of mercury between red blood cells and plasma shows interesting differences related to type of mercury compound. The short-chain alkyl mercurials and the phenyl mercurials are preferentially accumulated by the red blood cells (15, 21, 22). Lundgren et al (52) reported red blood cell to plasma ratios as high as 20 in humans exposed to methyl mercury fungicides. On the other hand, the levels in plasma and red blood usually do not vary by more than a factor of two in animals or in humans exposed to the elemental vapor and to salts of inorganic mercury (52–54). Vostal (55) has pointed out that differences in plasma-red cell distribution play an important role in determining the relationship between whole blood levels of mercury and urinary excretion.

The red blood cell to plasma ratio presents large species differences in

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the case of exposure to methyl mercury compounds. The highest cell to plasma ratio reported to date, about 300, was found in the rat (22). Lundgren et al (52) reported values in humans of approximately 20. Berglund & Berlin (21) refer to unpublished levels in the monkey and cat of approximately 10 and Norseth (56) reports a ratio in mice of 10. Differences in red blood cell-plasma ratios may account for species difference in brain accumulation of mercury after exposure to methyl mercury compounds (21). Whole blood-to-brain concentration ratios appear to be related to the red blood cell-to-plasma ratios. The rat, with the highest red blood cell-to-plasma ratio (approximately 300), also has the highest blood-to-brain ratio. Primates, including man, have the lowest blood-to-brain and red blood cell-to-plasma ratio. In other words, plasma-to-brain ratios do not show large species differences. Takahashi et al (57) have suggested from the autoradiographic observations that binding to the red blood cells retards the entry of ethyl mercury compounds into the brain. For a given concentration of the alkyl mercurial in whole blood, the greater proportion attached to the red cell, the less probability of penetrating the blood brain barrier.

The intra-organ distribution of mercury.—The distribution of mercury within the organ has been studied by various methods—by dissection and analysis of anatomical substructures of the organ, by radioautography, and by histochemical measurements. All the methods have certain drawbacks: Dissection gives a gross picture of distribution with no resolution of cell types involved in mercury deposition. Radioautography has a resolution between 5–15 μ, and so cannot resolve beyond the cellular level (58). Histochemical methods may distinguish mercury deposits down to intracellular sites but lack sensitivity and are subject to interference from other metals (59–61).

The intrarenal distribution of mercury following doses of mercuric salts, and mercurial compounds including mercurial diuretics, has been followed by workers interested in mechanisms of mercurial diuresis. No new evidence in this area has come to light since Cafruny's review (44). The intrarenal distribution of mercury is similar for the mercurials referred to above. The renal cortex contains the highest levels. Maximum concentrations of mercury are found in the cells of the proximal convoluted tubule, in the cytoplasm, and in the apical and basal membranes of these cells. Lower concentrations are observed in the straight portion of the proximal tubule, in the distal tubule, and in the loops of Henle. Mercury is close to background levels in the glomeruli and collecting ducts.

The diuretic chlormerodrin has a similar intrarenal distribution in humans to that in dogs (58), but its distribution in rats follows a different pattern. The highest accumulation is seen in the distal tubules. The acid-base balance of the animal, although having a marked effect on mercurial diuresis, produces little change in the intrarenal distribution following doses of labeled chlormerodrin. The administration of nonradioactive PCMB pro-

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duced no change in the renal autoradiograph in the dog given radioactive chlormerodrin despite the reversals of diuresis produced by PCMB.

The pattern of distribution of mercury in the brains of experimental animals is similar in its main features following exposure to vapor or after injection of the inorganic salts (62). Thus the greatly increased total uptake by the brain after exposure to vapor is not related to specialized areas or to special types of cells in the brain. Mercury is visible on the autoradiograph in all areas of the brain. However, the pattern of distribution changes with time after dosing. Mercury is eliminated from some areas of the brain more rapidly than from others. For example, levels in the cortex of the cerebrum are initially higher than those in the corpus callosum. However, mercury is lost from the cortex more rapidly than from the corpus callosum, so that at about 16 days after a single dose to guinea pigs, the corpus callosum level is higher. Mercury is eliminated more rapidly from the cortex than from the medulla in both the cerebrum and cerebellum. Slow elimination of the metal is also a characteristic of the nucleus dentatus. Concentrations of mercury in certain cells in the brain stem, designated by Nordberg & Serenius (62) as mercurophilic cells, may be 16 times higher than in neighboring cells. Elimination of the metal from the mercurophilic cell is very slow so that, with increasing time after exposure, differences between these and the other cells become even more pronounced.

The toxicological significance of differences in rates of elimination from different parts and cells of the brain is not known. As pointed out (62), long term exposure to mercury should result in accumulation in those cells from which mercury is released most slowly, thus giving rise to a potential for selective brain damage.

Differences in the average levels of mercury in anatomical substructures of the brain are much less pronounced than differences at the cellular level. Miyama et al (63) observed 96 hours after a single dose of mercuric chloride to rabbits that mean levels were on the order of brain stem > cerebellum > cerebral cortex > hippocampus, but that extreme levels did not vary more than a factor of two.

Some differences have been reported in pattern of brain deposition between exposure to vapor and exposure to the inorganic salts. Autoradiographs appear more "patchy" after exposure to the vapor. Nordberg & Serenius (62) interpret this pronounced patchiness, with localized concentrations of mercury around the blood vessels, as evidence for penetration of the vapor through the blood-brain barrier. Concentrations of mercury in the choroid plexus and the area postrema are much higher after injection of the inorganic salts and remain so at least up to 16 days after dosing.

Mercury was distributed to all parts of the brain when dogs were given a single large dose (30–60 mg/kg) of a methyl mercury salt (64). Mercury levels measured between the 2nd and 19th day after injection were highest in the calcarine tissue, with no consistent differences between white and gray matter. Other anatomical parts of the brain including the cerebellum,

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nucleus dentatus, frontal and temporal cortex, and the occipital cortex, had mercury levels usually not more than 50% lower than in the calcarine tissue. Berlin et al (65) also reported a fairly uniform distribution of mercury in brain tissue of monkeys within a few days of a single dose (approximately 0.25 mg/kg) of radioactive methyl mercury salt.

An autoradiogram of a monkey brain from an animal exposed for four weeks to a cumulative dose of approximately 5 mg/kg indicated a much less uniform distribution of mercury than was seen after single doses. High concentrations of mercury were present subcortically in the cerebrum. The concentration was highest in the occipital region. The concentration of mercury in the subcortical layer of the calcarine cortex was reported to exceed that in other parts of the brain by a factor of about sixteen. It is of interest to note that the findings of Yoshino et al (64) and of Berlin et al (65) indicate that mercury accumulated in the calcarine cortex and that visual disturbances were the most common symptoms noted in the experimental animals.

The pattern of distribution of mercury in the monkey brain at 20 hours after a single dose of radioactive ethyl mercury chloride (approximately 1 mg Hg/kg) had some similarities to the pattern associated with mercuric chloride (66). The autoradiogram indicated relatively high levels in the choroid plexus, the intracranial and extracerebral arteries and veins, and the sinus. The distribution pattern differs from the mercuric chloride patterns in that radioactivity was conspicuous in the cerebral and cerebellar cortices, subcortical gray matter, various nuclei of the brain stem, and gray matter of the spinal cord. Much less radioactivity was observed in the cerebral and spinal white matter, and levels close to background were found in the corpus callosum, anterior commissure, and optic nerve. The hypophyseal gland, particularly the anterior lobe, and the pineal body contained a high level of radioactivity. The distribution in cat brain was similar to that described for the monkey.

The brain distribution of mercury was dependent upon the time after giving a single dose of ethyl mercury chloride. The autoradiogram of a monkey brain taken only 60 minutes after dosing revealed most radioactivity in the blood vessels. However, at the end of 8 days, most mercury was observed in the cerebral and cerebellar cortices, especially in the occipital lobe and less pronounced in the white matter. Radioactivity in the blood vessels was close to background (57).

Autoradiograms of monkey brain taken at 20 hours after a single dose of phenyl mercuric chloride revealed a pattern of distribution similar to that after mercuric chloride (66).

The distribution of mercury between subcellular particles.—Several attempts have been reported to measure the subcellular distribution of mercury compounds using differential centrifugation. Unfortunately, most experimenters did not attempt to determine the purity of the centrifugal frac-

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tion. Norseth's studies (67–70) represent the most careful attempt to determine the proportion of different subcellular particles in centrifugal fractions using the enzyme marker technique of De Duve et al (71). As Norseth's (69) results well demonstrate, a knowledge of the particulate composition of the centrifugal fractions is essential for any quantitative measure of the binding of mercury to microsomes, lysosomes, and mitochondria.

The subcellular pattern of distribution after injection of mercuric chloride differs from that after dosing with methyl mercury compounds. With these two mercurials and with all other mercurials studied to date, mercury is found in all the centrifugal fractions, i.e., the so-called nuclear, mitochondrial, lysosomal, and soluble fractions (43, 64, 69, 72, 73). However, following a single dose of mercuric chloride to rats, mercury tends to accumulate with time after injection in the lysosome-peroxisome group of particles (69). After a single injection of methyl mercury dicyandiamide (MMDD), the fraction of mercury in the lysosome group was much lower. In the most recent report from this laboratory, inorganic mercury that had been released from the methyl mercury radical in vivo, also accumulated in the lysosomal fraction (70). Lysosomal accumulation of inorganic mercury has been demonstrated in the kidney by histochemical methods (74). The pattern of subcellular distribution of mercury after a single dose of methoxyethyl mercury chloride in rats is intermediate between the pattern given by salts of inorganic and methyl mercury (69). This compound has been shown to release inorganic mercury in animal tissues much more rapidly than compounds of methyl mercury (15, 47). Norscth & Brendeford (70) have suggested that differences in the lysosomal concentrations of mercury following doses of different organomercurial compounds are related to the in vivo stability of the carbon-mercury bond.

The reason why inorganic mercury is selectively accumulated by the lysosomal system is unknown. The lysosomal accumulation of mercury may represent a detoxication process (70). These authors also suggest that protein bound inorganic mercury excreted in bile may originate from the lysosomes. Plausible as these suggestions are, definitive studies have not yet been made.

No correlation between subcellular distribution and biological effects of mercury compounds has been possible. In their studies on the relationship between ATPase inhibition and mercurial diuresis, Nechay et al (73) found no correlation between micrsomal concentration of mercury and the diuretic effects. Studies by Grief et al (72) also revealed no obvious correlation with diuresis. Yoshino et al (64) found mercury in all subcellular fractions of the rat brain following a toxic dose of methyl mercury thioacetamide.

Mercury binding sites in tissues.—The fact that the kidneys accumulate the highest tissue levels of mercury and are the site of action of the mercurial diuretics and an important target organ for many other mercury compounds probably accounts for the choice of this organ in studies on

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tissue binding sites for mercury. The weight of evidence, both theoretical and experimental, favors the conclusion that mercury binds to thiol groups. The equilibrium affinity constant between the sulfur atom of a thiol group and mercury (either as the mercuric ion or as the organo-mercurial cation—R-Hg+) is many orders of magnitude above that for reversible binidng between mercury and other types of ligands (9a, 32). When the sulfhydryl concentration was increased in the cytoplasm of kidney cells by protein deprivation of rats, the levels of mercury in cytoplasm also increased as compared to normal rats injected with mercuric chloride (75). Cafruny et al (76) and Cafruny & Farah (77) demonstrated by histological methods that protein bound SH decreased in cells of the distal part of the convoluted tubule, loops of Henle, and collecting ducts following doses of the mercurial diuretic, mersalyl, to rats and dogs. DeMetry et al (78) also showed a fall in cortical SH concentration in animals receiving mercury.[1] Thiol compounds are the most effective complexing and chelating agents in removing mercury from kidney tissues. Non-thiol compounds capable of causing accelerated release of mercury from kidneys probably do so by initiating metabolic and permeability changes and by increasing the exfoliation of tubular cells (80).

Ligand groups other than thiols may play an important role in tissue binding. A two-point attachment to a receptor has been suggested for organomercurial molecules by Kessler et al (13) and for the mercuric ion by Weiner et al (81). The two-point receptor model usually envisages one point of attachment through a thiol group and the other through some unknown non-thiol ligand (81, 82). The ability of sodium maleate to enhance the effectiveness of penicillamine to remove mercury from kidneys has been interpreted by Stoytchev et al (83) as evidence for a two-point attachment of inorganic mercury. It was proposed that maleate binds to the non-SH ligands of the receptor, thus making the mercuric ion more readily removable by penicillamine.

Equilibrium dialysis of homogenates of kidneys of rats given mercuric chloride revealed that over 99% of the mercury was not diffusible (84). Jakubowski et al (85), using gel filtration analysis of water extracts of kidney, found only trace amounts of mercury bound to low molecular weight compounds (M.W. = 100–300). Fang (86), using similar techniques, also observed that all the mercury was protein bound in aqueous extracts of kidney of rats given ethyl mercury chloride, phenyl mercuric acetate, and mercuric acetate.

[1] Changes in tissue —SH levels following doses of mercury are not conclusive evidence that the metal binds to SH. Shore & Shore (79) have shown that changes in —SH in renal tissue induced by doses of $HgCl_2$, chlormerodrin, or PCMB are due to the inhibition of the enzyme S-S reductase. In any case one would not expect to see large changes in —SH levels in renal tissues due to stoichiometric reactions with mercury because kidney levels of the metal never account for more than 10% of the total —SH even after lethal doses of mercuric chloride (75).

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Clarkson & Magos (84) distinguished two different classes of nondiffusible binding sites in kidney tissue in terms of different affinity constants for mercury. The sites having the highest affinity for mercury had a maximal binding capacity of 1×10^{-7} moles of mercury per gram wet weight of tissue. Kidney levels of mercury associated with changes in kidney function are approximately 2×10^{-7} moles. The second class of binding sites had a maximal capacity of 30×10^{-7} moles as compared to the total nondiffusible —SH in kidney homogenates of 60×10^{-7} moles per gram wet weight of tissue.

Attempts have been made to identify the different protein fractions responsible for the binding of mercury. Yagi & White (87) reported "salting out" curves using ammonium sulfate. Mercury was found precipitated with both the "globulin" and "albumin"-like fractions. Protein deprivation of the rats, resulting in resistance to the lethal effects of mercuric chloride, was associated with an increase in the amount of mercury bound to the "albumin"-like fraction.

Jakubowski et al (85) used gel filtration to separate the protein components of an aqueous extract of kidney homogenate after various doses of mercuric chloride to rats. They observed that mercury is bound to both high and moderate molecular weight fractions. The high molecular weight fraction could be separated into two components with distinctly different molecular weights.

The moderate molecular weight fraction was identified as metallothionein (88). This protein was first isolated by Kagi & Vallee (89) and shown to be responsible for binding cadmium in kidney tissues. It is characterized by a molecular weight of approximately 10,000–11,000, absence of aromatic amino acids, and a high —SH content. Cysteine accounts for ¼–⅓ of all the amino acid residues.

The importance of metallothionein in the renal accumulation of mercury was first indicated by Pulido et al (90), who discovered that metallothionein contained almost as much mercury as cadmium when isolated from kidneys of humans treated with mercury diuretics.

Recent studies in the Piotrowski laboratory (91) point to an important protective role for metallothionein. Metallothionein accounted for the chief protein bound fraction of mercury in kidneys. Normal levels of metallothionein in rat kidneys are equivalent in mercury binding capacity to a level of renal mercury associated with the onset of toxic effects. Interestingly, this level of metallothionein compounds corresponds approximately with the maximum binding capacity of the high affinity site reported by Clarkson & Magos (84). Thus it is argued (91) that once the binding capacity of metallothionein has been saturated, toxic effects appear in the kidney.

Chronic dosing of rats with mercuric chloride over a period of three weeks induced an approximately six-fold increase in the renal metallothionein levels. This fact provides an explanation for the almost linear increase of mercury in kidneys over several weeks of daily exposure (Trojanowska,

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1971, cited in (91). Furthermore, a protective role of metallothionein would explain findings that the kidney can accumulate, during chronic exposure to mercury compounds, much higher levels of inorganic mercury than the toxic level observed after a single dose, and do so without any detectable deleterious effects.

In contrast to cadmium, mercury was able to induce synthesis of metallothionein in kidney tissue but not in the liver (91). This is consistent with the observation that whereas kidney levels of mercury rose on repeated dosing, the liver levels remained stable.

Fang (86) has applied gel filtration analysis of aqueous extracts of kidneys of rats dosed with organomercurial compounds. He observed that a substantial fraction of the mercury in animals exposed to phenyl or ethyl mercury salts was attached to the protein fraction corresponding to metallothionein. He also noted that the half-time of removal of mercury from the metallothionein complex was much longer than half-times observed for other protein fractions. Thus both Fang's and Piotrowski's studies point to the importance of metallothionein in the long-term accumulation of mercury in kidney tissue. The binding of mercury to metallothionein may account for the "albumin-like" fraction reported by Yagi & White (87) and its protective role in mercury poisoning observed in protein depleted rats.

Organs other than kidney and liver have received relatively little attention. Yoshino et al (64) have reported that all mercury in brain is protein bound following a single dose of methyl mercury salt to dogs.

Mercury compounds differ greatly in their ability to accumulate in red blood cells. Methyl mercury salts are accumulated to a high degree in red cells and, according to Takeda et al (92) the methyl mercury radical is bound to the cysteine residues of hemoglobin. Paper electrophoresis and ultrafiltration of solutions of hemolyzed red blood cells indicate that mercury becomes bound in part to hemoglobin and in part to diffusible compounds when red blood cells are exposed in vitro to mercury vapor (27). Extensive in vitro studies by Rothstein and his coworkers (93) indicate that mercury may bind to all the sulfhydryl groups in the red cells when mercuric chloride is added to the cell suspension. On the other hand, the highly polar organomercurial p-mercuribenzene sulfonate reacts chiefly with sulfhydryl groups on the outer surface of the cell membrane. Penetration into the cells takes many hours. The binding of both methyl and inorganic mercuric ions to the red cells is readily reversible as evidenced by the rapid fall in blood levels when exposed animals or humans are treated with agents that accelerate the excretion of mercury (94, 95).

The binding of mercury in biological fluids.—The concentration of mercury in plasma, cerebrospinal fluid, bile, and urine is usually much lower than levels observed in kidneys. Thus identification of biocomplexes of mercury is made very difficult and to date no unequivocal identification has yet been made. Nevertheless, several attempts have been made to identify the

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chemical form of mercury in plasma, urine, and bile. The results, although incomplete, have given rise to new ideas on pathways of distribution and excretion of mercury in the mammalian body.

Most observations revealed that virtually all the mercury in plasma is protein bound (96). Clarkson et al (27) reported complete binding to plasma protein when mercuric chloride was added to heparinized samples of human blood and when blood samples were exposed in vitro to radioactive mercury vapor. Berlin & Gibson (97) noted complete binding to plasma protein in rabbits injected intravenously with radioactive mercuric chloride. Hemolyzed samples of blood from rats given radioactive mercuric chloride yielded less than 0.5% ultrafiltrate mercury. Kessler et al (13) obtained similar results when a variety of organomercurial diuretics were injected into dogs. However, for theoretical reasons, one would expect that a fraction of the plasma mercury would be attached to diffusible thiol compounds. The observations referred to do not, in fact, exclude the possibility but merely indicate that binding to diffusible plasma thiols accounts for less than 1% of plasma mercury. Norseth (98) has detected a trace of a diffusible mercury compound in mice given a single dose of radioactive methylmercury chloride. The compound appears to be similar to that present in much higher concentrations in mouse bile.

Jakubowski et al (85) were able to identify three distinct fractions of protein bound mercury in plasma of rats injected with mercuric chloride. The highest proportion of mercury was found in the protein fraction containing mainly lipoproteins and benzidine-positive globulins. Susuki et al (99) reported that mercury was bound to both albumin and alpha-globulin according to paper electrophoretic separation of plasma from rats given subcutaneous dose of mercuric nitrate. The amount of mercury associated with the albumin band decreased at 24 hours as compared to 5 hours after injection. Mercury associated with the alpha-globulin band was unchanged. An association of inorganic mercury with globulin plasma proteins has also been noted by Cember et al (100). However, at high dose levels, there was a shift from alpha-globulin to the albumin band on paper electrophoresis.

Bile collected from rats injected with a single dose of radioactive methylmercury chloride contained two fractions of methylmercury compounds as distinguished by gel filtration chromatograph (101). Approximately 20% of the mercury was associated with proteins of unknown molecular weight. The second fraction consisted of a single low molecular weight compound tentatively identified as methyl mercury cysteine. However, subsequent tests indicate that the diffusible compound is probably a complex of methyl mercury with a thiol containing dipeptide (98). As the bile entered the small intestine, this diffusible complex of methyl mercury was rapidly and completely reabsorbed into the blood stream, thus giving rise to a large enterohepatic recirculation of methyl mercury.

Studies still in progress (98) point to species differences in the chemical form of methyl mercury in bile. The chief compound in mouse bile seen in

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the first 6 hours after a single injection may be glutathione, but a complicating factor is that binding of mercury in mouse bile changes with time.

When radioactive mercuric chloride was injected into rats, only a trace of low molecular weight mercury compounds was detected in the bile (101). The types of proteins involved in mercury binding in bile were not identified. Inorganic mercury released from methyl mercury in the animal tissues was also secreted in bile attached to protein. Intestinal absorption of protein bound mercury secreted in bile appeared to take place very slowly, if at all (101).

Urine contains mercury in a variety of forms. As pointed out by Cember (102), the exfoliation of tubular cells may account for some urinary excretion of mercury. This process may be important when toxic doses of mercury are given or when other nephrotoxic agents cause extensive exfoliation of cells (80).

Weiner & Müller (103) identified a cysteine complex of the diuretic mersalyl in the urine of dogs given a single dose. However, it is possible that other closely related thiol-containing amino acids and peptides might give similar polarographic waves. Weiner et al (81) claimed to have identified the cysteine complex of mercuric ion in urine of dogs injected with mercuric cysteine.

Gel filtration of urine from rats dosed with mercuric chloride indicated a high proportion of low molecular weight mercury compounds, being intermediate between mercuric cysteine and mercuric glutathione. The fraction of diffusible mercury was over 50% of the total mercury of some of the urine samples, but in some cases the proportion fell as low as 1%. The cause of these fluctuations was not determined but appears to be unrelated to dose. The binding of mercury to different protein fractions in mice was highly variable, but in some samples small amounts of the mercury-metallothionein complex were detected (85).

Mechanism of distribution and uptake.—Rothstein (104) has discussed from the theoretical point of view the important factors that may determine the transport of mercury from blood to tissues. Diffusible compounds of mercury have the opportunity to cross the capillary membrane and enter tissue spaces. Tissue uptake will be determined by chemical affinitives for cellular binding sites and the diffusible complex and by the ability of the diffusible complex to penetrate the cell membrane. Thus, not all diffusible complexes of mercury present in plasma will lead to tissue accumulation.

Another important factor will be the kinetics of equilibration of mercury from protein binding sites to the diffusible complexes. Diffusible complexes of mercury represent less than 1% of the total mercury in blood. It may be supposed that during a single passage of blood through an organ, all the diffusible complex diffuses to the tissues. If re-equilibration from protein bound to diffusible forms is a slow process as compared to the circulation time, a very small fraction of mercury will be lost to the tissue. Rothstein

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(104) has pointed to the observation that the red blood cell-to-plasma ratio does not become steady for some time after exposure to mercury vapor, inorganic mercury salts, and methyl mercury compounds as evidence that re-equilibration may be a slow process. Thus, the amounts of diffusible mercury in plasma and the rates of re-equilibration may be key factors in tissue distribution.

The diffusion of the short-chain alkyl mercurials from the red blood cell may be the rate-determining step, at least with regard to uptake by the brain. In interpreting their autoradiographic studies of the penetration of methyl mercury compounds into brain, Takahashi et al (57) concluded that binding to the red blood cells was delaying entrance into the brain. This would also explain species differences in brain uptake, discussed above.

The mechanism of penetration across the blood-brain barrier is unknown except in the case of exposure to mercury vapor where Magos (33) has presented evidence for diffusion of the dissolved elemental mercury from blood to brain tissue. The chloride salts of the alkyl mercurials are lipid soluble, and therefore could diffuse passively across the blood-brain barrier. However, the high affinity of alkyl mercury cation for sulfhydryl groups would indicate that extremely small concentrations of the chloride salt would exist in plasma (9a). Indeed, lipid solubility of the chloride salts cannot be the sole determining factor; otherwise, the brain uptake after doses of phenyl mercury compounds would be as rapid as after exposure to alkyl mercurials.

Rothstein (104) has calculated that even following toxic doses of mercurials less than 1% of the sulfhydryl groups in blood are attached to mercury. Thus, the distribution of mercury in blood will be determined by a small number of sulfhydryl groups with high affinities for the metal. The behavior, with respect to mercury, of the average protein sulfhydryl groups may be irrelevant. For example, about 95% of the total sulfhydryl groups in blood are found in the red blood cells, but the blood-to-cell ratios of the alkyl mercurials may be as high as 300:1. Inorganic mercury, on the other hand, gives a ratio of close to unity. Both inorganic and alkyl mercury penetrate the red cell membrane rapidly (102) so that these different steady state distribution ratios must reflect special binding properties of a small fraction of SH groups in plasma proteins and in the red blood cells.

In support of Rothstein's theoretical analysis (104), observations on the biliary secretion of complexes of methyl mercury point to the importance of diffusible forms of mercury in blood (98, 101). Thus, when the intestinal reabsorption of the diffusible complex of methyl mercury in bile is prevented by cannulation or ligation of the bile duct, the pattern of mercury deposition in tissues is changed. Norseth (98) has observed that the diffusible biliary complex of methyl mercury in the mouse is not the same as in the rat and that this may account for differences in red blood cell-to-plasma ratios in the two species. The distribution of mercury between diffusible and nondiffusible form is also influenced by biliary secretion. Thus, Norseth (98) was able to identify a diffusible compound in plasma of rats apparently

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similar to the diffusible complex in bile. The diffusible complex could not be detected in plasma from rats with ligated and cannulated bile ducts. Norseth's observations add a new dimension to any consideration of mechanisms of transport or distribution of mercury to tissues.

Renal cortical tissue possesses a remarkable ability to accumulate mercury no matter what type of mercurial compound is the source of exposure. The mechanisms of selective renal accumulation of mercury have been a continuing stimulus to research. Results of investigation up to 1970, as summarized by Clarkson & Magos (105) are conflicting, depending on the techniques used in the study. Dreisback & Taugner (113), who utilized most of the techniques used by others, concluded that mercury enters the tubular cells both through the brush borders after glomerular filtration and across the basal membrane.

The rate of renal accumulation of mercury following a single dose varies with the type of mercurial compound. Maximum levels in the rat kidney are attained in less than a day after doses of mercuric chloride (105), but after methyl mercury chloride, the maximum point is not reached until about 2 weeks after injection (22). The delayed maximum in renal levels after methyl mercury chloride is due to the accumulation in kidney of inorganic mercury from the slow breakdown of the organomercurial. Peak kidney levels of the intact methyl mercury radical is usually attained within a day following a single dose.

The rapid accumulation of mercury may be due to two possible processes. Mercury might attach to sites in kidney tissue possessing a uniquely high affinity for this metal. This possibility was discounted when Clarkson & Magos (84) reported that thiol groups in the kidney had no higher affinity for inorganic mercury than thiol groups in other tissues. The second possibility is that the renal cells may expend metabolic energy to accumulate mercury. In the case of uptake via glomerular filtration, the energy would be supplied by concentrative uptake of water by the tubular cell. If accumulation took place in the peritubular capillaries, a specialized cellular metabolic pathway may be involved. For example, Cafruny (44) has summarized evidence indicating that the mercurial diuretics, with the exception of chlormerodrin, are believed to undergo active secretion.

The renal accumulation of inorganic mercury is inhibited by DNP (105). No changes were observed in the glomerular filtration rate. The effect of DNP appeared to be on the uptake process because this metabolic inhibitor did not cause any release of mercury from the kidneys when given after maximum mercury levels had been attained. Of special interest is the observation that the reduced renal levels were not accompanied by significant increases in urinary excretion. This observation is not compatible with the hypothesis that DNP inhibits the reabsorption of filtered mercury from the tubular lumen. The observation is compatible with the alternative pathway to kidney tissue, viz., directly from the peritubular capillaries.

The degree of inhibition of uptake produced by DNP indicates that at least 26–34% of the total renal uptake follows the metabolic-dependent

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pathway from the peritubular capillaries. These are probably minimal values since it is unlikely that DPN produced a complete block of ATP synthesis. On the other hand, the observations do not exclude a substantial role for glomerular filtration.

Excretion

The kinetics of excretion of mercury is greatly influenced by the chemical compound of mercury to which the animal is exposed. Differences are apparent in both the absolute magnitude of excretion rate and the complexity of kinetic models necessary to simulate the excretion data. Furthermore, the rate of excretion of mercury from animals given the same mercurial compounds exhibits very large species differences. Absorption rates from the lung or gastrointestinal tract do not vary greatly from one animal species to another. Thus, variation in the steady state body burdens in animals chemically exposed to the same level of mercury in air or in the diet is due mainly to specics differences in cxcretion rates.

The rate of excretion of mercury from the whole animal is most accurately measured by making whole body counts of animals given the mercury labeled with the γ-ray emitting isotope ^{203}Hg. For any given animal species, the short-chain alkyl mercurials, as typified by methyl mercury salts, exhibit the simplest kinetic features and are excreted more slowly than any other compound of mercury. The daily excretion rate is directly proportional to the simultaneous body burden in experimental animals (14, 21, 41, 106, 107) and in human volunteers (12, 26). A semilogarithmic plot of the whole body counts against time yields a straight line. The half-time of excretion, calculated from the slope of the line, depends upon the animal species under study. Reported values vary from a half-time of 8 days in the mouse to approximately 70 days in man and other primates and to nearly 1000 days in some species of fish and shell fish (reviewed in 41).

The biological half-time in mice was the same after both a single dose and chronic exposure in food (41, 95). Tejning (cited in 4) reported a half-time of elimination of mercury from the blood of persons after cessation of high intake of methyl mercury in fish of approximately 70 days, the same as the biologic half-time reported in human volunteers after a single dose.

The virtually complete absorption of methyl mercury compounds from foodstuffs and the first-order kinetics of excretion lead to a simple equation relating the body burden, B, to the daily intake, d, as follows:

$$B = (d/k)[1 - \exp(-kt)]$$

where k, reciprocal days, is the rate constant of excretion and t is the time in days (23, 41, 106). At large values of t, the body burden B_∞ becomes steady and is given by $B_\infty = d/k$. Values of steady state body burden are proportional to the reciprocal of thc excretion constant. For example, man, with an excretion half-time of 70 days, would accumulate steady state tissue levels of methyl mercury approximately 10 times as high as those of the

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mouse, which has an excretion half-time of 7 days. Toxicological studies of the type reported by Fitzhugh et al (11), in which rats (excretion half-time of 16 days) were fed diets containing added mercuric salts, would lead to tissue levels of mercury considerably below those expected in humans given the same mercury levels in food.

In contrast to the short-chain alkyl mercurials, the rate of excretion of mercury is usually higher in experimental animals exposed to the elemental vapor or receiving doses of the phenyl and alkoxy-alkyl mercurials and the mercurial diuretics. In general, the decline in the body burden with time after cessation of exposure cannot be described by a single biologic half-time. Most investigators recognize at least three different phases in half-time excretion rates. Rothstein & Hayes reported that a single subtoxic dose of mercuric chloride given to rats was excreted in three phases as determined by whole body counting (108). The initial rapid phase, involving 35% of the dose, lasted for a few days; a slower phase (half-time 30 days) followed, involving 50% of the dose; and finally a slow phase became apparent, accounting for the remaining 15% of the dose and characterized by a half-time of 100 days. Clearly this slow component would play a predominant role in determining the cumulative body burden in clinically exposed animals. These findings suggest that rats exposed daily to mercury would gradually accumulate the metal throughout their life spans. The data of Fitzhugh et al (11) on rats fed mercury daily for up to 2 years are compatible with a gradual increase in tissue levels.

The initial rapid phase in the decline in the body burden was associated with high levels of mercury in the liver and high fecal excretion rates (108). The two slower phases of excretion corresponded to clearances from the kidney tissue, where most of the body burden was localized. At these times urinary excretion accounted for almost all of the total excretion from the body.

After brief exposure of rats (0.5–5 hours) to mercury vapor, the body burden declined with a half-time of 2 days, accounting for 30% of the dose, and subsequently with a half-time of 20 days (28). The rapid phase was due mainly to fecal excretion. In the slower phase, urinary and fecal excretion were approximately equal.

After chronic exposure to radioactive vapor, the size of the component rapidly excreted was very small. Only exposures during the last few days contributed to the fast component, whereas exposure of the last several weeks contributed to the slow component. Thus, excretion of mercury after multiple exposures to the vapor took place at the same rate as that reported for the slower component after a single brief exposure. Rothstein & Hayes (109) concluded from these and other data that each dose of mercury vapor in multiple exposures behaves independently of the other. In other words, the kinetics of excretion following a single dose may be used to estimate the rates of excretion after multiple dosing.

Mercury excretion from rats after single or long-term exposure to phenyl or methoxyl-ethyl mercury salts occurs at a similar rate and with simi-

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lar kinetic features as in the case of corresponding exposures to salts of divalent inorganic mercury (14, 106, 107).

In their 1968 papers, Swensson & Ulfvarson (14, 107) described the excretion of mercury from rats and poultry in terms of "second-order" kinetics, i.e., the daily excretion was assumed to be proportional to the square of the simultaneous body burden. While their model gave an adequate description of their excretion data for single doses of mercuric nitrate, phenyl mercuric hydroxide, methoxyethyl mercury hydroxide, and methyl mercury hydroxide, a multiple compartment model based on linear kinetics, such as that used by Rothstein & Hayes (108), would also adequately describe the data.

Vostal (55) has reviewed evidence relating to possible mechanisms of urinary excretion of mercury. As indicated in another section of this review, less than 1% of mercury in plasma must be expected to be in the diffusible form. A finite proportion of mercury in plasma must be expected to be diffusible for theoretical reasons since diffusible thiol groups exist in plasma (104). However, the weight of evidence indicates that glomerular filtration of diffusible mercury may not make an important contribution to urinary excretion. Berlin & Gibson (97) could find no correlation between glomerular filtration and excretion of mercury. Cho & Cafruny (110) demonstrated that mercurial diuretics were actively reabsorbed when injected with cysteine through a urethral catheter in the renal pelvis of the dog. Thus, small quantities of mercury may be filtered in the glomerulus and never go directly into the urine. The delay in excretion of mercury following an intravenous injection of inorganic, phenyl, or methyl mercury salt suggests tubular excretion rather than glomerular filtration (111). Vostal & Heller (112) demonstrated the ability of divalent inorganic mercury to pass across the tubular wall in the chicken kidney. Micropuncture measurements by Berlin & Vostal (cited in 55) favored the idea that accumulation of mercury in the tubular walls was more decisive for the transport of mercury into the nephron than filtration through the glomerulus. Norseth & Clarkson (22) reported that the proportion of inorganic mercury to total mercury in urine followed the proportion of inorganic mercury in kidney tissue and was not related to the porportion of inorganic mercury in plasma in rats given single doses of methyl mercury chloride.

Unfortunately, nothing is known of the processes involved in the release of inorganic and alkyl mercurials from renal tissue into the nephron. The presence of diffusible complexes of mercury in urine suggests that low molecular weight thiol compounds may play a role in urinary excretion. Doses of inorganic mercury capable of causing renal damage trigger acceleration release of mercury via exfoliation of renal cells.

Cafruny (44) reviewed evidence implicating the role of active tubular secretion in the urinary excretion of the mercurial diuretics. Chlormerodrin is an exception, probably because its molecule does not contain an acidic group. Cafruny suggests that the high rate of urinary excretion is due to concentration of the mercurial in renal cells, followed by passive release into the lumen of the nephron. The mechanisms involved are unkown.

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Weiner et al reported (81) that the rapid urinary excretion of mercurial diuretics appears to be an important mediator of the diuretic effect. Organomercurial compounds that do not undergo rapid urinary excretion are not capable of eliciting diuresis in dogs. Weiner et al were able to convert a nondiuretic mercurial into an active one by giving the compound with a molar excess of cysteine. The urinary excretion increased from less than 1% of the dose in 3 hours to 7%.

During the first 10 days after a single dose of methyl mercury chloride to rats, Norseth & Clarkson (101) observed that the biliary excretion of mercury exceeded fecal excretion by a factor of at least three. Sephadex chromatography analysis of biliary and intestinal contents revealed enterohepatic recirculation due to the biliary secretion of a low molecular weight complex of methyl mercury that underwent complete absorption in the intestine. The fecal mercury originated in part from the secretion of protein complexes of inorganic mercury and methyl mercury in the bile, and in part from the mercury attached to cells exfoliated from the intestinal epithelium. The small fraction of inorganic mercury in bile originates from biotransformation of the parent molecule, probably in the liver.

The fecal excretion of mercury was increased by more than 100% in mice given a single intraperitoneal dose of methyl mercury chloride after a polystyrene resin containing attached sulfhydryl groups was added to their food (95). Urinary excretion was unaffected. Thus, the resin probably acts by trapping the diffusible methyl mercury complex secreted in the bile and so diminishing enterohepatic recirculation. The resin may be used as an experimental tool to test for enterohepatic recirculation of methyl mercury in other species and for enterohepatic recirculation of other compounds of mercury. For example, the resin diminished the absorption of phenyl mercury compounds added to food given to mice, thus demonstrating the ability of the resin to form nonabsorbable complexes with phenyl mercury. The resin failed to elicit increased fecal excretion of mercury in mice given single intraperitoneal doses of phenyl mercury chloride, suggesting that enterohepatic recirculation is small with this mercurial.

Little is known about the pathways leading to fecal excretion of inorganic mercury. Its presence has been reported in bile of experimental animals (101, 111). Since it is not well absorbed across the intestinal epithelium, biliary secretion of inorganic mercury may be important to fecal excretion. It is also secreted in saliva (114); this process may also be important to fecal excretion. Autoradiographic observations of high concentrations of mercury in the epithelial cells of the large intestine have led to the suggestion that cellular exfoliation may contribute to fecal excretion (111).

Biotransformation

Elemental and inorganic mercury.—To react chemically with proteins and other molecules in the living organism, elemental mercury must undergo oxidation to the mercurous (Hg_2^{++}) or mercuric (Hg^{++}) ion. Since the general pattern of distribution in body tissues is similar following expo-

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sure to vapor and after injection of salts of divalent mercury, oxidation of the vapor probably takes place soon after absorption from the lungs (28, 108).

Stock et al (115) were the first to show that elemental mercury in aqueous solution is rapidly oxidized in the presence of air.

Horwitz (116) demonstrated that the toxicity of elemental mercury vapor to a variety of simple organisms, including green algae and yeast, was due to oxidation of the metal to the mercuric ion inside the cell.

Clarkson, Gatzy & Dalton (27) studied the equilibrium of mercury vapor in vitro with samples of whole blood, plasma, and hemolysates. They concluded that mercury vapor was oxidized rapidly enough in vitro to account for the build-up of toxic levels of mercury in the whole animals, from the following observations: (*a*) The levels of mercury found in whole blood, plasma, and toluene-extracted hemolysates of red blood cells were at least 70 times as high as the maximum solubility of mercury vapor in water. (*b*) The rate of uptake was influenced by oxygen tension. (*c*) The rate of uptake was dramatically influenced by the chemical composition of the solution. (*d*) Ultrafiltration experiments and electrophoretic measurement demonstrated that the mercury taken up by plasma and cell hemolysate was in a chemically combined form.

Comparison of the rate of oxidation of vapor by plasma, whole blood, and hemolysates indicated that most of the oxidation, at least 80%, took place inside the red cell and that some of the mercuric ion formed within the red blood cells discharged out into the plasma, causing an approximately equal distribution of mercury between cells and plasma.

Magos (33, 117) studied the in vitro equilibration of mercury vapor with blood using an isotope with higher specific activity, ^{203}Hg, to permit measurements of uptake on shorter exposure times. His observations confirmed the hypothesis that mercury vapor was oxidized by blood in vitro and that the rate of oxidation was dependent on the quantity of red cells in the suspension, as determined by measurements of hemoglobin. Magos also demonstrated that, despite the rapid oxidation, significant amounts of elemental mercury were dissolved in the blood sample. The amount of elemental mercury ($4–8 \times 10^{+3}$ μg/ml) dissolved in the blood sample is what would be expected for the known solubility of the vapor in water (118). Intravenous injection of elemental mercury resulted in the exhalation of vapor from the lung, thus demonstrating that the dissolved element persists in blood and that mercury crosses the pulmonary membranes in the elemental form. This small amount of unoxidized mercury plays an important role in the distribution of mercury to the brain after exposure to vapor.

Neilsen Kudsk (119) reported that small doses of ethyl alcohol (20–30 grams) inhibited the absorption of mercury vapor in the lung in humans. Alcohol at blood levels of 0.02–0.04% produced a 30% inhibition of absorption. Higher blood levels produced no further inhibition.

Nielsen Kudsk (120) reproduced the inhibitory effect by equilibrating

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mercury vapor with blood samples in vitro. Methyl and ethyl alcohol produced a maximum inhibition of 60% of the rate of uptake of vapor by whole blood. Isopropyl and n-butyl alcohol were ineffective. He was able to exclude methemoglobin as playing a significant role in oxidation of the vapor in the red blood cell. An aqueous solution of hydrogen peroxide and oxidized glutathione rapidly oxidized mercury vapor. A wide variety of metabolic inhibitors and substrates influenced the rate of oxidation of the vapor in whole blood and in hemolysates. Their effects could be explained at least qualitatively in terms of the known or suspected influence of these agents on the levels of hydrogen peroxide and oxidized glutathione in the red blood cells. Glutathione peroxidase, glutathione reductase, and metabolic processes affecting levels of NADPH may have an important indirect influence on the oxidation rate by regulating the levels of H_2O_2 and the ratio of oxidized to reduced glutathione in the cells. Nevertheless, these results do not conclusively demonstrate that an enzyme is directly involved in catalyzing the oxidation of mercury vapor in red blood cells.

In his most recent study, Nielsen Kudsk (121) was able to demonstrate that crystalline beef liver catalase stimulated the in vitro oxidation of mercury vapor in solutions of 3 mM glutathione and in the presence of hydrogen peroxide. Catalase-stimulated uptake was inhibited by 3-amino-1,2,4-triazole, a well known inhibitor of this enzyme and by 0.2% (w/v) ethyl alcohol. These results are of considerable interest in view of Magos' findings (33) indicating that mercury vapor diffuses across the bloodbrain barrier and undergoes oxidation in brain tissue. Oxidation of mercury vapor may also take place in other tissues. It remains for future studies to determine the role of the catalase-hydrogen peroxide complex in tissue oxidation and fixation of mercury. The oxidation of mercury vapor by erythrocytes is not inhibited by 3-amino-1,2,4-triazole. This may reflect differences between red blood cell and beef liver catalase. On the other hand, alternative pathways of oxidation may exist in the red blood cells. Thus the site of the inhibitory action of methyl and ethyl alcohol on vapor oxidation within the red cells is not yet known.

The reduction of divalent mercury to elemental mercury vapor probably accounts for the ability of certain commonly occurring micro-organisms to volatilize mercury for biological media (122). Loss of volatile radioactive mercury has been observed to take place from rats injected with salts of divalent mercury labeled with the ^{203}Hg isotope (123). Part of the volatile mercury was exhaled via the lungs, the remainder by way of the skin and fur. The volatile loss accounted for up to 20% of the total rate of excretion of mercury from the animals. The role of the fur in volatilization of mercury is not completely understood so that these findings cannot be safely extrapolated to man.

The mercurous ion is known to be unstable in the presence of proteins and to dissociate to one atom of elemental mercury and one mercuric ion. This dissociation has been observed to take place in mammalian tissues.

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Hand et al (124) injected mercurous chloride into rabbits and demonstrated the presence of both elemental and divalent mercury in various tissues.

Biotransformation of organomercurial compounds.—The organomercurial compounds discussed in this section have in common an organometallic bond linking mercury to a carbon atom. The other valence of mercury is free to combine reversibly with a wide variety of ligands found in biological tissues. The term "biotransformation" does not refer to the formation of these reversible complexes and chelates—a subject that will be discussed in another section. Biotransformation includes those reactions leading to chemical modifications of the organic moiety and the cleavage of the carbon-mercury bond.

Relative rates of biotransformation.—All compounds tested to date undergo some cleavage of the carbon-mercury bond in animal tissues (125). The exact rates of breakdown in the whole animal have not been determined. However, rough estimates from published data indicate that the short-chain alkyl mercurials undergo conversion to inorganic mercury much more slowly than other organomercurial compounds (126). Following a single injection of methyl mercury salts into experimental animals, the intact methyl mercury radical can be detected in tissues weeks after exposure. On the other hand, the phenyl and methoxyethyl mercuric salts and the mercurial diuretics are completely converted to the inorganic form within a few days after dosing the animal. Suzuki (127) reported that ethyl mercury compounds undergo biotransformation in human tissue. Inorganic mercury in proportions of total mercury ranging from 12–69% was detected in red cells, plasma, brain, spleen, liver, and kidneys, in a patient exposed for about 3 months to ethylmercurithiosalicylate. This is the only reported observation of biotransformation of an organomercurial in humans.

Mechanisms of biotransformation.—Few studies have been made on the biochemical processes involved in the cleavage of the carbon-mercury bond in mammalian tissues. Weiner et al (81) demonstrated that certain classes of organomercurial compounds would release inorganic mercury in vitro under conditions sufficiently mild to approximate an in vivo milieu. In their in vitro test system, breakage of the carbon-mercury bond required the presence of a thiol compound in a medium buffered at pH 4.0. The organomercurial diuretics rapidly released inorganic mercury. The phenyl mercury compounds and the alkyl mercurials were stable under these conditions. Daniel et al (47) have shown that methoxyethyl mercury salts also break down in vitro, in the presence of 3 mM cysteine, at a rate that is accelerated with lowering of the pH of the medium.

On the other hand, salts of mercuribenzoate exhibit the opposite effect of pH, being stable at pH 4.0 but labile at pH 8.0.[2] To what extent in vitro

[2] T. W. Clarkson and J. Vostal, unpublished data.

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mechanisms are involved in the in vivo breakdown of the carbon-mercury bond is not clear, although Daniel et al (47) have suggested that methoxyethyl mercury undergoes the same type of cleavage in vivo as in vitro. The chemical reaction believed to occur in vitro is the opposite of that used in synthesis (15). Thus, in the case of methoxyethyl mercury, the reaction would take the form:

$$CH_3 - OCH_2 - CH_2 - HgCl + HCl \leftrightarrows CH_2 = CH_2 + CH_3OH + HgCl_2$$

Presumably the presence of a thiol compound favors the reaction toward the right. Daniel et al (108) noted that the rate of breakdown in vitro of methoxyethylmercury in the presence of cysteine at pH 6.0 is about 1% per hour, a rate corresponding roughly to the rate of cleavage in vivo. Furthermore, these authors were able to isolate ^{14}C labeled ethylene from rats given methoxyethyl mercury chloride labeled with ^{14}C in the ethyl group.

Recent observations by Gage (15) indicate that microsomal enzymes may play an important role in the metabolism of phenyl mercury salts. Following the observation by Weiner et al (81) that the phenylmercury salts were stable in an in vitro test system but the p-hydroxy derivative was unstable, Gage followed the metabolic fate in rats of phenyl mercury salts having the benzene ring uniformly labeled with ^{14}C. Preliminary results suggest that the first step in the metabolism of phenyl mercury compounds is hydroxylation of the benzene ring by a microsomal enzyme in the liver, followed by a nonenzymatic cleavage of the carbon-mercury bond leading to phenol and inorganic mercury.

The biochemical mechanisms involved in the breakdown of the short chain alkylmercurials are not known. These compounds are stable in the in vitro system of Weiner et al (81). Methyl mercury chloride will release inorganic mercury at a rate of 0.4% per day (22) in the presence of 0.1 M cysteine at pH 7.0. The rate of breakdown in the rat following a single dose of methyl mercury chloride may be estimated from the data of Norseth & Clarkson (22) as between 3–5% per day. Thus it is not possible at this time to exclude the role of metabolic processes in the transformation of alkyl mercurials in rats.

Inorganic mercury as a mediator of the toxic effect of organomercurial compounds.—The acute LD_{50} of the organomercurial compounds are, on the average, about 10 times as high as the LD_{50} of inorganic mercuric salts (9a, 16, 125). Thus the conversion of as little as 10% of an LD_{50} dose of an organomercurial would release an amount of inorganic mercury equivalent to the LD_{50} dose of mercuric chloride.

Evidence that inorganic mercury is in fact a mediator of the toxic effects of organomercurials is indirect at best. Following a single dose of p-mercuribenzoate, given as the cysteinate salt, all the mercury in the rat kidneys is in the inorganic form within 60 minutes of injection (128). Thus the changes in excretion rates of salt and water that occur at 24–68 hours after a single dose of this mercurial are probably due to the inorganic mer-

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cury. Degenerative changes in kidneys of rats by the feeding of phenyl mercuric acetate for 1–2 years are probably due to inorganic mercury also (11). The renal changes induced by feeding phenyl mercury are the same as those inflicted by inorganic mercury salts and, as demonstrated by Miller et al (129) and Gage (130), phenyl mercury salts undergo complete breakdown to inorganic mercury within 24 hours in rat tissue. An LD_{50} dose of chlormerodrin in rats produced similar changes in urinary excretion rates to those produced by an LD_{50} dose of $HgCl_2$. The levels of inorganic mercury in kidney tissues were the same after chlormerodrin as after mercuric chloride (125).

That inorganic mercury released from organomercurial diuretics is the cause of diuresis is an idea that has been entertained almost from the time of introduction of these drugs (131). The so-called "mercuric ion" hypothesis received strong but indirect experimental support from Weiner et al (81). These investigators offered the following evidence in support of the mercuric ion hypothesis. First, inorganic mercury injected into dogs as the cysteinate salt induced diuresis at a lower dose (expressed as mg Hg/kg body weight) than was observed with organomercurial diuretics. Second, organomercurial diuretics rapidly released inorganic mercury in vitro in the presence of a thiol compound at an acidic pH. Organomercurials that did not release inorganic mercury in this in vitro test were nondiuretic. Third, the mercuric ion hypothesis offered a ready explanation for the fact that organomercurials are more potent diuretics in acidotic dogs whereas the potency of mercuric cysteinate is relatively independent of the acid-base balance of the test animal. This hypothesis received further support when the diuretic chlormerodrin was shown to deposit inorganic mercury in kidneys of rats, chickens, and rabbits (132) and of dogs (133). This indirect evidence prompted a more detailed quantitative study on renal levels of inorganic mercury and diuretic response in dogs (134). Two factors came to light that were unfavorable to the hypothesis. First a minimally effective diuretic dose of mercuric cysteine produced kidney levels of inorganic mercury much higher than levels of inorganic mercury found in the dog kidney after a diuretic dose of chlormerodrin or mersalyl. Second, a chosen dose of chlormerodrin or mersalyl was diuretically effective in acidotic dogs but ineffective in alkalotic animals. Nevertheless, the renal levels of inorganic mercury released from these diuretics were not increased under acidic conditions. It is difficult to design experiments to disprove the hypothesis conclusively, since it can always be argued that diuresis is produced by only a small fraction of the total inorganic mercury released to kidney tissue. Thus measurements of tissue levels of inorganic mercury in cortex and medulla do not necessarily indicate changes in minute amounts of mercury attached to the cortical renal receptor sites. Nevertheless, the observation that release of inorganic mercury is unaffected by acidosis, whereas diuretic potency is greatly increased, is difficult to accommodate to the mercuric ion hypothesis. If the total rate of release of inorganic mercury from diuresis is unaffected by acid-base changes in the animal, it seems doubtful that the rate of release at some localized receptor site would be increased.

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The short-chain alkyl mercurials cause symptoms of damage to the central nervous system following a characteristic latent period (16, 62, 135, 136). One explanation for the latent period might be an accumulation of inorganic mercury in the brain resulting from the slow rate of biotransformation of methyl mercury salts (137). However, the weight of evidence is against this possibility. Norseth & Clarkson (22) have shown that brain levels of inorganic mercury following single doses of methyl mercury chloride to rats never exceeded 2.8% of the total mercury in the brain and exhibited no tendency to accumulate over a period of 30 days. These findings suggest that inorganic mercury does not play an important role in CNS toxicity for other short-chain alkyl mercurials since all these compounds produce similar CNS disturbances. Ethyl mercury salts do, however, lead to much higher proportions of inorganic mercury in the brain of experimental animals (see review by Suzuki et al, 127) than was observed after methyl mercury salts. Levels of inorganic mercury were high in one human case of ethyl mercury poisoning (127). The toxicological significance of these high levels of inorganic mercury in brain following exposure to ethyl mercury compounds remains to be explored.

Role of biotransformation in the distribution and deposition of mercury.—Different classes of organo-mercurial compounds give rise to characteristically different patterns of distribution and deposition in organs and tissues as outlined in another section. However, such distribution patterns change with time and eventually approach a pattern similar to that of inorganic mercury. The rate of change in the pattern is affected by the rate of biotransformation of the mercurial and by the speed at which the intact mercurial is removed from the body. For example, chlormerodrin is both rapidly excreted and rapidly converted to inorganic mercury. Thus within 48 hours of a single dose of chlormerodrin, only inorganic mercury remains in the animal (132). A similar change in pattern is seen after injection of phenyl and methoxyethyl mercury salts (14, 107).

The change in pattern from that characteristic of the organomercurial to that of inorganic mercury takes place most slowly following doses of methyl mercury salts (22). The short chain alkyl mercurials and especially the methyl homologue are more slowly excreted and converted to inorganic mercury than all other classes of mercurials studied so far. Thus as long as 50 days after a single dose of methyl mercury chloride is administered to rats, significant quantities of the intact mercurial remained in the animal tissues.

The role of biotransformation in the excretion of mercury from the body.—The conversion of organic to inorganic mercury may increase or decrease the total rate of excretion of mercury from the body. If the intact molecule of the organomercurial is excreted more rapidly than inorganic mercury, biotransformation will decrease the overall excretion rate. This has been demonstrated in the case of chlormerodrin where the intact mole-

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cule is almost completely excreted within 24 hours, but inorganic mercury remains in the animals for a much longer period (132). The phenyl and methoxyethyl mercury compounds are excreted at an identical rate to that of inorganic mercury (14, 107). Thus biotransformation does not affect excretion rates. However, in the case of the short-chain alkylmercurials, biotransformation may play an important role in determining the rate of excretion of total mercury from the body. Methyl mercury compounds are excreted much more slowly than inorganic mercury (14, 107). Inorganic mercury accounts for approximately 50% of the total mercury in feces, the principal pathway of excretion following single or chronic doses of methyl mercury compounds (22, 130). Norseth & Clarkson (101) showed that the methyl mercury compounds undergo extensive enterohepatic recirculation in rats, but inorganic mercury does not. Thus a small rate of biotransformation in the liver leading to biliary excretion of inorganic mercury makes an important contribution to the fecal excretion of mercury.

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Effects of Mercury on Biochemical Processes

Previous attempts to elucidate the mechanisms of action at the cellular and subcellular level have encountered difficulties. First, to quote from a recent review by Rothstein (104):

> In studying the mechanisms of action of mercurials, we are faced with a severe logistics problem. Although mercurials are highly specific for sulfhydryl groups, they are highly unspecific in terms of proteins, almost all proteins contain sulfhydryl groups that are metal-reactive. Furthermore, because most sulfhydryl groups are important in most protein functions, mercurials can disturb almost all functions in which proteins are involved. Thus, almost every protein in the body is a potential target.

That is why the more than 1000 papers on mercury-protein interaction published between 1960 and 1965 (9a) are of little help in explaining the selective toxicity of mercury. Besides chemical specificity, the investigator must take into account the all-important distribution factors that determine whether or not mercury will reach a particular target protein and at what concentration.

A second difficulty is that the cell contains inactive binding sites (81, 96). Combination of mercury with these sites produces no detectable effects. The fact that minimum diuretically effective doses of chlormerodrin and mersalyl give kidney levels of mercury that differ by a factor of ten suggests that most of the metal may be bound to inactive sites. The observations of Pulido et al (90) and Piotrowski et al (91) indicate that metallothionein is probably one class of inactive binding sites. Furthermore, the concentration of these sites probably depends upon previous exposure to heavy metals, as discussed elsewhere.

A third difficulty arises from the fact that in common with other heavy metals, mercury binds reversibly to tissue ligands (96). The possibility is always present that the metal will redistribute from one protein to another when tissues are subjected to homogenization and extraction procedures.

For example, Nechay (138) explained the fact that Jones et al (139) observed inhibition of kidney ATPase whereas his own laboratory did not (73) as due to differences in procedures for the extraction and isolation of the ATPase enzyme system. In the original procedure of Nechay et al (73) the enzyme preparation was so diluted that mercury dissociated from it. There are many other possibilities. For example, mercury may dissociate from one enzyme to another, thus giving a completely false picture of the actions of the metal in vivo.

Another hurdle facing attempts to pinpoint enzymes directly inhibited in vivo is the probability that enzymic inhibition may be secondary to a more primary damage to the cell. The many studies attempting to implicate succinic dehydrogenase as a primary target for mercury were finally ended when Rodin & Crowson (140) reported that histologic changes preceded inhibition of the enzyme in kidney tissue.

It is therefore not surprising that progress has been slow toward pinpointing the biochemical lesions that may underly the cellular damage inflicted by mercurials. Yoshino et al (136) reported that inhibition of protein synthesis measured in brain slices taken from dogs given a single high dose of methyl mercury preceded overt neurological symptoms. Other biochemical changes came after inhibition of protein synthesis and occurred at the same time as CNS symptoms. Electron microscopic observations have indicated structural changes in the ribosomes of neuronal cells in the brain of poisoned animals (24).

The greatest research effort has been directed toward elucidating the biochemical changes associated with mercurial diuresis. In recent years most of this effort has been concerned with the $Na^+ + K^+$ activated ATPase, and publications up to 1968 have been reviewed by Cafruny (44). To judge from the most recent review from Nechay (138), there has been little progress in the past 3 years. In searching for a correlation between biochemical changes and pharmacological effects, investigators have generally taken the approach of comparing diuretic and nondiuretic mercurials, specifically, to attempt to differentiate between the inhibition of $Na^+ + K^+$ ATPase by diuretic and nondiuretic mercurials. Jones et al (139) and Bowman & Landon (141) observed ATPase inhibition in kidneys of rats injected with mercurial diuretics and absence of inhibition in animals treated with PCMB, a mercurial that does not produce diuresis. However, rats are unsatisfactory test animals for mercurial diuretics. The most recent studies in dogs (138) indicate that PCMB (*p*-chloromercuribenzoate) and mercurial diuretics give an equal depression of ATPase activity 1 hour after injection of the mercurial. Furthermore, a variety of in vitro tests on the ATPase system failed to distinguish between the mercurials. For example, Nechay tested the effect of PCMB and mercurial diuretics on isolated ATPase in the presence of varying concentrations of cysteine. According to Weiner et al (81), the diuretic enters renal tissue attached to the amino acid, and differences may exist between the cysteine complexes of diuretic and nondiuretic mercurials. However, Nechay could find no differences with respect to

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inhibition of $Na^+ + K^+$ inactivated ATPase isolated from dog kidney.

Unfortunately most investigators have chosen PCMB and PCMBS (*p*-chloromercuriphenyl sulfonate) as representative of the "nondiuretic" mercurials. It is in fact correct that these two compounds do not produce diuresis, but they do possess a unique and important property that sets them apart from all other nondiuretic mercurials. As Miller & Farah (142) were the first to show, these mercurials can prevent and reverse mercurial diuresis. In a subsequent paper (82) they suggested that PCMB competed with the mercurial diuretic for the same receptor site. However, there is no proof of this. When dogs treated with a mercurial diuretic were given a dose of PCMB, small amounts of mercury were displaced from the kidneys. However, this displaced mercury could have come from the inactive binding sites in the kidney.

Preconceived ideas on the mechanisms by which PCMB reverses mercurial diuresis have an important bearing on the interpretation of published data on inhibition of renal ATPase. It is perfectly compatible with the available evidence that PCMB and PCMBS attach to two classes of receptor site in renal tissue. One class is identical with the diuretic receptor site, combination with which results in diuresis. The combination of PCMB and PCMBS with the second class results in the prevention and reversal of mercurial diuresis. This second property was experimentally demonstrated by Miller & Farah (142). Thus the "nondiuretic" property of these mercurials is compatible with a reaction between these compounds and the diuretic receptor site in exactly the same way as the mercurial diuretic. In other words, if the diuretic receptor site happens to be the Na + K activated ATPase, one would expect to see the same degree of inhibition caused by PCMB and by the diuretic mercurials.

Future studies should include other nondiuretic mercurials than PCMB and PCMBS for structure activity studies in the ATPase system. Our ability to interpret the previous studies on the ATPase hypothesis will await a better understanding of the mechanisms by which PCMB and PCMBS reverse mercurial diuresis and, in particular, a better ability to distinguish between the one receptor attachment proposed by Miller & Farah (82) and the two-receptor theory discussed above.

The original idea of the biochemical lesion as propounded by Peters (143) involved the inhibition of a specific enzyme from which other deleterious changes in the cell ensued. An alternative point of view is that the cell membrane is the first point of attack by the heavy metals (96). The attachment of the metals to ligands in or on the plasma membrane may result in changes in passive permeability or to selective blockage of specific transport processes. Rothstein (104) has discussed the "membrane lesion" in a review of his studies on the action of a variety of mercurial compounds in the membrane of the red blood cell. Different mercurial compounds elicited widely different changes in the properties of the red cell membrane. The mercurials cross the membrane at markedly different rates. For example,

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PCMBS crosses the membrane slowly. Its rapid binding to the surface of the membrane involves only 2% of the total sulfhydryl groups in the cell membrane but results in complete blockage of glucose transport. As the mercurial slowly penetrates to compartments within the membrane, the permeability to $Na^+ + K^+$ increases, and the $Na^+ + K^+$ activated membrane ATPase is inhibited. As the mercurial passes through to the interior of the cell, the membrane recovers its original permeability properties. The more rapidly the mercury compounds penetrate the cell, the more transient the membrane effects.

The mercurial compounds follow distinct pathways through the membrane. PCMBS penetrates through two types of aqueous channels, one lined with positive fixed charged groupings, probably amino groups. Its movement through this channel can be blocked by agents reaction with NH_2 groups. Anions such as sulfate will competitively inhibit the transport of PCMBS through this channel. PCMB, on the other hand, diffuses through lipid regions of membrane in the undissociated form.

Most of our knowledge of the effects of heavy metals on cell membranes has come from studies in isolated cells and tissues (96, 104). Few investigations have been reported on permeability changes in the intact animal. White et al (144) claimed that mercurial diuretics increased the permeability to sodium of the basal membrane of the epithelial cells in the proximal tubule. However, Cafruny (44) suggested a different interpretation of their data. Bowman & Landon (141) observed a reduction in potassium permeability in kidney slices from rats treated with a mercurial diuretic. The $Na^+ + K^+$ ATPase and the active cellular accumulation of K^+ was also inhibited. Rats given PCMB did not show these changes. Bowman & Landon's paper is one of the few studies directly implicating a membrane action of mercurial diuretics.

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