on the principle that aqueous solutions of mercuric salts, but not mercury bound covalently to carbon, are capable of undergoing isotopic exchange with mercury vapour in contact with the solution. If animals are injected with methylmercury labelled with ^{203}Hg , the radioactivity of the mercury in the sample before exchange gives the total amount of mercury whereas the mercury taken up by the mercury globule in the inner well of a Conway micro diffusion unit after 4h incubation at 40°C gives the inorganic mercury. Norseth & Clarkson (1970a) have shown that, after injection of methylmercury to rats, up to 40% of the total mercury in the kidneys was in the inorganic form compared with 2.8% in brain and 11% in liver. More mercury was found in the erythrocytes than in the plasma, and mercury excreted in the faeces contained a higher proportion of inorganic mercury than that in the urine (Norseth & Clarkson, 1970b).

A non-isotopic technique applicable for the determination of total mercury and inorganic mercury in undigested biological samples has been developed (Magos, 1971); this, like the earlier one (Magos & Cernik, 1969), is a flameless atomic-absorption method that uses a very similar assembly. Tissue homogenates, urine or blood are diluted with saline to 20ml, and in the following order 1ml of 1% cysteine solution, 10ml of 8M- H_2SO_4 and either 1ml of SnCl_2 - CdCl_2 reagent (containing 500mg of SnCl_2 and 100mg of CdCl_2) or only 100mg of SnCl_2 are added to the reaction mixture. Air (2.5litres/min) is bubbled through the reaction vessel, and 20ml of 45% NaOH is then added. The SnCl_2 - CdCl_2 reagent releases all the mercury from the sample, but SnCl_2 alone releases only inorganic mercury.

Total mercury, inorganic mercury and, by difference, methylmercury have been determined with this method in rats treated orally five times a week with 0.84, 1.68 or 3.36mg of Hg (as methylmercury dicyandiamide)/kg body wt. (Magos & Butler, 1972). In the brain methylmercury invariably comprised 97% of the total mercury and in the liver about 92%. In the kidney the proportion of inorganic mercury to total mercury depended on the dose and averaged 65, 71 and 80% respectively. In the rats given 0.84mg/kg body wt. the proportion of inorganic mercury to total mercury increased with time, and after 25-30 doses the methylmercury content of kidneys actually decreased. This finding might indicate that the uptake and storage capacity for methylmercury decreased in the kidneys. By using 30cm gas-cell and reagent of low blank value it is possible to measure with this method mercury in normal blood samples containing less than 10ng of Hg/ml (Magos & Clarkson, 1972).

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The Biotransformation of Organomercury Compounds

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Organomercury salts have been extensively employed as fungicides in agriculture and in the paper industry, and in human medicine as diuretics. Although there is ample evidence that organomercurials are degraded to inorganic mercury in mammals, soil and micro-organisms, little is known of the mechanism(s) of this transformation. The development of simple and sensitive procedures for the differential analysis of inorganic and organic mercury compounds may, however, stimulate interest in this topic (Clarkson *et al.*, 1965; Gage & Warren, 1970; Clarkson & Greenwood, 1970).

The dissociation energy of the carbon-mercury bond for simple mercury dialkyls, e.g. $\text{Hg}(\text{CH}_3)_2$, has been estimated to be 54-218 kJ/mol (13-52 kcal/mol), and many organomercury compounds release Hg^{2+} readily in solutions of moderate acidity. Weiner *et al.* (1962) observed that all organomercury diuretics were acid-labile, and they suggested that pharmacological activity was the result of the non-enzymic liberation of Hg^{2+} at susceptible sites within the kidney. Daniel *et al.* (1971) showed that methoxyethylmercury was metabolized by rats to ethylene and Hg^{2+} . As the rate of formation of ethylene *in vivo* (30% in 24h) was similar to that found for the release of Hg^{2+} when the organomercurial was incubated *in vitro* at pH 6 in the presence of cysteine (2mM), the authors concluded that methoxyethylmercury was degraded non-enzymically in the tissues. Recent studies in rats with mersalyl {sodium *o*-[(3-hydroxymercuri-2-methoxy-

propyl]carbamoyl]phenoxyacetate} have, however, shown that, despite being acid-labile, it is excreted largely unchanged (J. W. Daniel, unpublished work).

Circumstantial evidence for enzymic cleavage of the carbon–mercury bond was obtained by Tonomura & Kanzaki (1969), who demonstrated that a cell-free extract of a mercury-resistant pseudomonad, in the presence of NAD(P)H and thioglycollate, converted phenylmercury, methylmercury and ethylmercury into metallic mercury and benzene, methane and ethane respectively. The authors represented the reaction as a reduction. Daniel & Gage (1971) found that rats converted phenylmercury into phenol and Hg^{2+} , and they suggested that the reaction probably involved the formation of *p*- or *o*-hydroxyphenylmercury, both of which are acid-labile. Subsequent studies with rat liver homogenates have established that phenylmercury is degraded initially to benzene, which is subsequently converted into phenol. Enzyme activity is present only in the soluble fraction and has no requirement for NAD(P)H. The enzyme catalyses the formation of Hg^{2+} from *p*-chloromercuribenzoate and methoxyethylmercury, but not from methylmercury or mersalyl. This reaction may be represented as a hydrolysis. It is possible that different mechanisms operate in the mammalian and bacterial systems, but an alternative hypothesis is that both reactions are hydrolytic but that in the microbiological preparation the Hg^{2+} liberated is rapidly reduced to metallic mercury by an NAD(P)H-dependent enzyme (Komura *et al.*, 1970).

Methylmercury, in contrast with phenylmercury, is only slowly degraded to Hg^{2+} in rats (Gage, 1964). Norseth & Clarkson (1971) suggested that most of the breakdown occurs within the large intestine, a view that is not supported from studies of methylmercury metabolism in germ-free rats (Norseth, 1971).

Kimura & Miller (1964) suggested that the degradation of phenylmercury in soil was the result of microbiological action. Whereas the loss of phenylmercury from soil was found to be due mainly to the formation of volatile metallic mercury, the loss of ethylmercury and particularly methylmercury salts was due to the volatility of the organomercurial. No evidence was obtained for the formation of either diphenylmercury or dimethylmercury, compounds that, on the basis of phytotoxicity experiments, were considered by Boorer (1944) to be the most likely products resulting from the interaction of phenylmercury and methylmercury salts with clay micelles.

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Permeability Effects of Organomercurials

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Organomercury compounds are well known as inhibitors of enzyme reactions and also of membrane transport processes, e.g. glucose transport across a variety of animal cell membranes (Stein, 1967), the phosphate carrier of the inner mitochondrial membrane (Tyler, 1969) and the $\text{Na}^+ + \text{K}^+$ -transporter adenosine triphosphatase of cell membranes (Skou, 1964). It has also been reported that *p*-chloromercuribenzoate and *p*-chloromercuribenzenesulphonate at rather high (0.5 mM) concentrations and after 75 min incubation decrease the water permeability of human erythrocytes tenfold (Macey & Farmer, 1970).

In contrast with these inhibitory effects, a completely different type of action has been observed in which certain organomercurial compounds catalyse the exchange of anions across biological membranes and artificial bilipid-layer membranes (Watling & Selwyn, 1970). In biological systems natural thiol groups act as inhibitors of the anion exchange by removing the organomercurial from the membrane or by immobilizing it by high-affinity binding in the membrane. Nevertheless under some conditions, such as during absorption of organomercurial compounds from the external environment, the anion exchange may proceed fast enough to have physiological effects and be involved in the toxic action of these compounds. Apart from its value as a model for natural transport processes, study of this anion exchange is valuable as it is related to the passage of the organomercurial across biological membranes. Further, a similar activity has been found with certain organolead and organotin compounds; the lead compounds have toxicological importance, and the tin compounds are of interest as biocides that may be degraded to comparatively harmless products. The toxicological properties of organic compounds of mercury, lead and tin have been compared by Barnes & Magos (1968).