Biochemical Pharmacology, Vol. 23, pp. 1903–1905. Pergamon Press, 1974. Printed in Great Britain.

SHORT COMMUNICATIONS

The chemical form of the methylmercury complex in the bile of the rat

(*Received* 5 *October* 1973; *accepted* 5 *January* 1974)

ENTEROHEPATIC-circulation of methylmercury is one of the factors responsible for the long biological half life of this compound.[1,2] Recently it has been shown that the reabsorption of methylmercury excreted into the gut with the bile can be prevented by the oral administration of non-absorbable mercury binding resin.[3] This has focused attention on the biliary excretion mechanism of methylmercury. Thus it has been shown that the biliary mercury excretion can be increased by penicillamine and dimercaptopropanol (BAL),[4] and phenobarbitone.[5]

In the present work the chemical nature of mercury in the bile of rats treated with methylmercury was investigated and compared with control bile supplemented *in vitro* with methylmercury.

Male white rats of Porton Wistar strain weighing 190–220 g were cannulated under light ether anaesthesia according to Cirkt.[6] After cannulation rats were injected with methylmercury chloride in a dose of 1·0 mg/kg Hg labelled with $Me^{203}HgCl$ (Radiochemical Centre, Amersham) in a volume of 1 ml/kg in 5 mM carbonate with saline. Controls were injected with the same volume without Hg. Bile samples collected from the same animal 60 to 300 min after injection were pooled and analysed. Bile obtained from control rats was supplemented with the same amount of mercury which was present in the bile of methylmercury injected rats.

Two ml bile samples were subjected to gel filtration either on Sephadex G-100 (2·5 × 80 cm) or Sephadex G-10 (2 × 40 cm) columns (Pharmacia G.B. Ltd., London) with an elution buffer of 0·01 M Tris–HCl, pH 8·0 containing 1 M NaCl and 0·02% sodium azide. Bile samples for G-10 were first deproteinized by two subsequent centrifugation in Centriflow membrane cones (Amicaon N. V., Costerhout, Holland). Fractions of 6·1 ml were collected at flow rates of 26 ml/hr for G-100 and 60 ml/hr for G-10. Figure 1 shows that approximately 20 per cent of the mercury in the bile was located in a large molecular fraction with an elution volume around bovine serum albumine and 50–60 per cent in a small molecular fraction. Analysis of samples by the method of Magos[7] have shown that 95 per cent of the mercury in the bile retained the metal to carbon bond and that inorganic mercury was mainly eluted with the large molecular fraction. Figure 2a shows that mercury was bound to a compound with a molecular weight between cysteine and glutathione. If rats were given 1·0 g/kg cysteine i.p. 40 min after mercury, depression

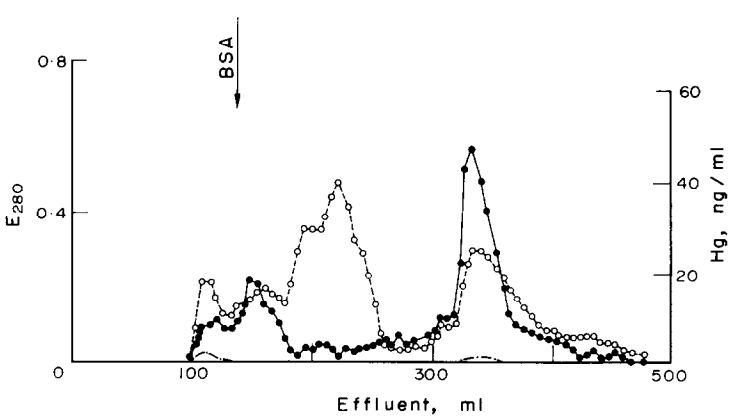

FIG. 1. Gel-filtration on Sephadex G-100 of the bile obtained from the rat injected with methylmercury-(^{203}Hg) chloride (1 mg Hg/kg i.v.). Extinction at 280 mm: (○) total mercury concentration in ng/ml: (●) inorganic mercury concentration in ng/ml: chain line. Arrow marks the elution of peak for bovine serum albumin (BSA).

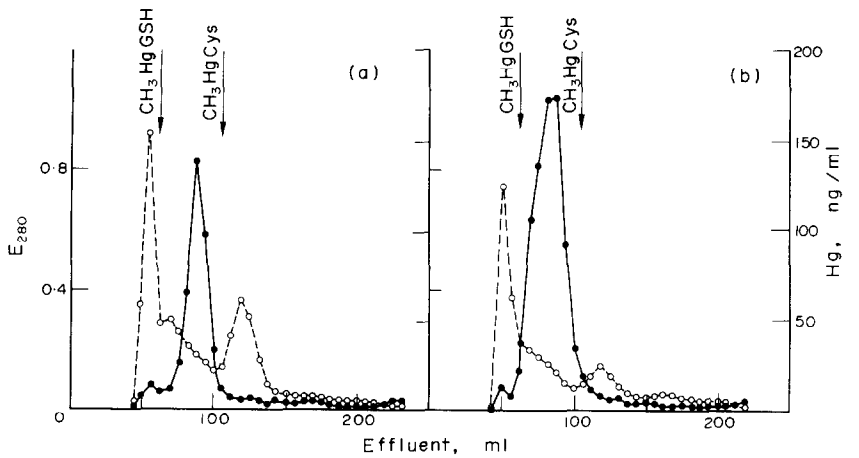

FIG. 2. (a) Gel-filtration on Sephadex G-10 of the bile sample after deproteinization from the rat injected with methylmercury-(^{203}Hg) chloride. The symbols are the same as on Fig. 1. The arrows mark the elution of peaks for methylmercury glutathione (CH_3Hg GSH) and methylmercury cysteine (CH_3Hg Cys). (b) Gel-filtration on Sephadex G-10 of the bile sample after deproteinization from the rat injected with methylmercury-(^{203}Hg) chloride and treated with cysteine (1 g/kg i.p.) 40 min after mercury. Symbols are the same as for (a).

in the biliary excretion of $MeHg^+$ lasting approximately for 1 hr was followed by approximately two fold increase in samples obtained between 120 to 240 min after mercury. Figure 2b shows cysteine treatment did not change the elution pattern. Pretreatment of rats with phenobarbitone was also without influence on the elution pattern. Moreover bile samples with added MeHgCl gave almost the same elution peak on Sephadex G-10.

The R_f value for MeHgCl added to bile (final molar concentration 10^{-5}) or mixed with different thiol compounds (2 thiol group per one Me^{203}HgCl in Tris-buffer) was estimated on aluminium backed thin-layer silica gel sheets. Twenty μl samples were used and after development with ethanol–34% aqueous ammonium hydroxide (70:30 by volume) up to 15 cm from the origin, strips of the dried chromatogram were analysed by counting ^{203}Hg. Table 1 shows that the R_f value for methylmercury in the bile was the same as for MeHg-cysteine or methylmercury-*N*-acetylcysteine. Separation on Sephadex G-10 already eliminated MeHg-cysteine as a possible form of biliary $MeHg^+$. Distribution between a solvent phase and water eliminated methylmercury-*N*-acetylcysteine. One ml benzene, chloroform, toluene or benzyl alcohol was added to 2 ml of the eluate with a low molecular weight MeHg complex from Sephadex G-10 or to 2 ml 5×10^{-4} M methylmercury compounds in 0·01 M Tris HCl buffer, pH 8·0, containing 1 M NaCl, or to 0·2 ml bile diluted to 2 ml with the same buffer. After shaking the samples vigorously for 20 sec with an electric mixer they were centrifuged at 600 *g* for 10 min. The organic layer was collected with a capillary. The same procedure was repeated twice with the remaining aqueous phase. Radioactivity

TABLE 1. THIN-LAYER CHROMATOGRAPHY OF THE BILE AND METHYLMERCURY COMPOUNDS

	R_f Value
Bile	0·82
Methylmercury chloride	0·03
Methylmercury cysteine	0·81
Methylmercury-*N*-acetylcysteine	0·81
Methylmercury homocysteine	0·70
Methylmercury glutathione	0·61

Mobility of mercury radioactivity is given in the table. Silica gel thin-layer chromatogram was obtained by developing aluminium backed sheet with ethanol–34% NH_4OH (70:30, v/v).

TABLE 2. DISTRIBUTION RATIO OF THE METHYLMERCURY-COMPLEX IN THE BILE AND SOME REFERENCE METHYLMERCURY COMPOUNDS

Compounds	Solvents	Distribution ratio*			
		Benzene	Chloroform	Toluene	Benzyl alcohol
Methylmercury chloride		17·5	6·94	24·6	165·7
Methylmercury cysteine		0·01	0·06	0·02	5·25
Methylmercury-*N*-acetyl-cysteine		0·01	0·02	0·01	2·50
Methylmercury glutathione		0·01	0·01	—	—
Methylmercury-complex in the fraction on Sephadex G-10		4·88	1·31	1·54	12·7
Methylmercury-complex in the bile					
Hg-injected		3·01	1·28	2·22	46·6
Hg-added		4·71	1·63	2·38	19·4

* The values were given as a ratio of the radioactivity in organic layer to that in buffer after three times extraction with a half volume of organic solvent.

in the pooled organic extract was counted. Distribution ratios on Table 2 besides cysteine and glutathione eliminates *N*-acetylcysteine. There were some differences in distribution ratios for eluate or bile with *in vivo* or *in vitro* added MeHg. This might be explained by the susceptibility of this method to interference by non-extractable material which concentrates in the aqueous phase. However, the distribution ratio for the complex is significantly higher than the distribution ratio for MeHg-cysteine, MeHg-*N*-acetylcysteine and MeHg-glutathione and lower than that of MeHgCl.

The present work has indicated that the $MeHg^+$ in the rat bile is mainly complexed with a compound which has a molecular weight between cysteine and glutathione. That it is not glutathione or homocysteine has been shown by thin-layer chromatography and that it cannot be cysteine, glutathione or any of their polar metabolites is indicated by the distribution ratios. This compound seems to be a normal bile constituent as indicated by the same elution pattern and distribution ratio for *in vivo* and *in vitro* administered MeHgCl. We have no idea of the quantity of the compound which complexes $MeHg^+$ in the bile, but *in vivo* experiments in progress seem to suggest that if the $MeHg^+$ concentration is increased above 10^{-3} M in the bile, the major part of $MeHg^+$ remains located at the origin of the thin layer sheet instead of migrating with the usual biliary methylmercury comples.

MRC Toxicology Unit,
Medical Research Council Laboratories,
Woodmansterne Road,
Carshalton,
Surrey,
England

M. OHSAWA*
L. MAGOS

REFERENCES

1. T. NORSETH and T. W. CLARKSON, *Arch. Environ. Health* **22,** 568 (1971).
2. T. NORSETH, in *Mercury, Mercurials and Mercaptans* (Eds. M. W. MILLER and T. W. CLARKSON), p. 264. Charles C. Thomas, Springfield, Ill. (1973).
3. T. W. CLARKSON, H. SMALL and T. NORSETH, *Fedn. Proc.* **30,** 543 (1971).
4. T. NORSETH, *Acta pharmac. toxic.* **32,** 1 (1973).
5. L. MAGOS and T. W. CLARKSON, *Nature, New Biol.* **246,** 123 (1973).
6. M. CIKRT, *Br. J. industr. Med.* **29,** 74 (1972).
7. L. MAGOS, *Analyst.* **96,** 847 (1971).

* Present address: Department of Occupational Diseases, National Institute of Industrial Health, Kizukisumiyoshi, Nakahara-ku, Kawasaki, Japan.