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In vitro Methylation and Demethylation of Mercury Compounds by the Intestinal Contents

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The idea that inorganic mercury ingested in small quantities with diet can be partly transformed into methylmercuric ion (MeHg) has been suggested by Edwards (1973) and Rowland (1984). Also our previous studies gave the evidence that the inorganic mercury can be converted into methylmercuric ion in various segments of gastrointestinal tract in the *in vitro* conditions (Ludwicki 1989). The conversion was time dependent, although in some cases after initially rapid increase in MeHg formation some decrease in this compound contents was observed in the intestinal loops. This could be due to the reverse processes resulting in MeHg decomposition and Hg° formation. Such reaction is possible and its biochemical basis were presented by Fox (1982) and Silver (1984). The toxicological importance of these two different processes i.e. methylation and demethylation can not be underestimated because of great difference in toxic action of MeHg and Hg°.

This study aimed at the investigation of the possibility of simultaneous existence of both methylation and demethylation processes in the contents of the gastrointestinal tract.

MATERIALS AND METHODS

In our previous experiments the intestinal loops were used to measure of MeHg formed as a result of methylation of inorganic mercury (Ludwicki 1989). This method however, occured to be unpractical for determination of mercury vapours (Hg°) formed as a result of reduction of mercuric chloride or demethylation of MeHg. Therefore, the experiments were based upon the incubation of the intestinal contents spiked with $HgCl_2$, MeHg and phenylmercuric acetate (PhHg).

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The precalibrated incubation flasks were used for this purpose. The flasks were specially designed to allow direct connection with the atomic absorption spectrophotometer (AAS). This approach allowed for simultaneous monitoring of methylation and/or reduction of inorganic mercury and also for demethylation of MeHg and PhHg.

The experiments were performed as follows. 0.5 g of the intestinal contents was removed from the jejunum of male rats and suspended in 10.0 ml of saline. 1.0 ml of the suspension was placed in the precalibrated incubation flasks filled with 10.0 ml of the broth according to Rowland (1974) and containing 0, 2.5, 5.0, 10,0 and 15.0 μgHg/ml added as $HgCl_2$. Similar concentrations of mercuric chloride were used in our previous work, where the intestinal loops served as an experimental model (Ludwicki 1989). After 24, 48, and 72 hours of incubation the concentration of elemental mercury was measured by flushing Hg^o with air stream (85 cm^3/min) directly from the incubation to the measuring cell of the atomic absorption spectrophotometer. Cold vapour technique was applied for this purpose. MeHg was extractred from the remaining liquid phase according to clean-up technique proposed by Edwards et al. (1975), and was quantitatively determined by gas- liquid chromatography with electron capture detector (EPA 1974).

In the separate experiments 100 μg Hg added as methylmercuric chloride and 100 μg Hg as phenylmercuric acetate were incubated at the above described conditions and the formation of Hg^o as a result of degradation processes was measured. The same amounts of mercury compounds incubated without the jejunal contents were treated as controls.

RESULTS AND DISCUSSION

Amounts of MeHg and Hg^o formed as a function of the initial concentration of $HgCl_2$ and the incubation time are shown in the Fig. 1 a,b,c,d,e. MeHg as well as Hg^o formed during the incubation were only a small fraction of the mercury introduced at the begining of the experiments. The simultaneous determination of MeHg and Hg^o allowed to demonstrate of considerable differences in formation of these two mercury species. In all experiments the amounts of Hg^o were higher than those of MeHg; the differece become greater with longer time of incubation and with the increase of the initial $HgCl_2$ concentration. The highest rise in Hg^o formation was observed between 48 and 74 hour of the incubation.

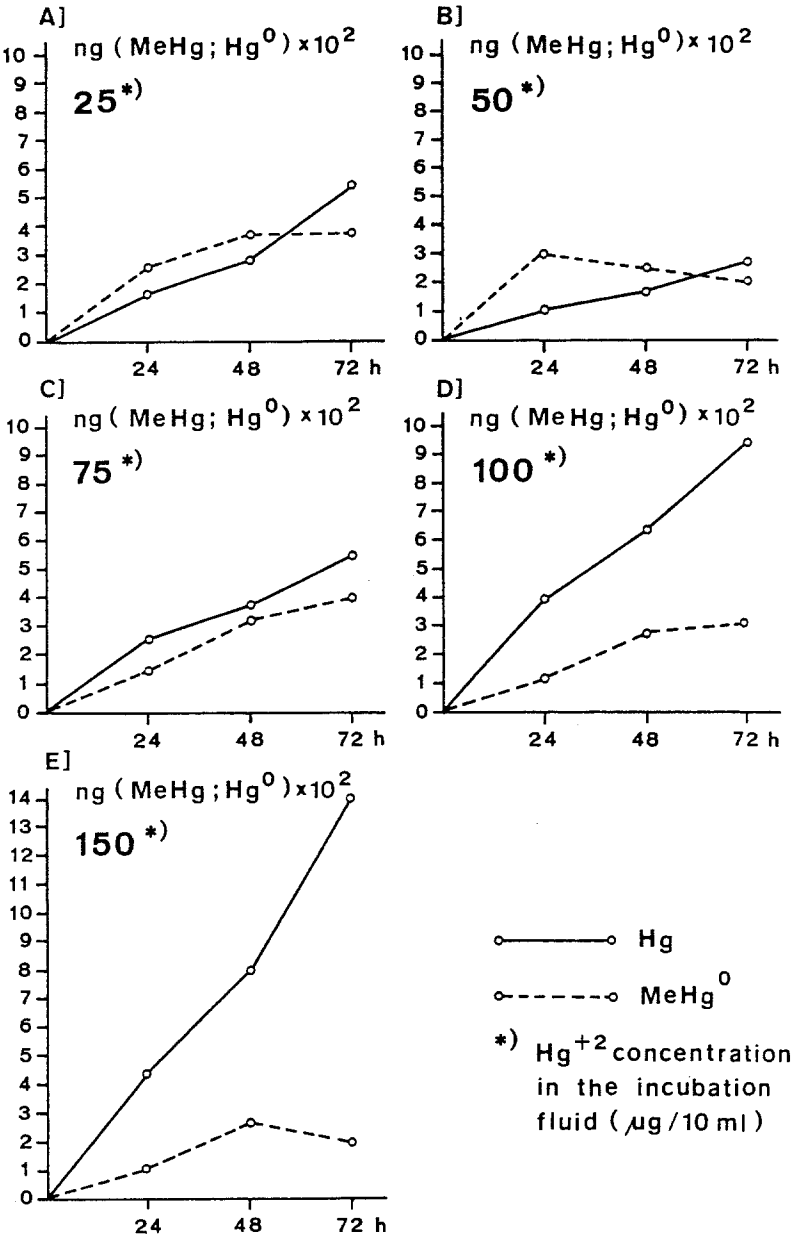

Figure 1 MeHg and Hg^0 formation from $HgCl_2$ under the influence of jejunal contents.

In case of MeHg the totally different formation pattern was observed when its concentration was analysed in the liquid phase. In contrast to the Hg^{o} the lowest relative increments of MeHg were observed at the end of incubation, and in one case the MeHg concentration was even lower after 72 than after 48 hours of incubation (Table 1).

This phenomena could be partly due to degradation of MeHg formed during the incubation. Such possibility was confirmed in a separate studies showing that both methylmercury chloride and phenylmercury acetate decomposed in the presence of intestinal contents to yield elementary mercury (Fig 2). The yield of decomposition was higher in case of PhHg than of MeHg, that could be explained by different susceptibility of mercury-carbon bond in these compounds (Silver 1984).

Table 1. The relative increments in MeHg and Hg^{o} formation after 24, 48 and 72 h of incubation (ng)

$HgCl_2$ concentration in the incubation mixture (μg/10 ml)	Incubation time (hours)					
	24		48		72	
	MeHg	Hg^{o}	MeHg	Hg^{o}	MeHg	Hg^{o}
25	49	49	77	49	83	183
50	242	80	45	47	11	177
75	169	204	71	82	29	233
100	99	324	156	230	27	283
150	89	457	191	409	-16	513

These experiments proved that the three types of reaction involving different mercury compounds are possible in the environment of gastrointestinal tract of rat i.e. methylation of inorganic mercury compounds, degradation of MeHg and PhHg, and reduction of some inorganic mercury compounds. The mechanism of the last reaction was explained by Fox (1982).

The aspects of methylation of inorganic mercury in the alimentary tract was discussed by Ludwicki (1989). The finding that the reverse process is also possible in the *in vitro* conditions needs some considerations on its possible effects in *in vivo* situation. It is quite possible that the role of demethylation in gut is a rather slow process comparing to the rate of MeHg absorption in the gastrointestinal tract.

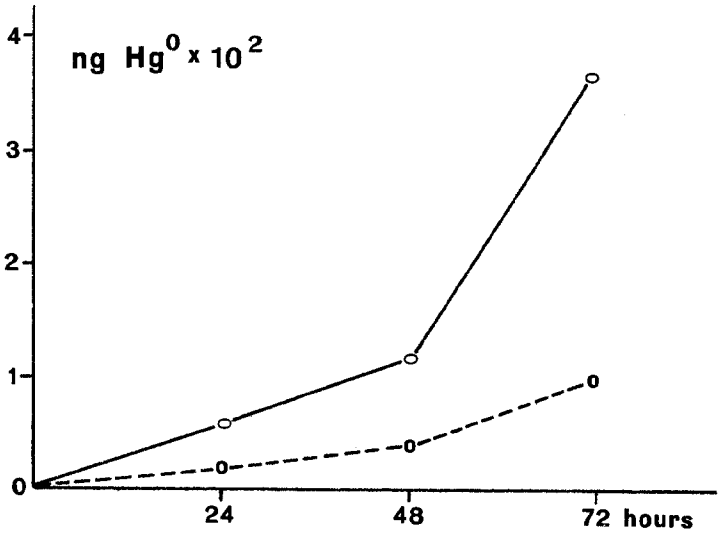

Figure 2. The influence of the intestinal contents on Hg° formation from MeHg and PhHg

According to the authors evaluating mercury intake from food (Buchet et al. 1983, Ludwicki 1987, Schelenz et al. 1973) or food contamination by this metal (Ludwicki 1987a) it seems that under normal conditions the amounts of MeHg derived from food would be minimal. However, the fact of the nearly complete absorption of MeHg, its toxicity and daily exposure to it through life, also this "intestinal source" of endogenous methylmercury, and also its possible decomposition should be considered in evaluation of the total exposure to mercury.

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