Bull. Environm. Contam. Toxicol. 22,151-158 (1979)

Effects of Three Diets on Mercury Excretion after Methylmercury Administration

T. D. Landry, R. A. Doherty, and A. H. Gates

Departments of Radiation Biology and Biophysics, Pediatrics and Obstetrics, Environmental Health Sciences Center, University of Rochester, School of Medicine, Rochester, N. Y. 14642

In contrast to adults, suckling mice excrete minimal amounts of their mercury body burdens until the approximate time of weaning to a solid diet, when there is an abrupt increase in mercury elimination (DOHERTY et al. 1977). Increased gut absorption of inorganic Hg in rats fed a milk diet has been reported (JUGO 1975). These observations suggest that dietary components may influence absorption and excretion of Hg compounds. This possibility was examined by determining the effects of (1) an evaporated whole milk diet, (2) a chemically defined liquid diet, and (3) a pelleted rodent diet on Hg retention in mice after administration of an apparently non-toxic dose of methylmercury (MeHg).[1] Whole body Hg elimination and fecal and urinary Hg excretion were measured daily for two weeks. Two weeks after MeHg administration, Hg concentrations in whole body, brain, liver, kidney and whole blood were determined. The diet groups showed differences in whole body Hg retention and fecal Hg excretion.

MATERIALS AND METHODS

Eight-month-old female BALB/c mice produced in our Inbred Mouse Unit, were housed in stainless steel metabolism cages. Each of the three experimental groups consisted of one cage of four animals. Mice were provided the liquid diets *ad libitum* beginning one week before MeHg administration. Prior to the experiment, all mice were fed the pelleted diet. The liquid diets were provided in calibrated glass feeding tubes. Water was available *ad libitum* to all mice. Individual mouse weights and volumes of liquid food intake were recorded daily. Total Hg content of each of the three diets was measured by atomic absorption spectroscopy (GREENWOOD et al. 1977). The pellet diet contained 32 ng/g and the liquid diets (milk and GIBCO 116 E.C.) contained less

[1]The sources for the diets were Pet, Inc., St. Louis, MO (evaporated whole milk); GIBCO, Grand Island, NY (GIBCO 116 E.C.); Agway, Inc., Hauppauge, NY (Charles River R-M-H 3000).

0007-4861/79/0022-0151 $01.60

than 20 ng total Hg/g of diet. Radioactive methylmercuric chloride ($Me^{203}HgCl$, New England Nuclear), determined to contain 91% organic Hg by triple benzene extraction (CAPON and SMITH 1977) was mixed with non-radioactive methylmercuric chloride (ethanol recrystallized, purity >99%, K&K Labs, Plainfield, NJ). The final specific activity was 0.05 mCi/mg Hg.

Each mouse received a single dose of methylmercuric chloride *per os*, average dose, 0.46 ± 0.02 mg Hg/kg (±1 SE) (0.01 mL/g of mouse) in 20 mM Na_2CO_3 on day 0. There were no significant differences between the groups, ($p > 0.05$). The dose was determined by counting each mouse in a whole body counter immediately after MeHg administration. For 14 days after MeHg administration, changes in radioactive Hg body burdens were determined daily with a whole body gamma spectrometer by placing each mouse in the 70 mm x 130 mm center well of a 135 mm diameter x 160 mm deep thallium-activated sodium iodide crystal. Hg body burdens were calculated after correction for radioactive decay and are expressed as Fractions of Initial Dose (FID) for each animal. Feces and urine were separately collected from each cage and were counted daily. The fecal samples were subsequently analyzed to determine the proportion of organic ^{203}Hg relative to total ^{203}Hg using a benzene extraction technique (CAPON and SMITH 1977), and Hg concentration (per gram of dry feces), using a heat-dried fecal sample. About one-half of the daily collection of feces from each cage was used for organic Hg determination; the other half, for Hg concentration determinations. Fourteen days after MeHg administration, necropsies were performed. ^{203}Hg concentrations in brain, liver, kidney and whole blood (per gram wet organ or tissue) were determined with a Beckman 300 gamma spectrometer.

RESULTS

During the 14 days of the experiment the mean weight of each group remained within ±3.5% of the respective group mean weight at the time of MeHg administration (day 0). On day 0, the group mean weights were 30.2 ± 0.4g (±1 SE) (GIBCO diet group), 28.1 ± 0.3g (pellet diet group), and 28.8 ± 0.8g (milk diet group). Mean food intake from day 0 until necropsy was 6.4 mL, GIBCO diet per mouse per day, and 7.7 mL milk per mouse per day. Pelleted diet intake in a subsequent experiment was measured to be 3.7g per mouse per day.

Whole body mercury elimination half-times were estimated by least squares linear regression analysis of log FID from day 1 through 14 (Figure 1). The decrease in total body radioactivity during the first 24 hours

was partly the result of loss of the portion of the MeHg dose that was not absorbed from the gut. Results summarized in Table 1 indicate that these diets differentially affected whole body elimination of Hg after MeHg administration.

The whole body elimination rates were confirmed by measuring cumulative fecal and urinary Hg excretion (Figure 2). Fecal excretion accounts for greater than 84% of the fecal and urinary Hg excretion of each group during the two week period. Fecal masses of the GIBCO diet

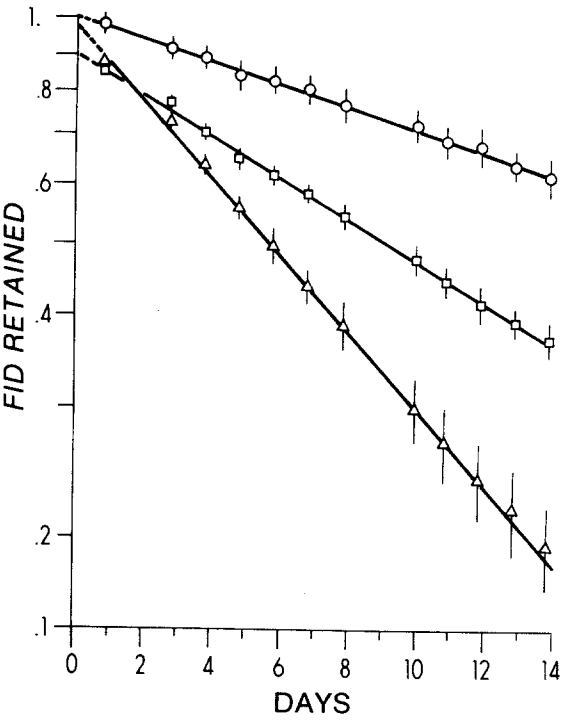

Figure 1. Changes in total body mercury calculated from whole body radioactivity and plotted as Fractions of Initial Dose (FID) of radioactive MeHg vs time (days after MeHg administration). GIBCO diet group (△), pellet diet group (□), milk diet group (○). Values are group means ± SE (vertical bars). The Hg elimination half-times (days 1 through 14), as estimated by least squares linear regression analysis (log FID vs day), and corresponding 95% confidence interval are: GIBCO diet, 5.8 days (5.3-6.3 days, 95% CI); pelleted diet, 10.6 days (9.9-11.5); and milk diet, 19.2 days (16.6-22.9).

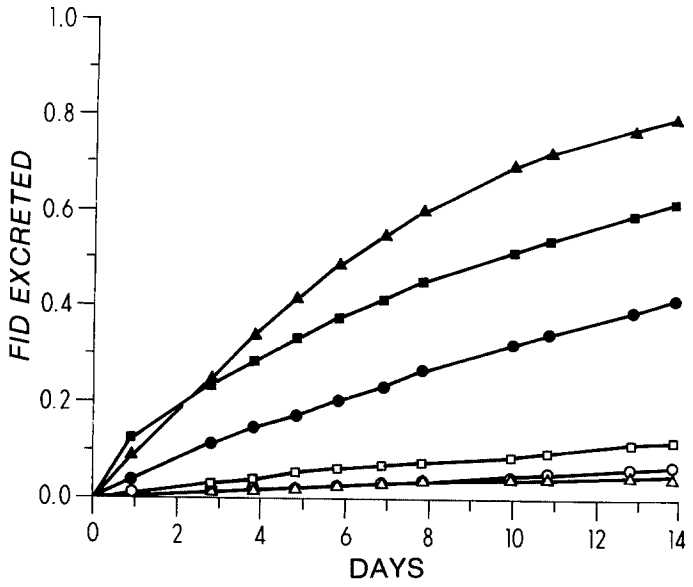

Figure 2. Cumulative mercury excretion in feces and urine. Feces: GIBCO group (▲), pelleted group (■), milk group (●). Urine: GIBCO group (△), pelleted group (□), milk group (○). Values are cumulative sums of total radioactive Hg of daily collections of separated feces and urine from each experimental group and are plotted as Fractions of Initial Dose (FID).

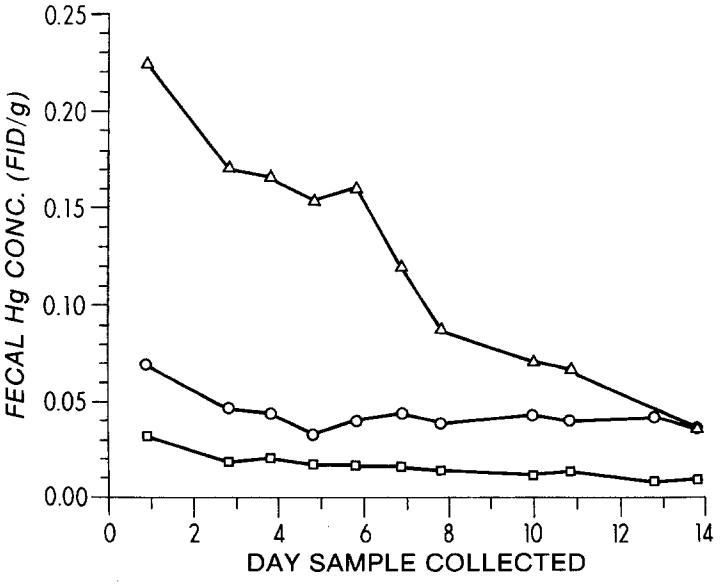

Figure 3. Fecal Hg concentration (FID/g dry mass). Group symbols are those specified in Figure 1.

and milk diet groups were much less than fecal masses of the pelleted diet group. The mean daily total fecal dry mass per cage of 4 mice (day 0 through day 14) was 0.53 ± 0.04g (±1 SE) in the GIBCO diet group, 2.38 ± 0.2g in the pelleted diet group, and 0.72 ± 0.04g in the milk diet group. This may account, in part, for the differences in fecal Hg concentrations among the three groups (Figure 3). The GIBCO diet group excreted a greater proportion of inorganic Hg (benzene non-extractable Hg) to total Hg than the other groups. Seven days after injection the ratios of inorganic Hg to total Hg were 0.91 (GIBCO diet group), 0.75 (pelleted diet group), and 0.72 (milk diet group).

Hg concentrations in organs and blood at necropsy generally correlated with whole body retention of Hg. Brain Hg concentrations were 149 ± 17 ng/g (±1 SE) (GIBCO diet group), 179 ± 8.5 ng/g (pelleted diet group), and 233 ± 19 ng/g (milk diet group). One way analyses of variance were calculated for brain, kidney, liver and whole blood Hg concentrations. The mean organ and blood concentrations of the three diet groups were found to be significantly different ($p < 0.05$).

Total Hg recoveries (whole body burdens of radioactive Hg immediately before necropsy plus all Hg excreted during the preceding 14 days) were calculated: 1.06 FID (GIBCO diet group), 1.11 FID (pelleted diet group) and 1.10 FID (milk diet group).

DISCUSSION

Hg compounds are introduced into the gut lumen in biliary and pancreatic and perhaps other gastrointestinal secretions, and in exfoliated intestinal epithelial cells. Fecal excretion of Hg is impeded due to recycling of Hg-containing compounds by reabsorption from the gut. Demethylation of MeHg to inorganic Hg affects Hg transfer processes, particularly reabsorption from the gut lumen (NORSETH and CLARKSON 1971). Because whole body elimination of Hg after MeHg administration depends largely on fecal excretion, the different diets in this experiment probably altered whole body elimination through one or more of these pharmacokinetic processes.

A milk diet has been reported to enhance gut absorption of certain compounds including lead (KELLO and KOSTIAL 1973) and inorganic Hg (JUGO 1975). Mechanisms have been proposed to explain the effect of a milk diet on absorption of heavy metals from the gut. It has been suggested that binding of heavy metal compounds to fatty acids from milk triglycerides enhances gut absorp-

tion of the metal (JUGO 1977). This mechanism is feasible, but effects of these factors on Hg kinetics in the gut have not been documented. Because ingested MeHg is almost entirely absorbed from the gut, the milk diet probably could not increase the initial absorption. MeHg is transformed *in vivo* (NORSETH and CLARKSON 1971) to Hg compounds (including inorganic Hg and Hg bound to proteins) that are not readily reabsorbed from the gut. Enhanced reabsorption of these compounds from the gut lumen due to the presence of ingested milk might account for the reduced fecal Hg excretion and the resultant longer elimination half-time.

The milk diet appears to decrease the proportion of benzene non-extractable (inorganic) Hg relative to total Hg in feces. Reduced demethylation of MeHg (in the gut lumen) would result in increased reabsorption from the gut, and would decrease the rate of whole body elimination. The GIBCO diet group differs markedly from the other groups in the proportion of inorganic Hg to total Hg in feces. Increased extra-intestinal transformation to inorganic Hg would be expected to enhance fecal and urinary Hg excretion. A large proportion of parenterally administered mercuric chloride (inorganic Hg) is excreted in the urine. Because there was relatively little urinary excretion of Hg in this experiment (Figure 2), it is unlikely that there was substantially increased inorganic Hg in the parenteral circulation. Increased demethylation in the liver or intestine might account for the large proportion of inorganic Hg in the feces from the GIBCO diet group.

Gut microbes also may play a role in transformation of organic Hg. It has been reported that germ-free mice given MeHg excreted Hg more slowly than control mice (NAKAMURA et al. 1977). It would be noted that Nakamura's findings do not agree with a prior study (NORSETH 1971) in which no differences in biotransformation of MeHg could be detected in germ-free vs control rats. It is possible that a milk diet reduces fecal inorganic Hg excretion by reducing transformation of organic Hg to inorganic Hg by gut microbes.

It is not possible to differentiate unequivocally the relative effects of specific dietary factors from this experiment alone because the three diets have numerous differences, some of which are summarized in Table 1. The relative content of lipid correlates inversely with the rate of Hg elimination. This is consistent with an hypothesized lipid effect (JUGO 1977) on absorption of Hg compounds. The relative carbohydrate intake is proportionate to Hg elimination rate. The GIBCO and the milk diets have a very low content of "fiber" (indigestible residue). Daily dry fecal mass

excreted by the mice fed the pellet diet was 4 to 5 times greater than the fecal mass from mice fed either of the liquid diets. The multivariate nature of this experiment does not rule out a fiber effect on Hg elimination, but does indicate that high fiber content is not essential for rapid fecal Hg excretion in mice as seen in the GIBCO diet group.

TABLE 1

DIETARY PROTEIN, CARBOHYDRATE, AND LIPID CONCENTRATION AND INGESTION

	PROTEIN		CARBOHYDRATE		LIPID	
	conc	ingest	conc	ingest	conc	ingest
GIBCO 116 EC	7.2	0.55	31.8	2.43	0.25	0.019
Pellet diet	22.3	0.82	51.5	1.89	5.3	0.19
Evaporated Milk diet	6.8	0.56	10.0	0.83	7.56	0.62

Major dietary components of the diets. Concentrations (conc) are expressed as g per 100 g of diet. Average ingestion (ingest) is expressed as g ingested per mouse per day based on measured consumed volumes of the liquid diets. Values for ingestion of the pellet diet are based on measured intake of the pellet diet in a similar subsequent experiment. Information on components of the diets was obtained from the following sources: Pet Milk, U.S. Government figures for canned whole evaporated milk; Charles River pelleted diet, Agway's "representative proximate analysis"; and GIBCO liquid diet, GIBCO's theoretical analysis (based on components added to the defined diet). The concentrations in the GIBCO diet were converted from g/100 mL to g/100 g after determining the specific gravity of the GIBCO diet.

Because Hg levels in the critical organ for MeHg toxicity (brain) were affected by the diets that were tested, it is possible that diet may influence MeHg toxicity. Large variations in Hg elimination half-times have been observed in human MeHg poisoning outbreaks (AL-SHAHRISTANI and SHIHAB 1974). It should be determined whether diet is influencing Hg elimination and affecting human Hg body burdens and risk of poisoning after human Hg exposure. It may also be possible to increase Hg elimination by altering diet, thereby more rapidly reducing Hg body burdens of exposed individuals or populations at risk.

ACKNOWLEDGEMENTS

Grateful acknowledgement is given to J. Hebberecht, F. Stoss, R.W. Kilpper, C.E. Sewell, R.B. Struthers, S. Demuth and N. Hanna.

Studies supported by USPHS-NIH Toxicology Training grant 5-T01-GM-01781, NIEHS Center grant (ES 01247), Program Project grant (ES 01248), and under contract with the U.S. Department of Energy at the University of Rochester Department of Radiation Biology and Biophysics and has been assigned Report No. UR-3490-1232.

REFERENCES

AL-SHAHRISTANI, H., and K.M. SHIBAB: Arch. Environ. Hlth. 28, 342 (1974).

CAPON, C. and J.C. SMITH: Anal. Chem. 49, 365 (1977). (The benzene extraction technique was from personal communication with the authors, and was based on this article.)

DOHERTY, R.A., A.H. GATES, and T.D. LANDRY: Ped. Res. 11, 416 (1977).

GREENWOOD, M.R., P. DHAHIR, T.W. CLARKSON, J.P. FARANT, A. CHARTRAND, and A. KHAYAT: J. Anal. Toxicol. 1, 265 (1977).

JUGO, S.: Environ. Res. 13, 36 (1977).

JUGO, S.: Ph.D. thesis, University of Zagreb [referred to in JUGO (1977)] (1975).

KELLO, D. and K. KOSTIAL: Environ. Res. 6, 355 (1973).

NAKAMURA, I., K. HOSOKAWA, H. TAMURA and T. MIURA: Bull. Environ. Contam. Toxicol. 17, 528 (1977).

NORSETH, T.: Acta Pharmacol. et Toxicol. 30, 172 (1971).

NORSETH, T. and T.W. CLARKSON: Arch. Environ. Hlth. 22, 568 (1971).