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have, however, shown that, despite being acid-labile, it is excreted largely unchanged (J. W. Daniel, unpublished work).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

- Boorer, J. R. (1944) *Ann. Appl. Biol.* **31**, 340
 Clarkson, T. W. & Greenwood, M. R. (1970) *Anal. Biochem.* **37**, 236–243
 Clarkson, T. W., Rothstein, A. & Sutherland, R. (1965) *Brit. J. Pharmacol.* **24**, 1–13
 Daniel, J. W. & Gage, J. C. (1971) *Biochem. J.* **122**, 24P
 Daniel, J. W., Gage, J. C. & Lefevre, P. A. (1971) *Biochem. J.* **121**, 411–415

- Gage, J. C. (1964) *Brit. J. Ind. Med.* **21**, 197–202
 Gage, J. C. & Warren, J. M. (1970) *Ann. Occup. Hyg.* **13**, 115–123
 Kimura, Y. & Miller, V. L. (1964) *J. Agr. Food Chem.* **12**, 253–257
 Komura, I., Izaki, K. & Takahashi, H. (1970) *Agr. Biol. Chem.* **34**, 480–482
 Norseth, T. (1971) *Acta Pharmacol. Toxicol.* **30**, 172–176
 Norseth, T. & Clarkson, T. W. (1970) *Arch. Environ. Health* **22**, 568–577
 Tonomura, K. & Kanzaki, F. (1969) *Biochim. Biophys. Acta* **184**, 227–229
 Weiner, I. M., Levy, R. I. & Mudge, G. H. (1962) *J. Pharmacol. Exp. Ther.* **138**, 96–112

Permeability Effects of Organomercurials

By M. J. SELWYN (*School of Biological Sciences, University of East Anglia, Norwich NOR 88C, U.K.*)

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).

The anion exchange was first observed as an exchange of Cl^- for OH^- across the mitochondrial membrane that was catalysed by tripropyltin chloride and allowed respiration-driven uptake of large amounts of Ca^{2+} (Selwyn *et al.*, 1970*b*). Much of the subsequent work, in association with Dr. A. P. Dawson, has been on the trialkyltin compounds, as these are otherwise much less reactive and are not bound by thiol groups. The anion exchange appears to be fundamentally similar in the mercury, tin and lead compounds. Although in principle the exchange of any pair of anions can be studied, it is experimentally most convenient to observe the exchange of any particular anion for OH^- ions, and it is also advantageous to avoid the complications caused by respiration. Two techniques, passive swelling in iso-osmotic media and pulsed titrations (Mitchell & Moyle, 1967), which have proved particularly useful (Selwyn *et al.*, 1970*a,b*; Watling & Selwyn, 1970), require the membrane in the form of the permeability barrier of a vesicular structure of microscopic size. This is conveniently provided by chloroplasts, mitochondria or erythrocytes or, in the case of artificial membranes, by smectic mesophases (also known as liposomes) (Bangham *et al.*, 1965). In the first technique the vesicles are suspended in a medium that is iso-osmotic with their contents; if both ions in the external medium can cross the membrane in a complementary fashion such that the overall process is electrically neutral and does not produce pH changes on either side of the membrane, then continued entry of both ions will occur. If the overall process is either electrically imbalanced or produces a pH difference across the membrane, then entry of both ions will be limited. Entry of ions is followed by entry of water to maintain osmotic balance, and the consequent swelling of the vesicles can be followed by recording changes in light-scattering by the suspension. In many experiments ammonium salts are used. If the external anion can exchange for an internal OH^- ion then extensive swelling will occur, since the other penetrant species is ammonia, which on re-forming NH_4^+ ions inside the vesicles effectively removes internal H^+ ions. Thus with certain ammonium salts rapid swelling is observed on the addition of the organometallic compounds that catalyse the anion- OH^- exchange. In other experiments sodium or potassium salts are used in conjunction with ionophores such as valinomycin and proton conductors such as 2,4-dinitrophenol. These provide similar information to that obtained from the experiments with ammonium salts, but in addition can be used to demonstrate the absence of an unspecific lytic activity and to show the compulsory nature of the exchange, i.e. the anion can cross the membrane only in exchange for an OH^- ion crossing the membrane in the reverse direction. In the technique of pulsed titrations a small amount of acid or alkali is added to the suspension of

vesicles. Titration of their contents is delayed unless H^+ or OH^- ions can cross the vesicular membrane in association with some process that balances their electrical charge. The anion- OH^- exchange mediated by organometallic compounds is an electrically neutral process, and this technique demonstrates the difference between these compounds and uncouplers that act as proton uniporters.

By using these techniques anion- OH^- exchange catalysed by organomercury and other organometallic compounds has been demonstrated with F^- , Cl^- , Br^- and I^- , and also the pseudo-halide ion CNS^- . The common characteristic of these ions, as well as the OH^- ion, is their ability to form a covalent bond with the metal. Ions such as NO_3^- , which form fully ionic salts with the organometallic cations, are not effective in the anion- OH^- exchange. Similarly quaternary ammonium compounds, even when lipid-soluble, do not catalyse an anion exchange. The relationship between ability to form covalent compounds and effectiveness in the anion- OH^- exchange is illustrated by the high conductivity of tetraethylammonium iodide and methylmercuric nitrate in dimethyl sulphoxide and the very low conductivity of methylmercuric halides in the same solvent (Gregory & Ingold, 1969). The relative rates for different anions are different for organomercury and trialkyltin compounds, and triethylgermanium chloride has little activity as an anion-exchange catalyst. A possible interpretation of these observations is that an insufficiently polarized covalent bond may have too low a ligand-exchange rate and that this can limit the overall transfer process. Another essential feature of these catalysts is a partition coefficient favouring concentration of the catalyst in the lipid membrane phase. In the series phenylmercury, ethylmercury, *p*-mercuribenzoate and *p*-mercuribenzenesulphonate the first two are highly effective whereas the last, which has a strongly hydrophilic group, is inactive in Cl^- - OH^- exchange.

Investigation of the effects of the anion- OH^- exchange, mediated by trialkyltins, on mitochondria has been made by Stockdale *et al.* (1970) and Rose & Aldridge (1972). The importance of the anion- OH^- exchange in the overall effect produced by the trialkyltins has been investigated by eliminating the exchange, in the earlier work by using a sucrose instead of a KCl medium and in the later work by the more satisfactory procedure of replacing the Cl^- ion by NO_3^- or isethionate ions. Neither of these ions is effective in the anion- OH^- exchange, and isethionate has the added advantage that it does not penetrate rat liver mitochondrial or erythrocyte membranes. Scott *et al.* (1970) have investigated the effects of *p*-mercuribenzoate and *p*-mercuribenzenesulphonate on heart mitochondria, but interpretation of the observed permeability changes is complicated by the extensive reaction of the former reagent with thiol groups.

From the work with trialkyltins it is clear that the anion- OH^- exchange collapses the pH gradient across the mitochondrial membrane, and under certain conditions this can cause inhibition of mitochondrial respiration: first, by lowering the intramitochondrial concentration of substrates of the citric acid-cycle enzymes (Manger, 1969), and, secondly, by shifting the intramitochondrial pH away from the optimum for some of the intramitochondrial enzymes (Dawson *et al.*, 1972). Under other conditions the anion- OH^- exchange can cause uncoupling of mitochondrial respiration or permit accumulation of Ca^{2+} (Stockdale *et al.*, 1970). Although much of the work so far has been on the trialkyltins, it is clear that under conditions where organomercurials are producing an anion- OH^- exchange this will have significant physiological effects in addition to the direct inhibitory actions of these compounds.

- Bangham, A. D., Standish, M. M. & Watkins, J. C. (1965) *J. Mol. Biol.* **13**, 238
 Barnes, J. M. & Magos, L. (1968) *Organometal. Chem. Rev. Sect. A* **3**, 137
 Dawson, A. P., Dunnett, S. J. & Selwyn, M. J. (1972) *Biochem. J.* **127**, 67P
 Gregory, B. J. & Ingold, C. K. (1969) *J. Chem. Soc. B* 276
 Macey, R. I. & Farmer, R. E. L. (1970) *Biophys. Acta* **211**, 104
 Manger, J. R. (1969) *FEBS Lett.* **5**, 331
 Mitchell, P. & Moyle, J. (1967) *Biochem. J.* **104**, 588
 Rose, M. S. & Aldridge, W. N. (1972) *Biochem. J.* **127**, 51
 Scott, K. M., Knight, V. A., Settlemire, C. T. & Brierley, G. P. (1970) *Biochemistry* **9**, 714
 Selwyn, M. J., Dawson, A. P., Stockdale, M. & Gains, N. (1970a) *Eur. J. Biochem.* **14**, 120
 Selwyn, M. J., Stockdale, M. & Dawson, A. P. (1970b) *Biochem. J.* **116**, 15P
 Skou, J. C. (1964) *Progr. Biophys. Biophys. Chem.* **14**, 131
 Stein, W. D. (1967) *The Movement of Molecules across Cell Membranes*, pp. 289–295, Academic Press, New York
 Stockdale, M., Dawson, A. P. & Selwyn, M. J. (1970) *Eur. J. Biochem.* **15**, 342
 Tyler, D. D. (1969) *Biochem. J.* **111**, 665
 Watling, A. S. & Selwyn, M. J. (1970) *FEBS Lett.* **10**, 139

The Biological Alkylation of Mercury

By LARS LANDNER (Swedish Water and Air Pollution Research Laboratory, Box 5607, S-114 86 Stockholm, Sweden)

The formation of alkylmercury compounds by micro-organisms was first described by Jensen & Jernelöv (1967, 1969). These authors reported that an unidentified micro-organism in sludge from aquaria and in lake sediments was able to methylate inorganic mercury.

Since these first reports on the biological methyla-

tion of mercury, studies on the biochemical pathways for the process have been made. Wood *et al.* (1968) showed that cell-free extracts of a strictly anaerobic methanogenic bacterium effectively convert inorganic mercury into methylmercury, with methylcobalamin as a substrate.

In an experiment *in vivo*, M. Yamada & K. Tonomura (personal communication) isolated an anaerobe, identified as *Clostridium cochlearium*, that was capable of producing methylmercury when it was incubated in a medium containing HgCl_2 , cysteine and vitamin B_{12} . However, no production of methylmercury from HgS was observed.

The emphasis on anaerobes for such biochemical experiments has sometimes led to the opinion that anaerobic conditions are required for the biotransformation of mercury in sediments contaminated by mercury. In natural waters and sediments, however, anaerobic conditions almost always mean that H_2S is present. Mercury thus will be bound as HgS , and in this form it is, even after transfer to an aerobic environment, 100–1000 times less available for methylation than Hg^{2+} is (Fagerström & Jernelöv, 1971). Thus, in natural aquatic ecosystems, anaerobic conditions, with H_2S present, should effectively inhibit the methylation of mercury. This is supported by recent results indicating a complete lack of yield of methylmercury in mud and soil under strict anaerobic conditions (Rissanen *et al.*, 1970).

On the other hand, high rates of mercury methylation have been observed in the upper, aerobic, layers of lake sediments (Jernelöv, 1970), as well as in dredged and air-dried mercury-contaminated sediments. Thus it is probable that the most important biomethylation of mercury (from an ecological point of view) is carried out by aerobic organisms.

Therefore it was decided to investigate the pathways used by aerobic micro-organisms for the methylation of mercury. When ordinary laboratory strains of *Neurospora crassa* were found to be capable of methylating mercury, a starting material for the demonstration of a different biochemical pathway was available, because vitamin B_{12} is not known to be involved in the metabolism of this ascomycete fungus (Dalal *et al.*, 1961).

The possibility that methylation of mercury *in vivo* is carried out by means of direct transmethylation involving methionine or *S*-adenosylmethionine was ruled out in preliminary experiments. Addition of L-methionine to the HgCl_2 -supplemented substrate of *Neurospora* cultures never increased the yield of methylmercury, but seemed to render the HgCl_2 more toxic to the cells. Increase of the toxicity of HgCl_2 was not observed in the presence of other methyl donors, such as choline or betaine. These observations suggested some kind of relationship between the methylation of mercury and the biosynthesis of methionine.