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The effect of various dietary fibres on tissue concentration and chemical form of mercury after methylmercury exposure in mice

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Abstract. The whole-body retention of mercury after exposure of BALB/c mice to methylmercury was measured in animals fed fibre-free, 5% pectin, 5% cellulose or 5, 15 or 30% wheat bran diets. The rate of elimination of mercury was dependent on the diet fed, with dietary bran increasing the rate of elimination. The incorporation of 15 or 30% bran in the diet of the mice decreased the total mercury concentration in the brain, blood and small intestine, although the effects were significant only in those animals on 30% bran diet. The fibres had little effect on mercury levels in other tissues. The proportion of mercury found in the mercuric form was significantly greater in liver, kidneys and gut of mice fed bran. The results suggest that dietary bran may reduce the levels of mercury in the brain after methylmercury exposure and may therefore reduce the neurotoxic effects of the organomercurial. We suggest that wheat bran exerts its effects on mercury retention and brain level via a modification of the metabolic activity of the gut microflora.

Key words: Methylmercury – Mercury – Gut microflora – Dietary fibre – Wheat bran

Introduction

The toxicity of a metal is a function of a variety of factors including absorption, excretion and biotransformation. These factors can be modified by diet which in turn may affect metal retention and toxicity. For example, the whole body retention of various inorganic metal salts, including lead, cadmium, mercury and plutonium, is greater in rats fed milk than in animals given a stock diet (Engstrom and Nordberg 1978; Kostial et al. 1981). In addition, there is evidence that qualitative and quantitative changes in dietary fats and protein influence the absorption of lead (Levander 1979; Barltrop and Khoo 1975; Baltrop 1976), the toxicity of which is also influenced by the levels of minerals and vitamins in the diet (Levander 1979; Mahaffey 1981). High dietary concentrations of fibre are known to reduce the utilisation of zinc, magnesium and calcium in man (Ismail-Beigi et al. 1977) and recently Kiyozumi et al. (1982) have shown that the addition of purified forms of fibre (cellulose, pectin or lignin) to a rat diet containing cadmium lowered the cadmium content of tissues. Much less is known about dietary modification of organometal retention and toxicity, although Landry et al. (1979) and Rowland et al (1984) have demonstrated differences in methylmercury retention and tissue concentration in mice given milk and stock diets. By comparison to inorganic mercury salts, methylmercury is rapidly and almost completely absorbed after ingestion (Miettinen 1973; Walsh 1982). A proportion of the absorbed dose is then secreted back into the gut via the bile, leading to an enterohepatic circulation (Norseth and Clarkson 1971; Norseth 1973). There is opportunity, therefore, for binding in the gut of the organomercurial, or its metabolites, to dietary fibre which may influence the absorption and tissue concentration of mercury. In addition, there is evidence that the intestinal microflora metabolise methylmercury to inorganic forms (Rowland et al. 1978) causing increased mercury excretion and decreased tissue mercury concentrations (Nakamura et al. 1977; Rowland et al. 1980, 1984). Since dietary fibre has profound effects on the metabolic activity of the gut flora (Wise et al. 1982; Rowland et al. 1983; Rowland et al. 1985), it is conceivable that fibre may also influence methylmercury retention indirectly via an effect on bacterial mercury metabolism in the gut.

In the present paper, we have studied the effects of different forms of fibre (cellulose, pectin, wheat bran) on the body burden, tissue concentration and chemical form of mercury after exposure of mice to methylmercury.

Methods

Animals

Male BALB/c mice (Olac 1976 Ltd., Bicester, UK), 3 weeks old, were housed in grid-bottom cages and fed either a control purified diet (Wise et al. 1982) or the same diet supplemented with cellulose or pectin (50 g/kg; Sigma Chemical Co., Poole, UK) or wheat bran (50, 150 or 300 g/kg; Jordan Cereals Ltd., Biggleswade, UK). The diets were formulated such that the ratio of energy to nutrients was not altered by addition of the fibre component. The animals were free fed the diets for 3 months and throughout the period of mercury exposure.

Materials

Methylmercuric chloride labelled with ^{203}Hg ($Me^{203}HgCl$), obtained from Amersham International plc (Amersham, Bucks, UK) was shown to contain 99% organic Hg by

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triple benzene extraction (Cappon and Smith 1977). Unlabelled MeHgCl was purchased from K&K Laboratories (Plainfield, NY). Dosing solutions of MeHgCl were prepared in 0.9% saline and stored at −18 °C for the duration of the dosing period.

Experimental design

1. Mercury retention studies. Three months after the start of the dietary regimes, each mouse (body weight range 25–32 g; five mice per dietary group) was given PO a single dose of $Me^{203}HgCl$ (5.0 mg/kg body weight; specific activity 0.02 μCi/mg). The dose was determined by whole body counting of each mouse in a well-shaped NaI crystal (well diameter 8 cm, well depth 8 cm, counting efficiency 43%) immediately after MeHgCl administration, and was taken as 100% of body radioactivity.

Changes in radioactive mercury body burdens were determined approximately twice weekly for 4 weeks. The results were expressed as percentage of the administered dose after correction for radioactive decay.

2. Determination of tissue mercury concentration. Three months after the start of the dietary regimes, groups of nine mice on each diet were given PO a dose of $Me^{203}HgCl$ (5.0 mg/kg body weight; specific activity 6.7 μCi/mg). On day 14 after dosing the mice were anaesthetised with pentobarbitone (60 mg/kg, IP), arterial blood samples taken into heparinised tubes and the animals killed by exsanguination from the aorta. The kidneys, brain, liver, small intestine (including contents) and large intestine (caecum and colon including contents), were removed, weighed and counted for radioactivity using a Packard autogamma scintillation counter. The total mercury concentration (μgHg/g) of the tissues was calculated from the count per unit weight of sample after correction for radioisotope decay. The mercury concentration in kidneys, liver and brain was corrected for the contribution of radioactivity in the residual blood in the tissues, using values of blood content in the brain given by Cremer and Seville (1983) and in liver and kidney by Zimmer and Carter (1978).

The proportion of mercuric mercury (HgII) in the tissues and gut of four of the nine animals in each group was determined by homogenisation of the tissues in deionised water, using an Ultraturrax tissue grinder and extraction of 1 ml of the homogenate with benzene as described by Cappon and Smith (1977). The benzene extractable radioactivity was termed organic mercury, the non-extractable portion, inorganic or mercuric mercury.

Statistical analysis

The rates of mercury elimination obtained by least squares regression analysis were compared as described in Geigy Scientific Tables (1982). The half-times of elimination and their standard errors were calculated as described by Snedecor and Cochran (1967). Mercury concentrations and proportions of inorganic mercury in tissues and gut were subjected to analysis of variance using the Minitab Statistical Package (Minitab Inc., Pennsylvania, USA), and values compared for significance using the least significant difference criterion (Snedecor and Cochran 1967).

Results

The presence of 5% cellulose in the basal diet slightly increased mercury retention in the BALC/c mice (Fig. 1, Table 1). In contrast, the feeding of diets containing 15 or 30% bran significantly lowered the retention of mercury, decreasing the half-time of elimination by approximately 43% (Fig. 1, Table 1). The feeding of the diet containing 5% pectin had no effect on mercury elimination.

Mice fed the cellulose diet had higher total mercury concentrations than animals fed the basal fibre-free diet in all tissues studied, but this trend was statistically significant only for the kidney (Table 2). Dietary pectin had no significant effects on mercury concentrations in tissues or gut (Table 2). The incorporation of bran, especially at the 30% level, into the basal diet generally decreased the mercury concentration in the tissues particularly in the blood small intestine and, highly significantly, in the brain where feeding of 30% bran was associated with a decrease of 24% in mercury concentration (Table 2). The amount of mercuric mercury present in the tissues is shown as a concentration (Table 3) and as a percentage of the total mercury in

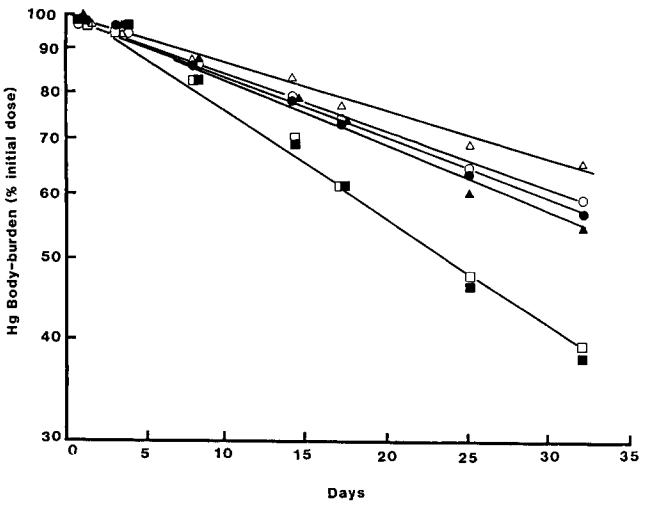

Fig. 1. Body burden of mercury (expressed as % of initial dose) in groups of male BALB/c mice given single oral doses of $Me^{203}HgCl$ (5.0 mgHg/kg). Values shown are the means for four animals. The mice were fed a basal fibre-free diet (○) or that diet containg 5% (●), 5% cellulose (△), 5% wheat bran (▲), 15% wheat bran (□) or 30% wheat bran (■)

Table 1. Rate and half-time of mercury elmination in BALB/c mice

Diet	Rate of Hg elimination (log % decrease in ^{203}Hg/day)	$T_{1/2}$ (days)
Control	−0.0165 ± 0.0006	41.3
5% Pectin	−0.0182 ± 0.0007	38.3
5% Cellulose	−0.0134 ± 0.0005	50.8
5% Bran	−0.0200 ± 0.0007*	35.3
15% Bran	−0.0301 ± 0.0011***	23.9
30% Bran	−0.0312 ± 0.0018***	23.3

The rate of Hg elimination is given as the slope ± SD of the line in Fig. 1 calculated by least squares regression analysis of the logarithmically transformed data. *Asterisks* denote that a slope differs significantly from that of the control; * $P<0.05$; *** $P<0.001$. Half-times of elimination of mercury were calculated from the formula for the line of the graphs

Table 2. Mercury concentration in tissues and gut of BALB/c mice given MeHgCl

Tissue/gut	Mercury concentration (μg/g tissue or gut)					
	Control (fibre-free)	5% Pectin	5% Cellulose	5% Bran	15% Bran	30% Bran
Blood	9.24	9.59	9.99	9.64	8.41	7.26**
Liver	13.99	13.88	15.33	14.60	13.50	12.60
Kidney	77.06	79.82	90.19*	89.88*	86.23	82.39
Brain	4.92	4.97	5.34	4.60	4.35	3.76***
Small intestine (wall & contents)	10.05	8.88	11.25	10.13	8.97	8.53*
Large intestine (wall & contents)	5.43	4.95	5.50	5.75	5.18	4.94

Mice (nine in each dietary group) were given a single *PO* dose of Me ^{203}Hg Cl (5 mg Hg/kg). Total Hg content of tissues and gut contents was determined by γ-counting. Results shown as means of nine animals and were subjected to analysis of variance (Minitab statistical package). Values marked with asterisks differ significantly from the control values (*$P<0.05$; **$P<0.01$; ***$P<0.001$)

Table 3. Mercuric mercury concentration in tissues and gut of BALB/c mice fed different diets

Tissue/gut	HG (II) (μg/g tissue)					
	Control (fibre-free)	5% Pectin	5% Cellulose	5% Bran	15% Bran	30% Bran
Blood	0.19	0.19	0.29*	0.20	0.17	0.13
Liver	0.78	1.00*	0.96	0.93	0.93	1.01*
Kidney	6.93	7.47	8.35	9.01	8.46	8.95
Brain	0.18	0.20	0.19	0.13	0.14	0.16
Small intestine (wall & contents)	0.37	0.28*	0.47**	0.40	0.31	0.35
Large intestine (wall & contents)	1.30	1.39	2.10*	1.92	2.28*	2.61**

Mercuric mercury (HG II) content of the tissues was determined by the benzene extraction of Cappon and Smith (1977). Values shown are means of four mice and those marked with *asterisks* differ significantly from the control values (Analysis of variance; *$P<0.05$; **$P<0.01$)

Table 4. Percentage of mercuric mercury in tissues and gut of BALB/c mice fed different diets

Tissue/gut	HG (II) as % of total Hg					
	Control (fibre-free)	5% Pectin	5% Cellulose	5% Bran	15% Bran	30% Bran
Blood	2.08	2.05	2.67	2.18	2.51	2.23
Liver	5.75	7.33***	6.15	6.54*	8.31***	9.16***
Kidney	9.74	10.37	9.02	10.52	10.95*	11.37*
Brain	3.76	4.35*	3.36	3.38	4.13	4.58**
Small intestine (wall & contents)	3.89	3.69	3.84	4.58*	4.49*	4.68**
Large intestine (wall & contents)	25.62	29.26	38.29*	35.97*	49.75***	53.64***

Mercuric mercury (HG II) content of the tissues was determined by the benzene extraction of Cappon and Smith (1977). Values shown are means of four mice and those marked with *asterisks* differ significantly from the control values (Analysis of variance; *$P<0.05$; **$P<0.01$; ***$P<0.001$)

the tissue (Table 4). Relatively few significant differences in concentration of mercuric mercury were apparent in the tissues, although the concentrations in the liver and kidneys of the animals fed pectin, cellulose or bran were higher than in the mice fed the fibre-free control diet. The concentration of mercuric mercury in the large intestine of the 15 and 30% bran fed mice was significantly greater than that in the control animals (Table 3). Calculation of the percentage of total mercury in the tissues present in the mercuric form (Table 4) provides information on proportion of methylmercury converted to inorganic mercury in the body. In animals fed cellulose, the percentage of inor-

ganic mercury in all tissues and gut preparations was similar to the controls, whereas pectin feeding was associated with significantly increased proportions of mercuric mercury in the liver and brain (Table 4).

The percentage of mercury present in the mercuric form in the brains of control animals and those fed 5 or 15% bran diets was similar, but mice fed 30% bran had significantly higher proportions of inorganic mercury in the brain (Table 4). Although the concentration of total mercury in liver, kidney and small and large intestine was not markedly affected by bran feeding, the proportion of mercury present in the inorganic form in these sites was significantly higher than in control mice particularly in the large intestine, where the percentage of mercuric mercury in mice fed 15 or 30% bran was increased twofold (Table 4).

Discussion

The present study has demonstrated that the retention of mercury by mice after a single oral dose of MeHgCl can be modified by diet. In particular, the presence of wheat bran in the diet significantly decreased the body burden of total mercury over a period of 32 days.

It seems unlikely that the influence of bran is attributable to its cellulose component since, at 5% in the diet, it actually decreased slightly the rate of mercury loss in BALB/c mice. It is possible that the lignin component of bran was important in reducing the mercury body burden since lignins have been shown to bind metals in in vitro studies (Kiyozumi et al. 1982). The rates of change in body burden of mercury observed in the present study were slow by comparison to those reported previously by Landry et al. (1979) and Rowland et al. (1984), since those authors reported diet-related differences in half-times of mercury elimination of 5–35 days.

The relative body burdens of mercury in mice fed the different diets were not always reflected in the mercury concentration of the various tissues studied, although it should be noted that previous studies (e.g. Rowland et al. 1984) have shown that in MeHg-exposed animals, the majority of mercury is located in the carcass which was not sampled in the present investigation. However, lower mercury concentrations were found in the brains of mice fed 5, 15 or 30% bran diets although the values were statistically significant ($p < 0.001$) only in animals fed the 30% bran diet. Again, the cellulose and pectin components were presumably not involved in the effect of bran on brain mercury concentration, especially since the feeding of cellulose was associated with an increase in the concentration of mercury in brain. The results imply that the toxicity of methylmercury, which is largely directed against the central nervous system, could be reduced by dietary wheat bran. A decrease in mercury concentration of this magnitude (24% in animals fed 30% wheat bran) has been shown to result in reduced incidence and severity of symptoms of methylmercury neurotoxicity in rats (Rowland et al. 1980).

Several mechanisms may be proposed to account for the effect of bran on methylmercury retention. It seems probable that certain components of bran can bind or adsorb mercury and so reduce its absorption from the gut. This has been proposed as a mechanism by Kostial et al. (1981) for the effects of some dietary components on inorganic mercury retention and by Kiyozumi (1982) to explain the effects of cellulose, pectin and lignin on cadmium absorption. Mercury absorption may also be decreased by a reduction in active transport sites in the intestine, a mechanism which has been proposed to explain fibre-related changes in intestinal fluxes of sodium and chloride ions (Schwartz et al. 1982). It is also possible that a bran-induced decrease in intestinal transit time may be responsible for the increased rate of elimination of mercury in animals fed the dietary fibre. A further mechanism which has been proposed to govern the rate of excretion of mercury after MeHgCl exposure is demethylation, the main site of which appears to be the gastrointestinal microflora (Seko et al. 1981; Rowland et al. 1984). Demethylation of methylmercury (either ingested or secreted into the gut via bile) converts it from the organic form, which is rapidly absorbed, to one (HgII) which is very poorly absorbed from the gut (Miettinen 1973), thus effectively increasing the rate of excretion of mercury. The mice fed diets containing wheat bran had a significantly higher proportion of mercuric mercury in the large intestine than the controls, thus lending support to the theory that increased bacterial demethylation is important in determining the higher rates of mercury excretion in bran-fed animals. As might be expected under these conditions, tissues which normally do not accumulate mercuric mercury, e.g. brain and blood, had similar levels of this form of mercury in bran-fed and control animals. In contrast, organs such as the liver and especially the kidney, which preferentially take up mercuric mercury, were found to contain a higher proportion of inorganic mercury in the bran-fed animals. Although methylmercury metabolism by the gut microflora of bran-fed animals has not been studied in vitro, it is known that rates of other microbially mediated reaction are raised by feeding bran to rats (Mallett et al. 1986). It seems possible therefore that wheat bran decreased mercury retention after methylmercury exposure mainly by increasing the rate of demethylation of the organomercurial in the gut.

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