Biochem. J. (1971) 121, 411–415
Printed in Great Britain

The Metabolism of Methoxyethylmercury Salts

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(*Received* 9 *September* 1970)

The metabolism of methoxy[^{14}C]ethylmercury chloride in the rat has been investigated. After a single subcutaneous dose a small proportion is excreted unchanged in urine and a larger amount in bile with some resorption from the gut. The greater part of the dose is rapidly broken down in the tissues with a half-time of about 1 day to yield ethylene and inorganic mercury. Ethylene is exhaled in the breath and the mercury migrates to the kidney and is excreted in urine. A small proportion of the dose appears as carbon dioxide in the breath and about 12% in urine as a mercury-free metabolite. It is possible that the breakdown of methoxyethylmercurychloride to ethylene and inorganic mercury is not catalysed by an enzyme system.

There is little information in the literature on the toxicity of alkoxyalkylmercury salts, but the available evidence suggests that they do not possess the marked neurotoxic action characteristic of the alkylmercury salts. In this laboratory (D. G. Clark, personal communication) it has been shown that mice remain unaffected after receiving 12 subcutaneous doses of MEMC* spread over 12 weeks, each dose equivalent to 4mg of mercury/kg body wt., whereas an equivalent dosage of methylmercury chloride produces a delayed paralysis. In the report of an International Committee on Maximum Allowable Concentrations of Mercury Compounds (1969) the conclusion was reached that the limit for methoxyethylmercury salts in the atmosphere should be 0.05mg/m^3 (as mercury) which is equivalent to the proposed values for inorganic mercury vapour and phenylmercury salts, and five times that of the alkylmercury salts.

The distribution of mercury in the body after administration of MEMC resembles that obtained with mercuric salts and phenylmercury salts (Ulfvarson, 1962). This suggests that methoxyethylmercury salts, like phenylmercury salts (Gage, 1964), are rapidly broken down in the body. The difference between the toxicity of methoxyethylmercury and methylmercury may well be due to the much greater stability of the latter, which is stored in the red cells and only very slowly degraded. The present investigation, which has in part briefly been reported elsewhere (Daniel & Gage, 1969), is concerned with the mechanism of the breakdown of MEMC in the body.

* Abbreviation: MEMC, methoxyethylmercury chloride.

MATERIALS AND METHODS

Synthesis of methoxy[^{14}C]*ethylmercury chloride.* Mercuric acetate (1.5mmol) and a solution of [U-^{14}C]ethylene (0.1mmol) (The Radiochemical Centre, Amersham, Bucks., U.K.) in methanol (3ml) were mixed and agitated under an atmosphere of non-labelled ethylene until absorption of the gas was complete. The solution was filtered and then evaporated to dryness. The residue was redissolved in methanol (5ml), mixed with a solution of NaCl (2mmol) in water (3ml) and again evaporated to dryness. The residue was recrystallized from water. The product, m.p. 68–69°C, specific radioactivity 285μCi/mmol, was obtained in 41% yield. Ascending paper chromatography in butan-1-ol saturated with water showed one radioactive component, the R_F of which (0.80) was identical with that given by an authentic specimen of MEMC.

Acid lability of methoxy[^{14}C]*ethylmercury chloride.* The acid lability in the presence of 2mM-cysteine was determined *in vitro* under the conditions used by Weiner, Levy & Mudge (1962). The reaction mixture was gassed continuously with N_2 (50ml/min) which then passed through two absorbers in series, each containing 6ml of mercuric perchlorate solution (0.25M) in $HClO_4$ (2M). The contents of the absorbers were removed at hourly intervals for radioactivity measurements and replaced with fresh solution. The experiments were made with 0.2M phosphate buffer prepared with Na_2HPO_4 and KH_2PO_4 at pH7.2 and 6.0, and with 0.2M-sodium acetate adjusted to pH5.0 and 4.0 with acetic acid.

Animal experiments. Male albino Wistar rats (180–220g) of the Alderley Park specific-pathogen-free strain were injected subcutaneously with an aqueous solution of methoxy[^{14}C]ethylmercury chloride (1.4mg/kg). Animals were then transferred to individual metabolism cages, which permitted the collection of urine and faeces, and in some experiments the extraction of radioactive

material from expired air. Unrestricted food and water were supplied and the excreta were collected daily.

Two procedures were used for investigating the expired air. In the first method the metabolism cage was ventilated by a current of CO_2-free air at 500ml/min. The air then passed through a vertical glass column (30mm×300mm) which was loosely packed with stainless-steel rings through which M-NaOH percolated from a reservoir at 30ml/h. The air that emerged from the top of the column was pyrolysed in the combustion apparatus described by Lubatti & Blackith (1956), and the CO_2 formed was then removed by scrubbing with M-NaOH as described above. In the second method the air was drawn through an absorber containing M-NaOH (6ml) and then passed through a column, packed as described above, through which a solution of mercuric perchlorate (0.25M) in $HClO_4$ (2M) percolated from a reservoir at 30ml/h. This reagent is specific for olefins (Keller, 1941) and was used by Young, Pratt & Biale (1952) for the trapping of ethylene.

The bile duct was cannulated in two rats that had been injected subcutaneously with methoxy[^{14}C]ethylmercury chloride and bile was collected for 24 and 48h. This experiment was repeated on a further pair of rats at intervals of 72 and 96h after dosing.

Measurement of radioactivity. Radioactivity in the extracts from expired air, and in urine, faeces, bile and tissues was determined as described by Daniel & Gage (1965).

Determination of mercury. Measurements of inorganic and total mercury in urine, bile, kidney and liver, and of total mercury in faeces, were made by the methods described by Gage & Warren (1970).

Analysis of expired air. The mercuric perchlorate solution used to extract the expired air was subjected to gas–liquid-chromatographic analysis. A portion (20ml) was transferred to a conical flask fitted with a rubber diaphragm seal and 2M-HCl (5ml) injected into the flask, which was then shaken for 5min. A portion of the vapour (2ml) was removed by a gas-tight syringe and injected on to a Porapak Q (150–200 mesh) column in a Varian Aerograph model 1522. The operating conditions were as follows: column dimensions, 5ft×1/8in; column temp. 30°C; detector, flame ionization; detector oven temperature, 35°C; injection temperature, 35°C; carrier gas, N_2 (30ml/min); chart speed, 40in/h. Under these conditions acetylene and ethylene had retention times of 3.65min and 3.4min respectively.

RESULTS

Excretion of radioactivity and mercury. Tables 1 and 2 show that after a single subcutaneous injection of ^{14}C-labelled MEMC to rats, about 25% of the radioactivity administered appeared in the urine within 4 days, and 50% in the expired air within 3 days. No significant amounts of radioactivity were excreted in faeces, and none was found at the termination of the experiment in liver or kidney tissue.

The total excretion of mercury over the experimental period was more variable; in the two experiments 48 and 28% of the dose was excreted, with the bulk in the faeces. The difference between

Table 1. *Excretion of radioactivity, inorganic and total mercury in the urine and faeces of rats injected with methoxy[^{14}C]ethylmercury chloride*

Five rats were used for each experiment. Results are mean values; pooled excreta were used except where standard deviations are given in parentheses.

		Expt. no.	Percentage of dose during day								
			1	2	3	4	5	6	7	8	Total
Urine	Radioactivity	1	10.8 (1.2)	8.4 (0.5)	2.6 (0.2)	1.1 (0.1)	0.4	0.2	—	—	23.5
		2	11.3	7.6	2.3	1.2	0.2	0.2	0.2	0.0	23.2
	Inorganic mercury	1	0.7 (0.3)	3.3 (1.7)	3.0 (1.5)	1.9 (1.0)	1.1 (0.3)	0.9 (0.2)	—	—	10.9
		2	0.9	1.0	1.0	1.9	2.3	2.4	2.3	1.0	12.8
	Total mercury	1	1.9 (1.1)	4.0 (1.7)	3.4 (1.5)	1.9 (1.0)	1.1 (0.3)	0.9 (0.2)	0	0	13.2
		2	1.8	1.3	1.7	2.0	2.9	2.5	2.3	1.0	15.5
Faeces	Total mercury	1	2.7	10.3	12.1	9.6	—	—	—	—	34.7
		2	3.7	3.4	4.6	0.4	—	—	—	—	12.1

Table 2. *Excretion of radioactivity in the expired air of rats injected with methoxy[^{14}C]ethylmercury chloride*

One rat was used for each experiment. Standard deviations are given in parentheses.

No. of experiments	Radioactivity recovered as	Percentage of radioactivity excreted during day			
		1	2	3	Total
3	$^{14}CO_2$	3.2 (2.1–3.8)	1.9 (0.9–2.4)	0	5.1
5	$^{14}CO_2$ after pyrolysis	27.0 (17.9–36.1)	16.7 (9.2–20.7)	0.5	44.2
3	Olefin	27.8 (22.9–33.3)	13.2 (11.8–14.9)	2.5 (1.1–4.0)	43.5

Table 3. *Excretion of radioactivity, inorganic and total mercury in the bile of rats injected with methoxy[^{14}C]-ethylmercury chloride*

Values are duplicate determinations on single rats.

	Percentage of dose excreted during day			
	1	2	3	4
Radioactivity	19.9, 25.1	5.9, 8.1	1.7, 1.1	0.4, 0.5
Inorganic Hg	0.0, 0.0	3.2, 2.1	1.5, 0.7	1.3, 1.0
Total Hg	14.1, 15.1	5.9, 5.0	2.3, 1.5	1.5, 1.4

Table 4. *Total and inorganic mercury content of the liver and kidneys of rats injected with methoxyethylmercury chloride*

Values are replicates on single rats.

Time after injection (h)	Kidneys		Liver	
	Total Hg (μg/g)	Inorganic Hg (μg/g)	Total Hg (μg/g)	Inorganic Hg (μg/g)
2	8.8	4.0	2.4	0.9
	7.0	4.4	2.0	1.2
	5.3	2.3	1.5	0.4
6	12.2	8.2	—	—
	11.9	7.9	—	—
24	14.0	14.0	1.0	0.7
	14.2	14.1	0.8	0.6
	14.8	13.9	1.1	0.8

the urinary excretion of total and inorganic mercury demonstrated that there was a small but significant excretion of organic mercury, which was equal to or greater than the excretion of inorganic mercury on the first day, but thereafter the organic excretion decreased whereas the inorganic excretion increased.

The radioactivity excreted in urine was considerably in excess of the organic mercury, indicating that urine contained a metabolite of MEMC in which the mercury was missing. Table 3 shows that there was a considerable biliary excretion of mercury, which was entirely in the organic form during the first 24h; the difference between the organic mercury and the radioactivity excreted in bile indicates that a mercury-free metabolite of MEMC was also excreted by this route.

Tissue storage. At the end of Expts. 1 and 2 in Table 1, an analysis of liver and kidney tissue showed mercury to be absent from the livers but that the kidneys contained 13–23% of the dose in Expt. 1 and 36.4% in Expt. 2. The mean total mercury recovered from the tissues and excreta in these two experiments was 67 and 64%.

In another set of experiments shown in Table 4, determination of the storage of mercury in the liver and kidneys was made at three time-intervals after dosing. The results show that initially more than half the mercury in liver and kidneys was in the organic form. After 24h the total mercury in liver

had appreciably decreased, with a smaller proportion of organic mercury. In the kidneys the organic mercury had completely disappeared after 24h, but a rise in inorganic mercury gave an increase in total mercury.

Identification of metabolites in expired air. Table 2 shows that there was a small excretion of labelled carbon dioxide after dosing, but that a very much larger amount was released after pyrolysis of the expired air. About the same amount of radioactivity was extracted from the expired air by means of mercury perchlorate solution, and could be readily released from it by the addition of mineral acid. Gas–liquid chromatography of this volatile material showed the presence of a single component, the retention time of which was identical with that of ethylene.

Identification of metabolites in urine. (*a*) Mercury compounds. Urine was extracted with the minimum quantity of dithizone [diphenylthiocarbazone, BDH (Chemicals) Ltd., Poole, Dorset, U.K.] in chloroform solution, and the extract after concentration was chromatographed on thin-layer plates of silica gel with hexane–acetone (4:1, v/v). Under these conditions the yellow MEMC–dithizone complex (R_F 0.42) could be separated from the orange mercury dithizonate (R_F 0.36).

(*b*) Mercury-free metabolites. Urine was collected for 48h from ten rats dosed with 1.5mg of MEMC/kg the total radioactivity administered being 3.2×10^5 d.p.s. A portion of the combined urine was acidified to pH 1 and extracted with ether, and the remainder passed at pH 4 through activated charcoal; neither of these treatments removed a significant amount of radioactivity. The urine was adjusted to pH 6 and passed down a column of DEAE-cellulose (Whatman DE 52; formate form); the radioactivity was not retained on the column. The eluate was concentrated under reduced pressure to give an oil, which was extracted with methanol, the extract was evaporated and the residue extracted again with ether. This extract, containing about one-half of the radioactivity in urine, was further purified by t.l.c. on silica gel with water-saturated butanol as solvent. Radioautography revealed a diffuse band (R_F 0.4–0.6), which was removed with methanol and rechromatographed with methanol–chloroform (1:3, v/v). Three radioactive bands were obtained, with R_F 0.45, 0.74 and 0.82. The middle band, containing most of the radioactivity, was removed with methanol.

A portion of the methanolic solution of the middle band was added to a mixture of ethylene glycol, 2-chloroethanol and 2-methoxyethanol, three possible metabolites of MEMC. The mixture was analysed on a Pye Series 104 model 64 gas–liquid chromatograph. The three peaks were trapped as they emerged in tubes cooled in liquid nitrogen, but none contained a significant amount of radioactivity.

Table 5. *Acid lability of methoxy*[^{14}C]*ethylmercury chloride in the presence of* 2mM-*cysteine*

pH	Percentage breakdown during 1h		
	0–1h	1–2h	2–3h
4.0	24.9	22.8	12.0
5.0	7.8	7.9	6.6
6.0	0.9	1.3	1.0
7.2	0.3	0.2	<0.1

The fraction was too impure to admit molecular-weight determination by mass spectrometry, and attempts to further purify it were unsuccessful.

Degradation of MEMC in vitro. Table 5 demonstrates that ethylene was rapidly released when MEMC was incubated at 37°C at pH 4 in the presence of cysteine; this is in agreement with the observations of Weiner *et al.* (1962). There was a considerable release of ethylene at pH 5, but this was much less at pH 6 and at pH 7.2 it was negligible. No breakdown occurred if cysteine was omitted from the reaction medium.

DISCUSSION

The results show that MEMC is rapidly degraded in the rat to yield mainly ethylene and inorganic mercury. The results in Table 2, showing the excretion of ethylene, are probably a good guide to the rate of breakdown in the body, and suggest that the half-life of retained MEMC is about 1 day. This is in contradiction to the view of Ulfvarson (1962) that MEMC is relatively stable. Shortly after administration, MEMC is found in the liver and kidneys, and at this stage it is excreted through the liver into the bile and through the kidneys into the urine. Thereafter, the MEMC content of the liver and kidneys decreases whereas the inorganic mercury content of the kidneys increases. This accumulation of mercury in the kidneys resembles that reported by Rothstein & Hayes (1960) after the administration of inorganic mercury. These observations suggest that initially the MEMC is distributed in the tissues, where it is progressively broken down to release inorganic mercury, which migrates to the kidneys, from which it is slowly excreted. The MEMC content of the kidneys did not exceed 3% of the dose; this is very different from the specific accumulation of chlormerodrin in the kidneys, in which 70% of an intravenous dose is found within a few hours after administration (Clarkson, Rothstein & Sutherland, 1965). No radioactivity was detected in faeces, so the mercury excreted in bile, which was almost entirely in the

organic form, must have undergone degradation in the gut. In the two experiments undertaken the faecal excretion of mercury was very different, but it seems likely that the daily faecal excretion is appreciably less than the daily excretion in bile. This suggests that there is some reabsorption in the gut, which will presumably involve organic mercury, as inorganic mercury is known to be poorly absorbed from the gut (Swensson, Lundgren & Lindstrom, 1959).

The production of ethylene *in vitro* from MEMC in the presence of acid and cysteine involves the reverse of the reaction used in synthesizing the compound:

$$CH_2{=}CH_2 + CH_3OH + HgCl_2 \rightleftharpoons CH_3O{-}CH_2{-}CH_2{-}HgCl + HCl$$

This reaction proceeds to the left if H^+ ions are supplied and if cysteine or some other thiol compound is present to remove mercuric ions. The total breakdown *in vivo* is about 27% in 24h, or a little over 1%/h. There is considerable uncertainty about the interpretation of measurements of intracellular pH values, but Carter, Rector & Seldin (1967) have indicated that the pH of skeletal muscle may be as low as 6. At this pH value the degradation of MEMC *in vitro* is about 1%/h, a rate similar to that observed *in vivo*, so it is possible that the degradation *in vivo* is not mediated by an enzyme. This hypothesis is attractive, as MEMC, like other organic mercurials, is an enzyme inhibitor.

A small excretion of radioactive carbon dioxide in the breath has been recorded after administration of MEMC, and it may be concluded that there is some incorporation of the ^{14}C into the carbon pool. The major mercury-free metabolite in urine has not been identified; this failure is due to the difficulty in separating and purifying the small quantities involved, as the toxicity of the MEMC limits the dose that can be administered. This metabolite does not appear to be acidic, nor to be any of the obvious oxidation products of ethylene. One possibility is that ethylene is first oxidized to ethylene oxide, and that the ring is then opened by reaction with acids to form compounds of the type HOC_2H_4R. An investigation into the metabolism of ethylene, which does not yet appear to have been undertaken, might throw light on the nature of this metabolite.

The authors acknowledge the assistance of Mr G. H. Walker, who prepared the sample of [^{14}C]MEMC.

REFERENCES

Carter, N. W., Rector, F. C. & Seldin, D. G. (1967). *J. clin. Invest.* **46**, 920.

Clarkson, T. W., Rothstein, A. & Sutherland, R. (1965). *Br. J. Pharmac.* **24**, 1.

Daniel, J. W. & Gage, J. C. (1965). *Fd Cosmet. Toxicol.* **3**, 405.

Daniel, J. W. & Gage, J. C. (1969). *Biochem. J.* **111**, 20P.

Gage, J. C. (1964). *Br. J. ind. Med.* **21**, 197.

Gage, J. C. & Warren, J. M. (1970). *Ann. occup. Hyg.* **13**, 115.

Keller, R. N. (1941). *Chem. Rev.* **28**, 229.

Lubatti, O. F. & Blackith, R. E. (1956). *J. Sci. Fd Agric.* **7**, 149.

Report of an International Committee. Maximum Allowable Concentrations of Mercury Compounds (1969). *Archs envir. Hlth*, **19**, 891.

Rothstein, A. & Hayes, A. D. (1960). *J. Pharmac. exp. Ther.* **130**, 166.

Swensson, A., Lundgren, K-D. & Lindstrom, O. (1959). *A.M.A. Archs ind. Hlth*, **20**, 467.

Ulfvarson, U. (1962). *Arch. Gewerbepath. Gewerbehyg.* **19**, 412.

Weiner, I. M., Levy, R. I. & Mudge, C. H. (1962). *J. Pharmac. exp. Ther.* **138**, 96.

Young, R. E., Pratt, H. K. & Biale, J. B. (1952). *Analyt. Chem.* **24**, 551.