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SPECIES DIFFERENCE IN BILIARY EXCRETION OF METHYLMERCURY

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Abstract—Species difference in the biliary excretion of methylmercury was studied in male rats, mice, rabbits and guinea pigs. The rates of mercury excretion (% dose/2 hr) into the bile of the rats, mice, rabbits and guinea pigs during the 2 hr from 2 to 4 hr after the administration of methylmercury were 0.61, 0.091, 0.036 and 0.019, respectively. These results suggest that biliary excretion and enterohepatic circulation of methylmercury in the latter three species may not influence the fate of this compound as significantly as in rats. Most of the methylmercury excreted into the bile of rats was bound to glutathione (GSH). In the mouse bile, 40% of the methylmercury was bound to GSH and the rest was found in a fraction eluted at the void volume of the Sephadex G-15 column. However, in the case of the rabbits and guinea pigs, methylmercury–GSH was scarcely detectable in the bile and almost all of the methylmercury was eluted at the void volume of the column.

Many studies on the metabolism of methylmercury have so far been carried out by using rats as the experimental animal. However, the species difference in the behaviour of methylmercury within the erythrocytes, which has recently been confirmed by us [1, 2], indicates that the information from such studies as above is not directly applicable to other mammals than rats, and requires the thorough investigation of the metabolism of this metal alkyl in other species as well. The factor of species difference may explain the discrepancy in sensitivity to methylmercury evident in the various species.

Rats treated with methylmercury have been observed to excrete a considerable amount of methylmercury into their bile [3]. Although the importance of enterohepatic circulation, namely biliary excretion and intestinal reabsorption, of methylmercury in its metabolism has been emphasized [3–5], most available information on the biliary excretion of methylmercury has been acquired by studies in which rats were used as experimental animals [1, 6–16], and only a few studies with animals other than rats have been carried out [17, 18]. The present study was undertaken to examine the rate of biliary excretion of methylmercury and its form in the bile of four different species: rats, mice, rabbits and guinea pigs, in order to obtain more precise information on the role of biliary excretion in the methylmercury metabolism in animals.

MATERIALS AND METHODS

Biliary excretion. Male rats (Wistar, 170–230 g), rabbits (Japan White, 1.6–2.0 kg), mice (ICR, 26–30 g) and guinea pigs (Hartley, 240–260 g) were used as the experimental animals. The animals were anaesthetized with sodium pentobarbital (100 mg/kg for rats, 33 mg/kg for rabbits, 70 mg/kg for mice and 33 mg/kg for guinea pigs, with additional administration if necessary), and kept at constant body temperature with warming lamps throughout the experiments. The bile ducts were cannulated with polyethylene tubing (i.d. 0.58 mm, o.d. 0.96 mm for rats; 1.00 mm, 1.50 mm for rabbits; 0.20 mm, 0.50 mm for mice; and 0.86 mm, 1.27 mm for guinea pigs), after which $Me^{203}HgCl$ (New England Nuclear, Boston, MA) was injected i.v. into these animals at a dosage of 250 μCi/3 μmole/kg. After the administration of $Me^{203}HgCl$, samples of bile were taken at 2 hr intervals during the first 4 hr. A separate experiment was carried out whereby the animals were operated under pentobarbital anaesthesia 22.5 hr after the injection of $Me^{203}HgCl$, and the bile ducts of these animals were also cannulated. Following this, samples of the bile were taken over a 2 hr period 23 hr after the administration of methylmercury. The content of the ^{203}Hg present in the bile was then determined by the Aloka Auto Well gamma system.

Column chromatography. Two hours after the administration of $Me^{203}HgCl$, samples of bile excreted over the following 1 hr were collected in vials kept on ice. These were taken from the bile duct near the liver to prevent the contamination of pancreatic juice which contains methylmercury–GSH converting enzymes [15]. The bile samples (0.5–3.0 ml) were applied to a Sephadex G-15 column (19 × 450 mm) which was eluted with 50 mM Tris–HCl buffer (pH 7.6) at a flow rate of 30 ml/hr, and the eluate was fractionated into 2.0 ml portions.

In vitro reactions. Bile (1.0 ml) obtained from untreated animals was incubated with 3×10^{-7} M $Me^{203}HgCl$ (30 nCi/ml bile) or 3×10^{-7} M [^{203}Hg]-methylmercury–GSH at 37° for 5 min. [^{203}Hg]-Methylmercury–GSH was prepared by mixing 10^{-4} M $Me^{203}HgCl$ and 10^{-4} M GSH, and was isolated by Sephadex G-15 chromatography.

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RESULTS

As shown in Table 1, species difference was observed in the rate of ^{203}Hg excretion into bile (% dose/2 hr). The ^{203}Hg excretion rates of mice, rabbits and guinea pigs were found to be markedly lower than that of rats.

The form of the low-mol. wt methylmercury complex in the bile of rats [11, 15] and mice [17] has been identified as methylmercury–GSH. In fact, in the present study *ca* 95% of the ^{203}Hg in the bile of rats receiving $Me^{203}HgCl$ was identified as methylmercury–GSH by the use of a Sephadex G-15 column (Fig. 1). In the mouse bile, ^{203}Hg was found in two fractions: 40% of the ^{203}Hg was found as methylmercury–GSH and the rest was found in a fraction eluted at the void volume of the Sephadex G-15 column. The methylmercury–GSH in the bile of rats and mice was further analysed by ion-exchange chromatography using CM-Sephadex C-25. In the bile of rabbits and guinea pigs, however, methylmercury–GSH was hardly detectable, and more than 90% of the ^{203}Hg was eluted at the void volume (Fig. 1). The amount of inorganic mercury which is likely to have been formed by the degradation of methylmercury seems to be negligible, as more than 98% of the ^{203}Hg excreted into the bile of these four animal species was extractable with benzene after acidification.

Radioactive mercury of $Me^{203}HgCl$ (3×10^{-7} M) added to the bile of untreated rats or rabbits was

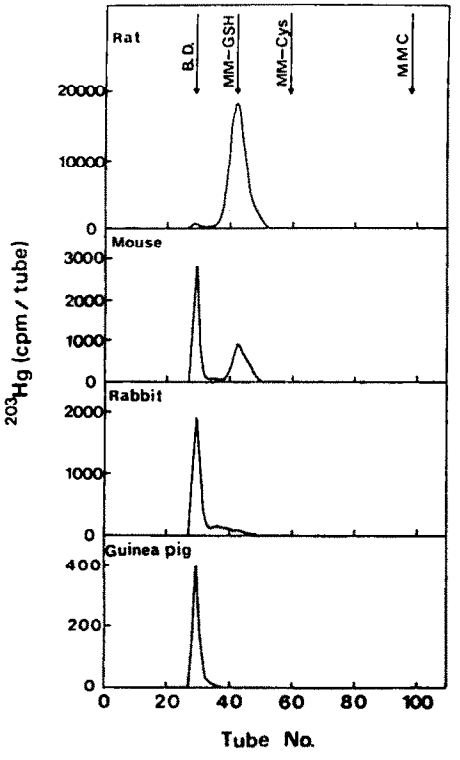

Fig. 1. Sephadex G-15 chromatography of bile from rats, mice, rabbits and guinea pigs 2–3 hr after i.v. administration of $Me^{203}HgCl$ (3 μmole/kg). B.D., blue dextran; MM–GSH, methylmercury–glutathione; MM–Cys, methylmercury–cysteine; MMC, methylmercuric chloride.

Table 1. Mercury excretion into the bile of rats, mice, rabbits and guinea pigs after i.v. administration of $Me^{203}HgCl$

	Bile flow (ml/100 g body weight/hr)	Concentration (nmole/ml) Time after injection (hr) 0–2	2–4	23–25	% dose 0–2	2–4	23–25
Rat	0.29 ± 0.06	2.33 ± 0.33	2.84 ± 0.86	3.08 ± 0.43	0.47 ± 0.11	0.61 ± 0.30	0.57 ± 0.14
Mouse	0.07 ± 0.03	0.72 ± 0.49	1.92 ± 0.66	1.73 ± 0.64	0.023 ± 0.013	0.091 ± 0.063	0.076 ± 0.021
Rabbit	0.28 ± 0.12	0.31 ± 0.09	0.31 ± 0.18	0.14 ± 0.08	0.049 ± 0.017	0.036 ± 0.012	0.019 ± 0.010
Guinea pig	0.59 ± 0.22	0.059 ± 0.006	0.054 ± 0.010	0.167 ± 0.038	0.024 ± 0.009	0.019 ± 0.007	0.069 ± 0.028

Values are average of four animals (mean ± S.D.).

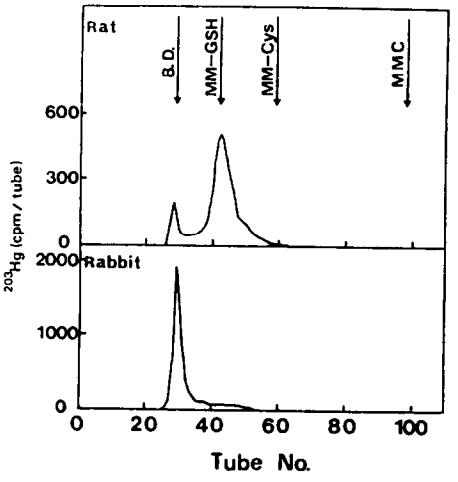

Fig. 2. Sephadex G-15 chromatography of rat and rabbit bile after the addition of Me^{203}HgCl (3×10^{-7} M).

seen to behave similarly in chromatography as when Me^{203}HgCl was injected into the respective animals (Fig. 2). This indicates that the difference in the biliary ^{203}Hg elution profile between rats and rabbits (Fig. 1) is not due to a lower ^{203}Hg concentration (*ca* 3×10^{-7} M) in the bile of rabbits injected with Me^{203}HgCl (Table 1). The component(s) of the rabbit bile eluted at the void volume of the gel may have a higher affinity to methylmercury than to GSH. Previous studies have indicated that the form of methylmercury secreted from the liver into the bile may be methylmercury–GSH in rats [13, 16]. In rabbit bile, however, most of the ^{203}Hg did not form a complex with GSH but was converted to a complex eluted at the void volume of the Sephadex G-15 column even when the synthesized [^{203}Hg]methylmercury–GSH complex was mixed with the bile of untreated rabbits (Fig. 3).

DISCUSSION

Approximately 10% of the injected methylmercury was excreted in rat bile during the 24 hr following its administration [3, 7], and approximately 90% of this methylmercury excreted in the bile was then reabsorbed from the gut [1, 19]. It has been considered from these experimental results that biliary excretion and enterohepatic circulation of methylmercury are important factors in determining the behaviour and toxicity of methylmercury in mammals. However, Vostal and Clarkson [18] have reported that the 5-hr biliary excretion of methylmercury after a single i.v. injection was higher in rats than in rabbits, guinea pigs, chickens, monkeys and dogs. The possibility that bile secreted shortly after the treatment might have been diluted by the bile stored in the gall bladder cannot be overlooked as all the species, apart from the rats, have a gall bladder. In the present study, the rates of excretion of methylmercury into the bile of mice, rabbits and guinea pigs were markedly lower than that of rats, at least during the 24 hr after the injection (Table 1). This clearly indicates that biliary excretion of methylmercury in species other than rats is not as influential in the fate of this compound as for rats. Norseth [7] indicated that the methylmercury concentration in rat bile reflected its hepatic concentration. In the present study, however, the concentration of ^{203}Hg in the rat liver was not significantly higher than that in the liver of mice, rabbits and guinea pigs (data not shown).

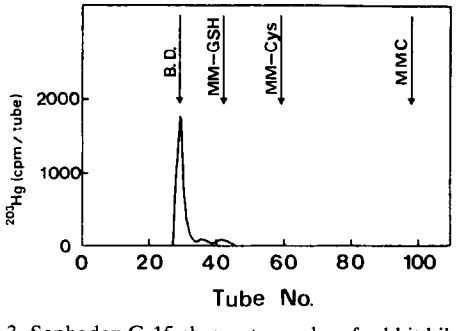

Fig. 3. Sephadex G-15 chromatography of rabbit bile after incubation with [^{203}Hg]methylmercury–GSH (3×10^{-7} M).

Methylmercury in rat liver has been thought to form a complex with GSH [20], which is then secreted into the bile [11, 15]. The portion of the methylmercury–GSH excreted into the intestines via the bile is probably reabsorbed as it is [14], and the rest reabsorbed as methylmercury–cysteine after digestion by the pancreatic enzymes [15]. In the present study, however, methylmercury–GSH was hardly detected in the bile of rabbits and guinea pigs, and most of the methylmercury in the bile of these species was found in a fraction eluted at the void volume of the Sephadex G-15 column (Fig. 1). The biliary component(s) eluted at the void volume was found to have a higher affinity to methylmercury than did GSH, and could deprive the methylmercury–GSH complex of methylmercury (Fig. 3) even when the methylmercury is initially secreted into the bile of rabbits as methylmercury–GSH, as in the case of the rat. About 60% of the methylmercury in the mouse bile was found in the fraction eluted at the void volume while the rest was found as methylmercury–GSH (Fig. 1). Norseth [17] claimed that a higher fecal excretion rate of mercury in mice than in rats indicated lower reabsorption rates in the intestinal tracts of mice. Species difference in the chemical form of methylmercury in bile, observed in the present study, could be responsible for the variations in the rates of reabsorption and fecal excretion of methylmercury in these species.

Oral administration of non-absorptive mercury-binding materials has been used to increase the fecal excretion of methylmercury in some mammals [19, 21–24]. These materials interrupt the enterohepatic circulation of methylmercury by trapping the mercury secreted into the bile. The efficiency of this treatment for quick mercury excretion may practically depend on the species difference in the rate of biliary excretion of methylmercury and its chemical form in bile.

Millburn *et al.* [25] have suggested that the extent of biliary excretion of chemicals in the rats depends on their molecular weight and polarity. Klaassen [26]

has described that an apparent correlation between the polarity of the compound and biliary excretion exists in rats, but not in other species as indicated by the biliary excretion of quabain. Marked species difference was observed in the rate of biliary excretion of drugs, especially when their molecular weights were 300–500 [27]. Abou-El-Makarem *et al.* [28] have reported that the order of biliary excretion of phenolphthalein glucuronide (mol. wt 495) is rat > rabbit > guinea pig. This has been verified by the present experiments on methylmercury excretion during the 4 hr after the administration (Table 1), and is explainable by the assumption that methylmercury is secreted as methylmercury–GSH, which has a mol. wt of 522.

Thus the species difference in the biliary excretion of methylmercury indicates, together with that in the methylmercury behaviour in erythrocytes [1, 2], that the data obtained from experiments using rats may not be directly applicable to species other than rats.

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