Direct Evidence for the Presence of Methylmercury Bound in the Thyroid and Other Organs Obtained from Mice Given Methylmercury; Differentiation of Free and Bound Methylmercuries in Biological Materials Determined by Volatility of Methylmercury

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Peroxidase in mouse thyroid was inhibited by mercuric chloride but not by methylmercury in *in vivo* and *in vitro* systems (Nishida, *et al.*, *J. Histochem. Cytochem.*, 37, 723 (1989)). To identify the reason for the difference, the present study was conducted to examine whether methylmercury is indeed bound within cells or tissues. Mice were given radioactive methylmercury by intubation for 18 d and the tissues were dissected out and vacuum-dried. With this procedure, free methylmercury was evaporated off and the bound mercury remained. The thyroid, liver, kidney and fats examined showed no loss of radioactivity under the vacuum, indicating that the mercury was bound to the thyroid, as well as the other tissues. Radioactive mercuric chloride was nonvolatile regardless of the presence or absence of the tissues. The preferential affinity of methylmercury for SH-containing materials was re-confirmed by this method.

Keywords methylmercury; mercuric chloride; thyroid; thyroid peroxidase; mercury binding; SH-compound

In the previous report,[1] we showed that mouse thyroid peroxidase was substantially inhibited *in vivo* and *in vitro* by mercuric chloride, but not be methylmercury. To assess the effects of the mercurials on the peroxidase, it was necessary to determine whether or not methylmercury indeed exists in the cells or tissues. The metabolic fate of methylmercury has been discussed by many authors[2-4] and the current understanding is that demethylation gradually occurs in bacteria[5] and animal tissues,[6,7] although little is known about the metabolic fate of methylmercury in the thyroid. It has also been reported that methylmercury is the least sensitive to demethylation of organic mercurials.[4,8] Therefore, it is necessary to ascertain that methylmercury is present in the thyroid before concluding that methylmercury does not inhibit thyroid peroxidase. For this purpose, we made use of the volatility of methylmercury under vacuum. In our method, free methylmercury is evaporated off, but methylmercury bound to biological materials is not.

Materials and Methods

Mercurial compounds and amino acids were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Bovine serum albumin (BSA), human serum albumin, bovine serum globulin mixture and boving thyroglobulin were products of Sigma Chemical Co. (St. Louis, U.S.A.). Oxidized and reduced forms of glutathione were from Boehringer Mannheim GmbH (W. Germany) and Kohjin Co., Ltd. (Tokyo, Japan), respectively. Tissue solubilizer for liquid scintillation counting was obtained from Amersham Corp. (Ill. U.S.A.). [^{14}C]CH_3HgCl (0.013 mCi/g) and [^{203}Hg]$HgCl_2$ (0.10 Ci/g) were from New England Nuclear (MA, U.S.A.) and Amersham Corp (Ill., U.S.A.), respectively.

ddY Male mice weighing 25—27 g were given 1 μCi of either [^{14}C]CH_3HgCl or [^{203}Hg]$HgCl_2$ by intubation for 18 d. The period of the treatment was determined from the result of a preliminary test as the period required for the onset of ataxial dysfunction with CH_3HgCl in mice. At autopsy, animals were injected with 100 U of heparin, then lightly anesthetized with ether. The thorax was opened and a cannula was inserted into the arch of the aorta *via* the left ventricle. Pre-warmed physiological saline containing 10 U/ml of heparin was injected through the cannula. The pressure was released by cutting the right atrium and the blood was eliminated. Portions of selected tissues were quickly removed, placed in a vial and put under vacuum in a desiccator connected to a trap cooled with dry ice–acetone, and to a vacuum pump. The samples were completely dried under vacuum, then aliquots of water and two volumes of tissue solubilizer were added and incubated overnight at 37 °C. The remaining radioactivity was measured on an Aloka liquid scintillation counter. The counting efficiency was calculated by using an external standard device and the activity was expressed in dpm. The result was expressed as the ratio of the radioactivity in a vacuum-dried sample to that of the unvacuumed sample. Specimens from animals treated with [^{203}Hg]$HgCl_2$ were also dried under vacuum and directly measured for gamma radioactivity on an automated well-type gamma scintillation counter with a counting efficiency of 55%. The same procedure as that used in animal tissues was applied to study the binding with amino acids, purified proteins and isolated thyroid epithelial cells. Isolation of porcine thyroid epithelial cells was carried out by the method described previously.[9]

Results and Discussion

Before the present procedure was applied to animal tissues, its reliability was tested on biological materials of simpler structures such as amino acids, proteins and isolated cells. [^{14}C]CH_3HgCl in solution was fairly stable but when it was dried under vacuum, the redioactivity was lost. When the mercury reacted with biological materials, the radioactivity remained in residues after the solution was dried. Cysteine completely prevented the loss of the radioactivity. Cystine (61% of the applied radioactivity), reduced glutathione (76%) and tyrosine (34%) retained the radioactivity in part; other amino acids did not react with the mercury. BSA retained 26% of the radioactivity of [^{14}C]CH_3HgCl but human serum albumin (3.5%), globulin mixture (3.3%) and thyroglobulin (4.5%) retained very little radioactivity of [^{14}C]CH_3HgCl under vacuum. Thus, purified proteins do not bind much with mercury except for BSA. This is presumably due to a low content of amino acids having high affinity for mercury in these proteins. In the isolated cells, however, 80% of [^{14}C]CH_3HgCl was retained. [^{203}Hg]$HgCl_2$ was also tested with the same materials, but no radioactivity was lost under vacuum.

Table I summarizes the results on the thyroid, brain, liver, kidney and fat from mice given [^{14}C]CH_3HgCl or [^{203}Hg]$HgCl_2$. Vacuum-treated specimens of the thyroid as well as other tissues did not lose radioactivity. The results indicate that methylmercury in the thyroid, liver and some other tissues of the mouse was retained as methylmercury. Whether or not free methylmercury inside cells can evaporate through the biological structures of the cells is not always clear. However, after tissues containing [^{14}C]-CH_3HgCl, as well as isolated cells, were dried to steady

TABLE I. Remaining Radioactive Mercury in Tissues of Mice under Vacuum

Tissues	No. of animals	$^{14}CH_3HgCl$			$^{203}HgCl_2$		
		Control (a) (dpm/mg)	Vacuum-treated (b) (dpm/mg)	Ratio (b/a)	Control (a) (dpm/mg)	Vacuum-treated (b) (dpm/mg)	Ratio (b/a)
Brain	6	136± 44	143± 46	1.06±0.11	20± 5	22± 2	1.16±0.28
Thyroid	4[a)]	86± 28	85± 17	1.03±0.21	47± 11	56± 14	1.17±0.04
Liver	6	219± 34	233± 56	1.02±0.11	180± 27	187± 26	1.04±0.03
Kidney	6	1795±365	1750±420	0.97±0.08	1975±252	2044±425	1.06±0.14
Fat	6	51± 16	52± 10	1.06±0.20	12± 3	19± 17	1.10±0.29

a) Number of specimens for thyroid represents the number of vials. Since an individual specimen was not sufficient to measure the radioactivity, thyroids from 3—4 animals were pooled in each vial. Data are expressed as mean±S.E.

levels of radioactivity, the remaining activity was unchanged by further repeated vacuum treatments, indicating that the residual materials no longer contained free radioactivity. Thus, we consider that free $[^{14}C]CH_3HgCl$ and free $[^{14}C]CH_3$-residue, if any, are completely eliminated and the remaining radioactivity represents the radioactive mercury bound to components having -SH or other groups of high affinity. It appears that after oral administration to mice, most methylmercury binds to tissues, and very little free methylmercury remains. This simple method is very useful to examine the ratio of free and bound methylmercury in biological materials. However, it should be noted that this procedure would not be adequate to differentiate primary binding and translocation of $[^{14}C]CH_3$-residue, if it occurs in tissues, because if the radioactive methyl group is transferred to an other location in tissues, there would be no detectable change in radioactivity in a dried sample.

As stated before, peroxidase in the thyroid gland was not inhibited by the administration of methylmercury.[1] The present study shows that methylmercury is indeed present in the thyroid, so the lack of susceptibility of the enzyme to methylmercury is not due to the absence or decomposition of methylmercury but is due to the inability of methylmercury to inhibit the enzyme.

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