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

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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.

Two further considerations were concerned in the basis of the search for a mechanism of methylation of mercury. First, it seems improbable that micro-organisms, which have been exposed to inorganic mercury in Nature at all times, should produce a more toxic compound out of a less toxic one. (Ionized methylmercury is more toxic than is inorganic mercury to *Neurospora*, as well as to many other organisms.) Therefore it is attractive to assume that the methylmercury, at the moment of its formation in the micro-organism cell, is complexed in a way that makes it biologically inactive. For the micro-organism itself the methylation might then signify a detoxification of inorganic mercury. The other consideration was inspired by the finding by Miller & Harmon (1967) that genes determining the resistance towards Hg^{2+} and genes determining enzymes involved in the synthesis of methionine are closely associated in one complex gene in *Staphylococcus*. This suggested the possibility of a functional relationship between resistance to (e.g. detoxification of) mercury and methionine biosynthesis.

To investigate the connexion between resistance to Hg^{2+} and methylation, a series of selection experiments was carried out after induction of mutations by X-ray irradiation (Landner, 1971). Irradiated conidia were plated on Hg^{2+} -containing media, and tolerant mutants were collected. Isolates tolerant to 225 p.p.m. of Hg^{2+} yielded about ten times more methylmercury than did the original strain, when grown aerobically in liquid minimal medium with HgCl_2 and to which a thiol was added. Also, in comparison with other unselected strains, the selected ones showed a much higher methylation efficiency. These results strongly indicate that the methylation of mercury in *Neurospora* does imply a detoxification.

The positive correlation demonstrated here between methylation efficiency and resistance towards high concentrations of mercury in the substrate might (if it is of general occurrence among micro-organisms) have fundamental ecological consequences. In regions with industrial mercury pollution high concentrations of inorganic mercury may be built up in the sediments of lakes and rivers. In such environments micro-organisms with an increased tolerance to Hg^{2+} are favoured to the detriment of those with low tolerance. This results in a higher efficiency to methylate mercury and the relative rate of mercury mobilization will increase. Thus contamination of lake sediments with inorganic mercury might cause a change in the selective pressure on the microbial community, favouring those that methylate at a higher rate, resulting in a remaining higher recirculation rate for mercury and a maintenance of higher methylmercury concentrations in water-living organisms.

The finding that addition of certain thiols enhanced the yield of methylmercury in tolerant as well as non-

tolerant strains was further studied. Addition of DL-homocysteine slightly in excess of Hg^{2+} (on molar basis) gave the most efficient synthesis of methylmercury. A large surplus of homocysteine decreased the efficiency, and a surplus of Hg^{2+} prevented growth. No such relationship was obtained when L-cysteine replaced homocysteine, even though cysteine also stimulates the methylation of mercury. Other thiols tested, such as mercaptoacetic acid, dimercapto-propanol and GSH, did not cause an increase in methylmercury concentration in the cells. These findings suggest, first, that the detoxicating methylation of mercury involves one or more steps of the methionine-biosynthesis pathway, and, secondly, that the stimulation of the methylation of mercury by thiols is not simply due to a more efficient uptake of Hg^{2+} to the cells. Homocysteine certainly is the last precursor for methionine in *Neurospora*, whether it is formed by cleavage of cystathionine, which in turn is synthesized from O-acetylhomoserine and cysteine (Flavin & Slaughter, 1964; Kerr & Flavin, 1968), or by condensation of homoserine and H_2S (Wiebers & Garner, 1960, 1966). Normally the homocysteine is methylated to methionine, which exerts feedback repression of the enzymes of the pathway.

If mercury is bound to homocysteine, the sulphur is masked, and in the transmethylation reaction the methyl group might be transferred to the mercury atom. The methylation of mercury may thus be regarded as an 'incorrect' synthesis of methionine. As the methylmercury-homocysteine complex presumably cannot execute the feedback control of the methylating enzyme(s), the synthesis continues until unloaded homocysteine molecules in sufficient amount have been methylated to methionine, this molecule being able to inactivate the enzyme or repress the synthesis of it. This seems to happen only when a large surplus of homocysteine over Hg^{2+} is available. The above interpretation is also compatible with the observation that the addition of methionine to the Hg^{2+} -containing media leads to a break in the detoxification of mercury by methylation (a strong decrease in yield of methylmercury) when non-tolerant strains are used.

A very plausible hypothesis would be that the mutants showing higher resistance to Hg^{2+} actually are mutants constitutive for the biosynthesis of methionine. The control of one or several enzymes of the methionine-biosynthetic pathway would then be impaired, giving rise to a continuous methylation of Hg^{2+} .

Mutants constitutive for the biosynthesis of methionine are well known in several organisms (Lawrence *et al.*, 1968; Su & Greene, 1971). To test the validity of the above-mentioned hypothesis the mercury-resistant mutants should be compared with such constitutive mutants, their ability to accumulate and/or excrete methionine should be investigated and

their resistance to different concentrations of methionine analogues, e.g. methylmethionine, ethionine or norleucine, should be tested.

- Dalal, F. R., Rege, D. V. & Sreenivasan, A. (1961) *Biochem. J.* **81**, 317
- Fagerström, T. & Jernelöv, A. (1971) *Water Res.* **5**, 121
- Flavin, M. & Slaughter, C. (1964) *J. Biol. Chem.* **239**, 2212
- Jensen, S. & Jernelöv, A. (1967) *Biocidininformation*, p. 10, Nordförs, Stockholm
- Jensen, S. & Jernelöv, A. (1969) *Nature (London)* **223**, 753
- Jernelöv, A. (1970) *Limnol. Oceanogr.* **15**, 958
- Kerr, D. & Flavin, M. (1968) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **27**, 799
- Landner, L. (1971) *Nature (London)* **230**, 452
- Lawrence, D. A., Smith, D. A. & Rowbury, R. J. (1968) *Genetics* **58**, 473
- Miller, M. A. & Harmon, S. A. (1967) *Nature (London)* **215**, 531
- Rissanen, K., Erkama, J. & Miettinen, J. K. (1970) *Conference on Marine Pollution, Rome, FAO FIR*, MP/70/E-61
- Su, C.-H. & Greene, R. C. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 367
- Wiebers, J. L. & Garner, H. R. (1960) *J. Bacteriol.* **80**, 51
- Wiebers, J. L. & Garner, H. R. (1966) *Biochim. Biophys. Acta* **117**, 403
- Wood, J. M., Kennedy, S. F. & Rosén, C. G. (1968) *Nature (London)* **220**, 173

Mercury in Food

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The cause of the so-called 'Minamata disease' in Japan during the period from 1953 into the 1960s was traced to the consumption of fish containing high concentrations of methylmercury compounds. Several workers then reported elevated concentrations of mercury in fish from Swedish rivers, lakes and coastal waters (*Methyl Mercury in Fish*, Report of an Expert Group, 1971). Following the lead of Sweden and Japan, other countries such as the U.K., U.S.A. and Canada also made studies of the mercury content of fish, and it became apparent that mercury concentrations were increased in fish from waters subject to certain industrial discharges such as those from chloroalkali plants and pulp and paper mills. Steps were therefore taken in some countries to limit the consumption of fish from or fishing in some waters. For example, in the U.S.A., fishing was prohibited in waters where average mercury concentrations in the fish exceeded 0.5mg/kg body wt.; Sweden prohibited fishing in areas where the mean mercury concentrations in fish were greater than 1.0mg/kg, and advised that only two meals a week should be consumed of fish containing between 0.2 and 1.0mg/kg.

Another dimension was added to the problem

towards the end of 1970, when it was found in the U.S.A. that canned tuna purchased from a retail outlet contained mercury concentrations of about 0.5mg/kg. In this country a survey by the Laboratory of the Government Chemist confirmed these results, the mean concentration in some 60 samples analysed being about 0.4mg/kg and most of the mercury was found to be in the form of methylmercury compounds (Ministry of Agriculture, Fisheries and Food, 1970). The Pharmacology Subcommittee of the Department of Health and Social Security considered these results and advised that, whereas there was no need to limit the sale or consumption of canned tuna in this country, more information was needed about the concentrations of mercury in canned and fresh fish as well as in other foods in the national diet. Accordingly an extensive survey of the mercury content of food was undertaken, and a report on the survey, together with the views of the Pharmacology Subcommittee and the Food Additives and Contaminants Committee, was published (Ministry of Agriculture, Fisheries and Food, 1971a,b).

Although the broad details of organization and results of the survey have been published, the aims are worth repeating here. These were: (a) to assess the intake of mercury by the average consumer in this country; (b) to assess the mercury contents of major foods in the national diet; (c) to determine whether any class of food might present special features with respect to the content and form of mercury present; (d) to identify any local areas of potential contamination that might affect the mercury content of food and to make special checks in these areas.

The mercury concentration in the national diet was reported to be less than 0.0065mg/kg and the mercury intake by the average consumer to be less than 10µg/day. These values were estimated by making the worst assumption that results of less than 0.005mg/kg for any food group were equal to 0.005mg/kg. However, if values reported to be less than 0.005mg/kg are taken as zero, then the estimated mercury concentration in the diet is about 0.003mg/kg and the intake by the average person is in the region of 4.5µg/day. On the basis of this latter value, fish would thus contribute almost 50% of the mercury to the average diet, since the daily intake of mercury from fish was estimated to be about 2.0µg. Generally, therefore, it appears that some 20–50% of the mercury in the diet results from fish. One estimate of the mercury intake from fish in Sweden was 2.1µg/day, on the basis of daily intakes of 3.5µg from whole diets without fish and 5.6µg from all the diets examined (Norden *et al.*, 1970). However, a high proportion of the diets examined in that study contained no fish, and fish consumption in Sweden is double that in the U.K.

There is no clear indication of what the normal background concentrations of mercury in food are,