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Studies on the Role of Gastrointestinal Tract Contents in the Methylation of Inorganic Mercury Compounds

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The toxic action of the mercury compounds and their bioavailability depends on the chemical stucture of the compound. It is well known, that mercury compounds can be transformed into metalic mercury or to alkyl mercury compounds in the environment. This transformation, caused by microorganisms was observed in the soil (Tonomura and Kanzaki 1967), water and biological media (Magos et al. 1964) and human faeces (Edwards et al. 1975).

Therefore, the idea that inorganic mercury ingested in small quantities with daily meals can be partly transformed into alkyl mercury compounds can not be rejected without prior experiments. Result of such studies should be of special importance, because of the exceptionaly high toxicity of methylmercury ion (MeHg) and its delayed neurotoxic action especially when *in utero* exposure is concerned (Bartholome et al. 1984).

This study aimed at the investigation of the fate of inorganic mercury compounds influenced by the contents of the gastrointestinal tract.

MATERIALS AND METHODS

Our preliminary, unpublished experiment showed that the trace amounts of methylmercury ion (MeHg) can be found in the contents of gastrointestinal tract of rats receiving 50 micrograms of inorganic mercury ($HgCl_2$) directly to the stomach by a probe. These small quantities of MeHg (5–6 ng/ml of the intestinal contents) suggested the possibility of methylmercury formation in the jejunum and caecum contents. They also suggested that even if this compound is formed in bigger amounts its detection or quantitative analysis can be

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interferenced by its rapid absorption from the gastrointestinal tract (Bernard and Purdue 1984). For this reason the experimental approach established the use of the intestinal loops in *in vitro* conditions.

The experiment was carried out on Wistar male rats (200±10g b.w.) kept before the experiments on the standard laboratory diet for three weeks in the 12 h light dark regime and constant temperature 21±1°C. After 12 h of starvation the animals were killed with light ether anasthesia, and the part of the gastrointestinal tract from duodema to caecum was removed.

The intestinal loops were prepared as follows. The whole caecum and aprox. 12 cm of the other intestinal segments were isolated and placed into 50 ml erlenmayer flasks filled with 25 ml of buffered glucose in Ringer solution according to Rowland (1974). 1 ml of the sterile $HgCl_2$ solutions (5 and 10 ugHg/ml) was introduced into the inner space of the loops by a specially designed probe to provide the equal distribution of the solution along the loop. The control loops contained 1ml of 0.9% NaCl solution instead of $HgCl_2$.

The intestinal loops were suspended in the flasks in such way that their 2-3 cm endings emerged over the liquid surface. The flasks were tightly closed and their contents were incubated in 37°C for 16, 24 and 48 hours. Jejunal loops were also incubated in anaerobic conditions.

After incubation the MeHg concentration in the intestinal contents and in the incubation solution were measured. MeHg from the intestinal contents was extracted using the method proposed by Edwards (1975), and determined by gas-liquid chromatography with electron capture detector according to EPA (1974).

RESULTS AND DISCUSSION

MeHg was found in all the intestinal segments used in these experiments. No MeHg was found in the incubation solutions. The concentration of MeHg in various intestinal segments after 16, 24 and 48 hours of incubation are shown in Table 1.

It was found that approximately 0.05 to 0.26% of the introduced mercury was converted into MeHg. In the control samples from the duodema and jejunum MeHg was

not detected, and in all cases of the controls from caecum 2-3 mm peaks of the same as MeHg retention time were observed. In table 1 these peaks were defined as traces.

The results show that relationships between the initial $HgCl_2$ concentrations, incubation time and the amounts of MeHg formed in the loops were similar in all the examined segments of the gastrointestinal tract. However, there are also some differences that are clearly visible when interpreted graphically (Fig. 1).

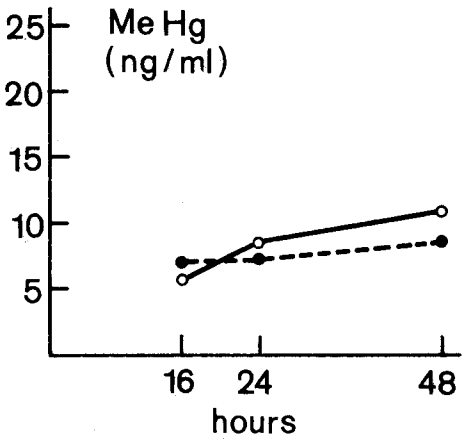
Table 1. Concentration of methylmercury (MeHg) in the contents of the intestinal loops (ng/ml ± SD)

Intestinal segment	$HgCl_2$ introduced (µg)	Incubation time (hours) 16	24	48
Duodemal	0	(4) n.d.	(4) n.d.	(4) n.d.
	5	(6) 6.6 ± 4.2	(5) 7.2 ± 5.1	(5) 8.0 ± 6.2
	10	(6) 5.1 ± 4.4	(5) 7.6 ± 4.9	(5) 11.2 ± 5.8
Jejunal (aerobic conditions)	0	(4) n.d.	(4) n.d.	(4) n.d.
	5	(6) 6.0 ± 3.8	(5) 7.7 ± 6.3	(5) 9.6 ± 5.8
	10	(6) 8.1 ± 4.8	(5) 12.6 ± 7.1	(5) 14.3 ± 7.9
Jejunal (anaerobic conditions)	0	(4) n.d.	(4) n.d.	(4) n.d.
	5	(5) 7.1 ± 4.3	(5) 7.2 ± 5.8	(5) 10.2 ± 6.9
	10	(5) 7.9 ± 4.6	(5) 9.9 ± 7.1	(5) 11.3 ± 6.8
Caecal	0	(4) traces	(4) traces	(4) traces
	5	(6) 5.2 ± 6.1	(5) 12.5 ± 12.0	(4) 13.1 ± 14.3
	10	(6) 11.1 ± 9.2	(5) 22.4 ± 16.7	(4) 18.4 ± 16.8

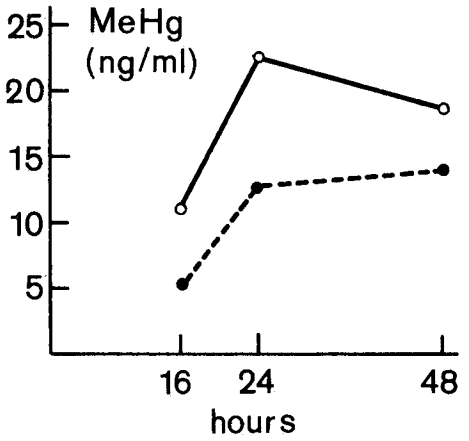
SD - standard deviation, n.d. - not detected
Numbers of samples are placed in brackets

The following similarities can be observed:
- higher MeHg concentrations in the experiments with the higher initial $HgCl_2$ concentrations,
- higher MeHg concentrations after longer incubation time,
- higher MeHg increase between 16-24th hour of incubation than between 24-48th hour of incubation.

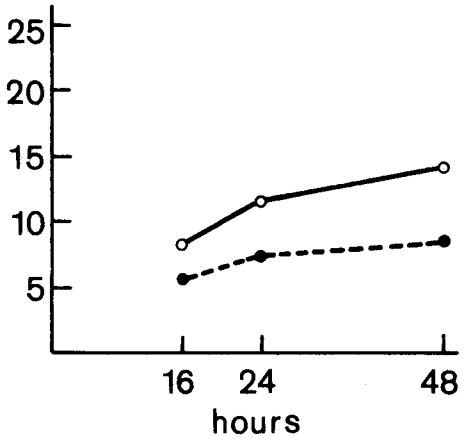
The amounts of MeHg formed in the duodemal loops (Fig.



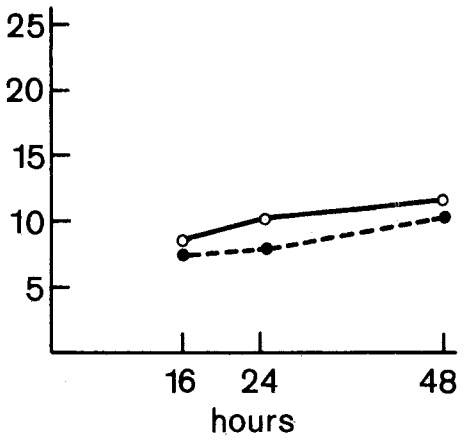
1a. Duodenum



1b. Caecum



1c. Jejunum
(aerobic conditions)



1d. Jejunum
(anaerobic conditions)

10 µg $HgCl_2$

5 µg $HgCl_2$

Figure 1. The influence of the incubation time and $HgCl_2$ concentration on the methylmercuric-ion [MeHg] formation in the intestinal loops.

1a) were lowest and they did not reflect the differences between the initial concentrations of inorganic mercury. However, the upward tendency was observed when the incubation time was prolonged.

Larger differences were observed in the jejunal loops (Fig. 1c), although even in this case twice as large $HgCl_2$ concentration at the begining of the experiment did not result in twice as large MeHg formation.

In the caecal contents relatively rapid increase in MeHg concentration was observed after 24 hours of incubation followed by much slower increase or even decrease after 48 hours (Fig. 1b). No differences in MeHg formation in aerobic and anaerobic conditions were shown (Fig. 1c,d). These results are difficult to explain. However, it is quite possible that some role can be played by the different volumes of the intestinal contents in the loops prepared from various segments of the intestine (Rowland 1984). In these experiments the volume of the intestinal contents of duodenum was always the smallest, and in the caecum the largest.

An attention should be given to the slower increase in MeHg formation in the second stage of incubation (between 24th and 48th hour). This might be due to the possible degradation processes, which was also suggested by the results of Edward's (1985) and Rowland's (1984) studies. The answer to the question whether both methylation and demethylation processes take place simultaneously in the same intestinal environment could throw more light on the fate of mercury in the organism. This problem is now under study in our laboratory.

These results suggest that the methylation of inorganic mercury compounds in the gastrointestinal tract is possible. On the other hand, it is well known that the mean mercury intake with food ranges within wide limits and exceeds the value of 130 µg Hg/week (Ludwicki 1987, Buchet et al. 1983, Schelenz and Diehl 1973). Therefore, the intensity of the methylation processes should be born in mind during the risk assessment procedures when the mercury intake is concerned. This problem was also stressed by Bartholome (1984), who discussed the risk of the possible microintoxications caused by methylmercuric ion.

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