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Pathological Effects of Mercury

By W. H. BUTLER (*Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey, U.K.*)

For centuries mercury has been recognized as toxic. The lesions induced are related to the class of mercury compound administered and to a smaller extent to the species. Inorganic mercury (HgCl_2) causes acute damage to the gastrointestinal tract when given by mouth and, if the animal survives, necrosis of the proximal tubule of the kidney. After a single dose the acute renal damage is rapidly repaired, but repeated treatment causes fibrosis of the kidney (Dieckmann & Butler, 1970). In man repeated doses of the mercurial diuretic mersalyl {sodium *o*-[(3-hydroxymercuri-2-methoxypropyl)carbamoyl]-phenoxyacetate} may induce a nephrotic syndrome (Riddle *et al.*, 1958). The effects of methylmercury on the central nervous system have received much attention since the description of Minimata disease (Harada, 1968) and also poisoning from dressed grains (Jalili & Abbasi, 1961). Earlier Hunter & Russell (1954) described cortical and cerebellar necrosis as a result of industrial exposure. In the rat a similar pattern of damage is seen, affecting the cerebellum and sensory pathways, although, however, cortical lesions are not seen. The distribution of lesions differs from that of organophosphorus compounds, in that the entire sensory pathway is involved whereas in poisoning by organophosphorus compounds a dying back of sensory nerves from the periphery is seen.

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The Biological Properties and Distribution of Mercury

By T. W. CLARKSON (*Departments of Radiation Biology and Biophysics and of Pharmacology and Toxicology, The Medical School, University of Rochester, Rochester, N.Y. 14620, U.S.A.*)

The high affinity of mercuric cations for thiol groups imposes a severe logistics problem in considering the biochemical mechanisms of action of mercurial compounds. To quote from a recent review by A. Rothstein (paper presented at the 4th. Rochester Conference on Toxicity at the University of Rochester, Rochester, N.Y., U.S.A., in 1971) 'although the mercurials are highly specific to -SH groups, they are highly unspecific in terms of proteins. Almost all proteins contain -SH groups that are metal reactive. Furthermore, because -SH groups are important to 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'. In other words the mercurials are potent but unspecific enzyme inhibitors. Thus the view has developed that biochemical and cellular injury will be seen wherever mercury accumulates in sufficient concentrations, a view that has influenced much of the experimental work on the toxicology of mercury compounds. Thus considerable emphasis has been placed on studies of the kinetics and mechanism of transport and distribution of mercury. It is of interest to see to what extent this approach has been useful in leading to a better understanding of the toxicology of mercury.

Quantitative description of the processes of absorption, distribution and excretion have been greatly facilitated by the introduction of γ -ray scintillation counting making use of the isotopic tracer ^{203}Hg (Rothstein & Hayes, 1960). Compartmental analysis of the data revealed an important general property applicable to all mercury compounds studied to date, namely that, in experimental animals given repeated single doses of radioactive mercury, each dose behaved independently of the previous dose with respect to its kinetic parameters. This property greatly simplified mathematical treatment of the data. It allowed calculation of the cumulative body burden of mercury during chronic exposure, the determination of biological half-times of mercury and the efficiency of pulmonary and gastrointestinal absorption. Thus Rothstein & Hayes (1964) were able to determine the pulmonary absorption of mercury vapour to be 100%. The gastrointestinal absorption

of methylmercury and phenylmercury compounds has been determined to be in the range 80–100%, whereas salts of inorganic mercury undergo 15% absorption or less (T. W. Clarkson, paper presented at the Workshop on Mercury in the Western Environment at Oregon State University, Corvallis, Oreg., U.S.A., in 1971).

The importance of absorption and distribution processes in determining the biological effect of mercury compounds is well documented. Phenylmercuric acetate and HgCl_2 produce similar toxic effects in the kidney when given daily in the diet (Fitzhugh *et al.*, 1950). Ingestion of these compounds leads to generally similar patterns of organ distribution and notably to high kidney concentrations of mercury. Phenylmercury compounds are rapidly converted into inorganic mercury in the body (Gage, 1964). The fact that much lower dietary doses of phenylmercury than of inorganic mercury can lead to the same degree of damage can be quantitatively accounted for by the difference in efficiency of gastrointestinal absorption of the two compounds.

The much greater potential of mercury vapour, compared with equivalent doses of inorganic mercury, to inflict damage to the central nervous system is undoubtedly related to the fact that brain contents after exposure to the vapour are an order of magnitude higher than after inorganic mercury (Berlin *et al.*, 1966; Magos, 1967, 1968).

The unique biological properties of the short-chain alkylmercury compounds appear to be related to the ability of these compounds to cross cell membranes and to the fact that they are only slowly converted into inorganic mercury in the body. The practically complete absorption of these compounds from food and their rapid passage across the blood–brain and placental barriers accounts for the potential of these compounds to damage the central nervous system in both adult and foetal life. Species differences in the toxicities of the alkylmercury compounds are paralleled by differences in biological half-lives and the differences in distribution of these compounds between erythrocytes and plasma.

Despite the importance of processes of transport and distribution, very little is known about the underlying biochemical mechanisms. The rapid transport of mercury vapour across pulmonary membranes is probably due to the high diffusibility and lipid-solubility of the mercury atom (Hughes, 1957). Uptake by brain tissue involves the diffusion of the dissolved mercury vapour across the blood–brain barrier and its oxidation to Hg^{2+} ion (Magos, 1967, 1968; L. Magos, T. W. Clarkson & M. R. Greenwood, unpublished work). The vapour is rapidly oxidized by suspensions of erythrocytes by metabolically dependent processes (Clarkson *et al.*, 1961; F. N. Nielsen-Kudsk, paper presented at the 4th. Rochester Conference on Toxicity at the Uni-

versity of Rochester, Rochester, N.Y., U.S.A., in 1971).

The efficient absorption of methylmercury and phenylmercury compounds from food may be due to the lipid-solubility of their chloride complexes. Methylmercury is protein-bound when ingested in food, but a small fraction of the chloride complex may be formed in the gastric juices as a result of digestion of the protein and the high concentration of HCl present. Diffusion of the chloride complex across the gastric membrane into the blood should lead to further dissociation of the protein–methylmercury complex until all the mercurial has been absorbed. Studies on the equilibration of methylmercury in model systems with polythiol resins support this conclusion.

The ability of the kidney to accumulate high concentrations of inorganic mercury is dependent in part on metabolic processes (Clarkson & Magos, 1970). 2,4-Dinitrophenol produced a 26–34% inhibition of renal uptake of inorganic mercury. These values give a minimal estimate of metabolically dependent accumulation, since it is unlikely that 2,4-dinitrophenol produced a complete block of ATP synthesis. However, the observations do not exclude a substantial role for glomerular filtration.

Metallothionein, a protein first isolated by Kagi & Vallee (1960), may play an important role in protecting kidney tissue from damage from inorganic mercury (J. Piotrowski, B. Trojanowska, J. J. Wisniewska & W. Bolanowska, paper presented at the 4th. Rochester Conference on Toxicity at the University of Rochester, Rochester, N.Y., U.S.A., in 1971). Its strong affinity for mercury is due to the high thiol content of this protein. Metallothionein accounts for the chief protein-bound fraction of mercury in kidneys. Normal metallothionein contents in rat kidneys are equivalent in mercury-binding capacity to a concentration of renal mercury associated with the onset of damage. Moreover, this metallothionein content corresponds approximately to the maximum binding capacity of the 'high-affinity' sites reported by Clarkson & Magos (1966). Thus, once the binding capacity of metallothionein is exceeded, toxic effects appear in the kidney. Interestingly, chronic dosing of rats with HgCl_2 over a period of several weeks induced an approximately sixfold increase in renal metallothionein content (J. Piotrowski, B. Trojanowska, J. J. Wisniewska & W. Bolanowska, paper presented at the 4th. Rochester Conference on Toxicity at the University of Rochester, Rochester, N.Y., U.S.A., in 1971).

The formation of small-molecular-weight (diffusible) complexes with thiol compounds in the liver appears to be an important process in the distribution of methylmercury (Norseth & Clarkson, 1971; T. Norseth, paper presented at the 4th. Rochester Conference on Toxicity at the University of

Rochester, Rochester, N.Y., U.S.A., in 1971). Approx. 80% of methylmercury in the bile of rats dosed with methylmercuric chloride was in the form of a small-molecular-weight thiol complex, probably a dipeptide. Most if not all of the complex is re-absorbed back into the blood stream, where it significantly influences the distribution of methylmercury to organs and tissues.

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Analytical Procedures Used in the Determination of Mercury in the Body

By L. MAGOS (*Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey, U.K.*)

The uniqueness of mercury rests on its liquid state at room temperature, and its volatility at room temperature makes it unique among the metals as a health hazard. The same quality makes it suitable for its determination by flameless atomic-absorption spectrophotometry. However, when this elegant technique was used for biological material, tedious treatment of samples was found to be necessary. In the method of Jacobs *et al.* (1960) the sample had to be digested and the mercury extracted with dithizone. The dithizone was evaporated and the residue burnt. A balance between the preparative technique and measurement has been established in the method of Magos & Cernik (1969). SnCl_2 is added to the acidified sample, which can be urine, blood or tissue homogenate; cysteine is added to ensure that no appreciable amount of mercury is lost before the reaction is triggered by the introduction of excess of NaOH. By this process all the inorganic mercury is reduced and carried as atomic mercury from the reaction vessel to the gas-cell of an atomic-absorption spectrophotometer or mercury-vapour detector in a stream of air.

The advantage of this method rests not only in the very simple preparative work, but, as Lindstedt & Skare (1971) pointed out, in the prevention of the formation of interfering complex ions of high redox potential.

The method can also be used for the determination of mercury, after digestion of, for example, blood or hair samples, with the precision of neutron activation (Takemoto *et al.*, 1971). Moreover, if the technique is used for digested and undigested portions of the same sample, total mercury, inorganic mercury and by difference organomercurials can be determined (Suzuki *et al.*, 1970). Gage & Warren (1970) made use of the different labilities of organomercurials in the presence of acid cysteine and were able to distinguish phenylmercury, methoxyethylmercury or ethylmercury from inorganic mercury when analysing pretreated and non-treated portions of the same sample with the Magos & Cernik (1969) method.

The use of SnCl_2 for the conversion of inorganic mercury into atomic mercury in the course of injection has been used to learn why more mercury accumulates in the brain after exposure to the vapour than after the injection of a mercury salt. According to Berlin (1966), mercury vapour does not reach the brain unchanged but is first taken up and oxidized by erythrocytes and later regenerated again as metal. However, Magos (1967) showed that *in vitro* blood takes up mercury, which is oxidized only after a certain delay. To investigate the problem *in vivo* a technique has been developed for injection of atomic mercury intravenously in aqueous solution (Magos, 1968). When ^{203}Hg was used and the expired air was passed through a hopcalite absorber immersed into the well of a scintillation counter it was possible to demonstrate that animals exhaled approx. 20% of the dose, confirming the time-lag in the oxidation of mercury in blood. The distribution of mercury followed the same pattern as after exposure to the vapour (Magos, 1968). When mercury was injected, by using this technique, into rats pretreated with ethanol, which is known to inhibit uptake of mercury from inhaled air (Kudsk, 1965), the exhaled amount of mercury increased, exhalation time was prolonged and less mercury was taken up by heart, lung and brain (L. Magos, T. W. Clarkson & M. R. Greenwood, unpublished work). That, in contrast with inorganic mercury, placenta does not form a barrier against atomic mercury was demonstrated also with this technique (T. W. Clarkson, L. Magos & M. R. Greenwood, unpublished work).

Although mercury vapour has remained the main form of occupational exposure to mercury, methylmercury has attracted more attention recently because of its importance as an environmental pollutant. This resulted in a search for simple analytical methods.

One method (Norseth & Clarkson, 1970a) is based