Biotransformation of Methyl Mercury in the Guinea Pig

F. IVERSON and S. L. HIERLIHY

Pharmacology Division, Research Laboratories Health Protection Branch, Department of National Health and Welfare, Ottawa, Canada, K1A, 0L2

Introduction

Initial studies on the toxicity of methyl mercury suggested that the carbon-mercury bond remained intact in the animal body. Recently, MAGOS (1971), and NORSETH (1972), GAGE and WARREN (1970) and NORSETH and CLARKSON (1970) have shown that the rat and mouse can cleave methyl mercury to produce inorganic mercury (Hg^{++}). Moreover the biotransformation appears to be inversely related to dose. Extensive biotransformation could reduce the total body burden of organomercurial, the agent responsible for CNS damage. On the other hand increasing concentrations of inorganic mercury may cause renal damage.

Recently it was shown that biotransformation of methyl mercury also proceeds in the quinea pig IVERSON et al. (1973). After 71 days of oral dosing with methyl mercury (400 µg Hg/kg), 42% of the total kidney mercury level was inorganic. This level is consistent with that found in the rat (NORSETH, 1972). Other tissue Hg^{++} levels, (liver 5% and brain <5%) were also similar to those found in the rat. The present communication reports on the accumulation of inorganic mercury in the liver and kidney of guinea pigs dosed for 71 days with methyl mercuric chloride at levels of 4, 40 or 400 µg Hg/kg.

Methods

Female guinea pigs from the Canadian Communicable Diseases Centre, Ottawa, were housed in stock banks with free access to food and water. Each day for 71 days radio labelled methyl mercuric chloride (New England Nuclear, Boston Mass.) was administered orally in 5 mM sodium carbonate. Control animals received sodium carbonate only. Two or 3 animals at each dose level were sacrificed 6, 12, 22, 36, 50 and 71 days after dosing commenced. Total mercury levels were determined by liquid scintillation counting and have been published previously (IVERSON et al. 1973).

Bulletin of Environmental Contamination & Toxicology, Vol 11, No. 1 © 1974 by Springer-Verlag New York Inc.

Inorganic mercury was determined by flameless atomic absorption employing a modification of the procedure reported by MAGOS (1971). Duplicate samples of kidney or liver tissue (600-800 mg wet weight) were homogenized 1:1 with distilled water and a 1.0 gm aliquot of homogenate weighed into a suitable flask. Three ml of concentrated nitric acid was added and rapidly mixed with the homogenate. After a one hour predigestion at room temperature, the acid homogenate was diluted with distilled water to a final volume that would allow convenient analysis.

The reduction of the mercury was accomplished using the reagents and apparatus of UTHE et al. (1970). A 1.0 ml sample of the diluted homogenate was placed into the reaction flask followed by 4.0 ml of the reducing reagent. The reaction was allowed to proceed with stirring, for 90 seconds before the mercury vapor was read at 253.7 nm on a Perkin Elmer Model 306 atomic absorption unit.

The acid predigestion step results in cleavage of a small portion of methyl mercury. We have found that within a range of 0.25 to 250 ppm total tissue mercury that 2.2% of the organic mercury present is cleaved. On the other hand, the acid step is required to ensure a quantitative recovery of inorganic mercury. Since the inorganic levels in kidney and liver tissue are quite high, the inorganic mercury released by the acid cleavage does not introduce a large error in the determination. However, the method is not suitable for accurate determinations of inorganic mercury where levels are <5% those of the total mercury content. Nevertheless, an upper limit of inorganic mercury may be obtained in these cases. The inorganic mercury concentrations presented in this report have been corrected for the small, but consistent amount of Hg^{++} released from methyl mercury -by acid hydrolysis.

Results

The accumulation of inorganic mercury levels in guinea pig kidney is shown in Figure 1. The total mercury levels, published previously (IVERSON et al 1973), are also included for reference. After 71 days dosing, inorganic mercury comprises 43, 58 and 64% of the total mercury levels at the 400, 40 and 4 µg Hg/kg. dose level respectively. The percent of inorganic mercury and the total inorganic concentration found at each sacrifice date are shown in Table I. Where animal numbers permit, levels of significance have been determined between dose levels at each sacrifice date and indicate a significant difference ($p < 0.05$) in 6 of the 8 data sets.

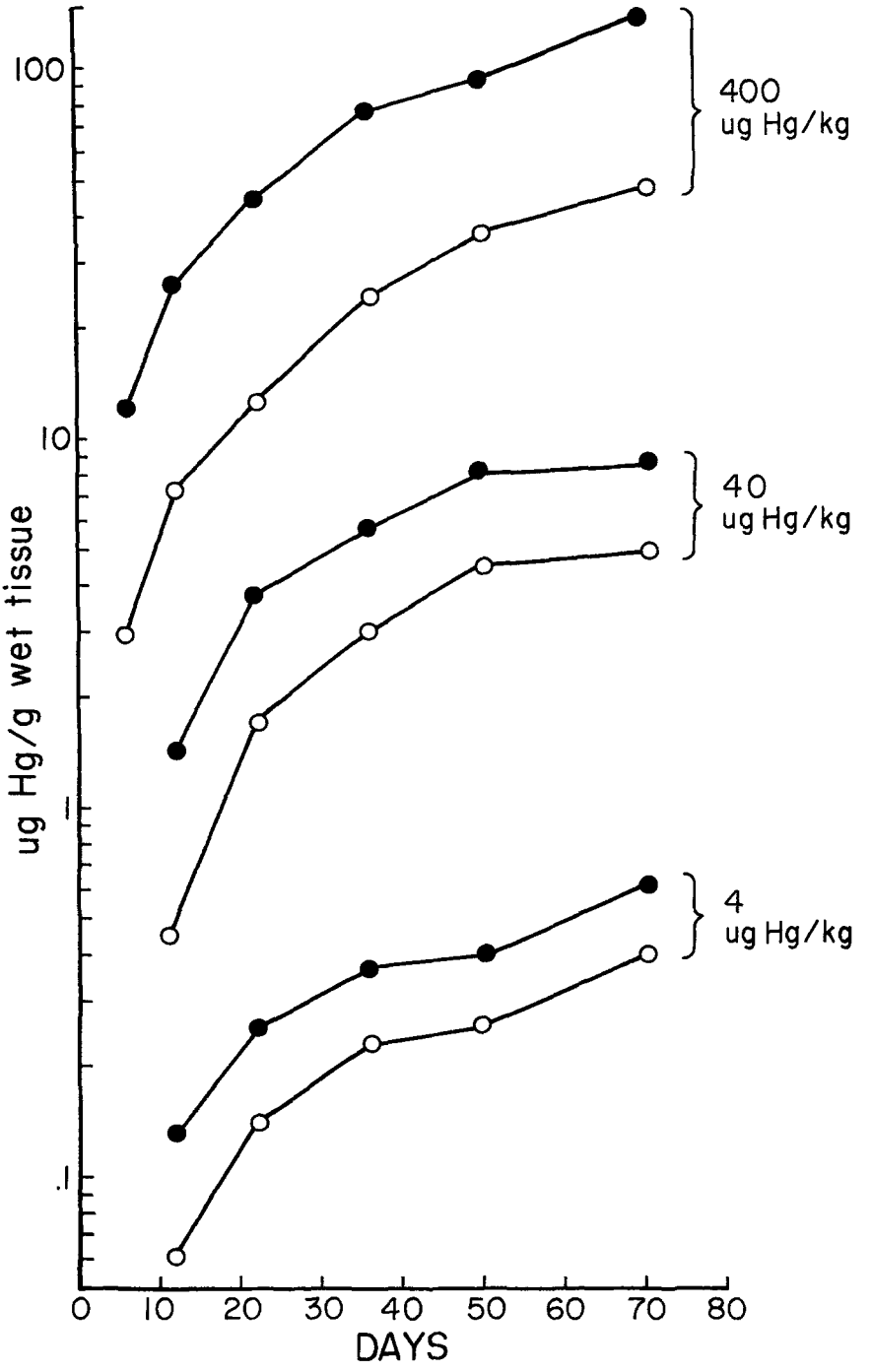

Figure 1. The accumulation of total (●) and inorganic (○) mercury in guinea pig kidney after dosing with methyl mercuric chloride at the levels indicated.

TABLE I

The level of inorganic mercury (Hg^{++}) in kidney and liver of guinea pigs dosed orally for 71 consecutive days with methyl mercuric chloride

Tissue	Dose mg Hg/kg	Hg^{++}[a]	DAY 6	12	22	36	50	71
Kidney	400	%	23.8	27.1	28.8	39.1	39.1	41.7
		Hg^{++}(µg)	2.87 ± .10	7.20 ± .85	12.72 ± .97	30.77 ± 2.04	36.36 ± 1.58	46.7 ± 1.56
				N.S[b]	*	*	*	
Kidney	40	%	-	31.2	44.6	53.0	56.1	57.9
		$Hg^{++}(10^{-1}$µg)	-	4.46 ± .27	16.94 ± 1.74	29.68 ± 1.40	44.71 ± .64	50.83 ± 1.49
				*	N.S.	*	*	
Kidney	4	%	-	53.9	54.8	62.1	62.6	63.9
		$Hg^{++}(10^{-2}$µg)	-	7.00 ± .56	14.08 ± 1.51	22.85 ± 1.10	24.72 ± 1.86	40.51 ± .89
Liver	400	%	3.3	3.6	3.8	4.1	5.9	3.5
		Hg^{++}(µg)	.24 ± .02	.49 ± .03	.73 ± .10	.93 ± .11	1.72 ± .15	1.15 ± .13
					N.S.	*	*	
Liver	40	%	-	-	5.9	10.8	14.1	11.0
		$Hg^{++}(10^{-1}$µg)	-	-	1.05 ± .30	2.49 ± .23	3.89 ± .63	4.28 ± .54
Liver	4	%	-	-	-	-	-	15.1
		$Hg^{++}(10^{-2}$µg)	-	-	-	-	-	3.61 ± .12

[a] The inorganic mercury levels are given as percent of total mercury levels and as µg Hg^{++}/ g wet tissue ± S.E.

[b] Levels of significance of percent inorganic levels between dose groups are indicated as *, ($P < .05$) or N.S. (not significant).

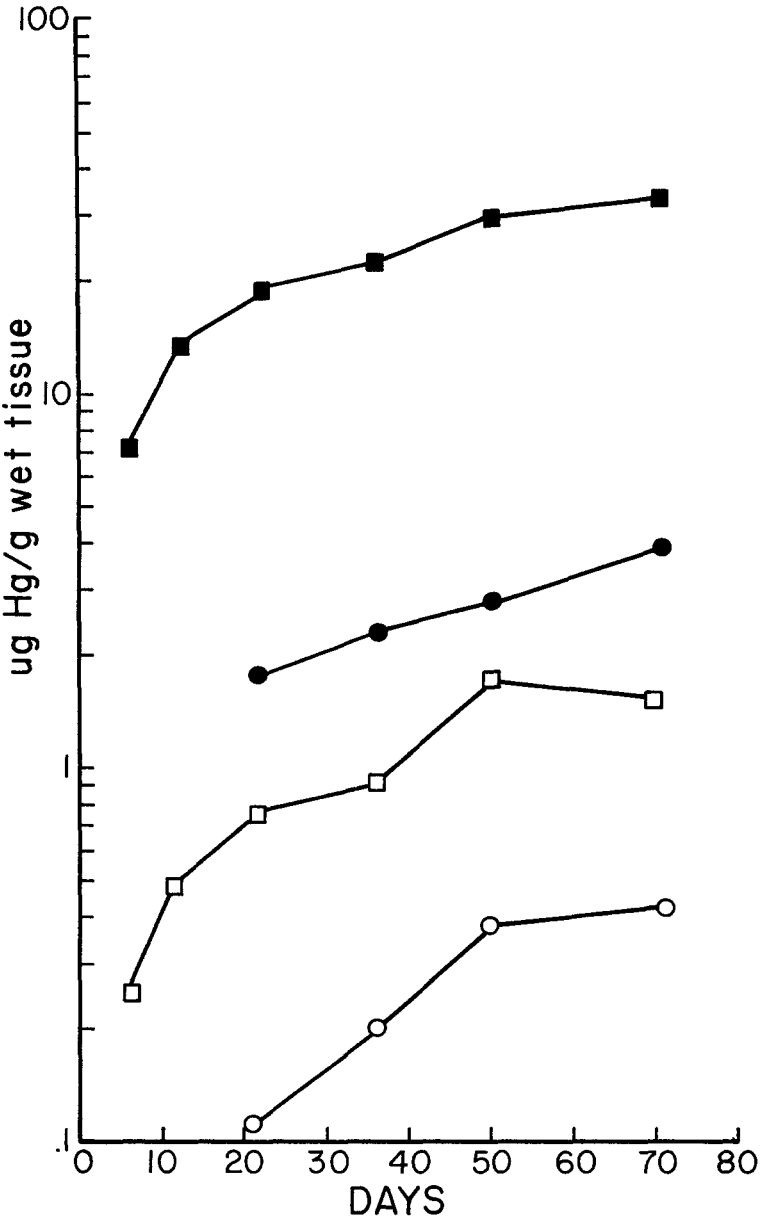

Figure 2. Mercury accumulation in guinea pig liver after daily oral dosing for 71 days with methyl mercuric chloride. (■) total mercury (□) inorganic mercury, 400 ug Hg/kg dose level. (●) total mercury (○) inorganic mercury, 40 ug Hg/kg dose level.

Figure 2 shows the accumulation of total and inorganic mercury in guinea pig liver at the 40 and 400 µg Hg/kg dose levels. In contrast to kidney, the liver inorganic levels were low, but again tend to be higher with a lower dose level. Table 1 indicates that after 71 days dosing, liver inorganic levels were 3.5, 11.0 and 15.1% of total mercury levels at the 400, 40 and 4 µg Hg/kg dose levels respectively. There was a significant difference ($p < .05$) between dose levels at days 36 and 51 but not at day 22. Levels of significance at other sacrifice dates could not be determined.

Discussion

The results suggest that the amount of biotransformation is inversely proportional to the dose level. Biotransformation of methyl mercury in the guinea pig appears to be quite similar to the pattern found in the rat (NORSETH, 1972) with kidney Hg^{++} levels approximating 50% and liver Hg^{++} levels 5%, of the total mercury levels.

After 71 consecutive doses at 400 µg Hg/kg the inorganic mercury concentration was 50 µg/gm wet weight As in other rodent species the kidney binds the bulk of the inorganic mercury. Although the Hg^{++} levels approach 50 µg/gm this is only a small portion of the total body burden of total mercury, the greater part being bound to the muscle as methyl mercury, (IVERSON et al., 1973).

The results do not allow a detailed analysis of the mechanism of biotransformation. The inverse dose dependency may arise from inhibition or saturation of an enzymatic mechanism or through an effect of mercury on a non-enzymic breakdown pathway which may involve cysteine, (NORSETH and CLARKSON 1970). It has been suggested that biotransformation of methyl mercury occurs in the liver with subsequent transport of Hg^{++} to the kidney, (NORSETH 1972). If the transport mechansims were inhibited then one might expect a higher percentage of inorganic mercury in the liver as the dose increases. The results obtained for the present study are not consistent with this possibility.

References

GAGE, J.C., and WARREN, J.M., Ann. Occup. Hyg. 13, 115 (1970).

IVERSON, F., DOWNIE, R.H., PAUL, C., and TRENHOLM, H.L., Tox. Appl. Pharmacol. (In press).

MAGOS, L., Analyst 96, 847 (1971).

NORSETH, T., Acta pharmacol, et toxicol. 31, 138 (1972).

NORSETH, T., and CLARKSON, T.W., Arch. Environ. Health 21, 717 (1970).

UTHE, J.F., ARMSTRONG, F.A.I., and STAINTON, M.P., J. Fish. Res. Bd. Canada, 27, 805 (1970).