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Carbon–Mercury Bond Cleavage in Blood of Rats Fed Methyl Mercuric Chloride[1,2,3] (38044)

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The release of inorganic mercury in animals after exposure to methyl mercuric salts is one of the important biotransformation reactions that may determine the degree of toxicity (1). Norseth and Clarkson (2) reported that as much as 50% of the mercury in feces was inorganic when methyl mercury chloride was injected into rats. Similarly, an appreciable proportion of inorganic mercury was found in kidney and other organs (1–6) of rats after an exposure to organic mercury salts. A small but detectable amount of inorganic mercury was also found in the bile (3, 4) and gastrointestinal tract (2–5) of rats after organomercurial intoxications.

The biotransformation of organomercury to inorganic mercury is also affected by the level of mercurial incorporation and its release from the target tissue and by the rate of splitting of the carbon–mercury bond (4). Researchers have used radioisotope dilution technique (1–4, 7), measurement of expired $^{14}CO_2$ (6, 8), double-label method (6), and the determination of extractable organic and total mercury in the tissues (5, 6, 9, 11, 12) to estimate the C–Hg bond split.

In order to obtain more insight regarding the stability of the carbon–mercury bond of methyl mercuric chloride, experiments were designed to quantitate the breakage of the bond in blood of rats fed tracer doses of $^{14}CH_3{}^{203}HgCl$. Since one of the factors that may influence the rate of the split is the rate of incorporation and release of the mercury compound from the tissue, the blood from the rat was fractionated into several components and ^{14}C and ^{203}Hg activities determined.

Procedures. Our working hypothesis is that, if the $^{14}CH_3{}^{203}HgCl$ remains intact after feeding to rats, the tissue ^{14}C to ^{203}Hg activities should have the same ratio as that in the original compound fed. If there is a breakage of the carbon–mercury bond, the ratio will probably change and should indicate the magnitude of the split unless the ^{14}C and the ^{203}Hg are deposited in tissues in the same proportion as the original compound. In this case one would be unable to predict from this ratio the breakage even if it should occur.

A differential deposition in comparable tissues after administration of single-label methyl mercuric chloride (^{14}C labeled or ^{203}Hg labeled) should also indicate breakage of the bond. These two hypotheses were tested by using mature female Sprague-Dawley rats averaging 373 g body weight. These rats were divided into three equal groups of 3 each and were force-fed either $^{14}CH_3HgCl$ or $CH_3{}^{203}HgCl$ or $^{14}CH_3{}^{203}HgCl$. The double-labeled compound was prepared by mixing an aliquot of $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$. Force-feeding was done by stomach tubing of the methyl mercuric chloride dissolved in corn oil so that 1 g contained the predetermined radioactivities.[2]

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[2] 30 μCi $^{14}CH_3HgCl$ or 30 μCi $CH_3{}^{203}HgCl$ or 30 μCi $^{14}CH_3{}^{203}HgCl$.

[3] $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$ were purchased from New England Nuclear, Boston, Massachusetts.

The specific activity of the $^{14}CH_3HgCl$ was 7.5 μCi/mg Hg while $CH_3{}^{203}HgCl$ was 3030 μCi/mg Hg.[3]

Twenty four hours after force-feeding, blood from each rat was obtained by heart punctures and heparinized. A small portion of the heparinized blood was used for hematocrit determinations and the remainder centrifuged at 2000 rpm for 15 min to separate the red blood cells and plasma. The red blood cells were washed three times with a 1.2% NaCl solution each time centrifuging at 2000 rpm for 10 min. To hemolyze the washed red blood cells 10 parts of distilled water were added for each part of erythrocytes. CO_2 was bubbled into the red blood cell solution to dissolve any precipitated hemoglobin (13). The red blood cell solution was centrifuged at 17,000*g* for 10 min to separate red blood cell ghost (cell walls) and hemoglobin. The hemolyzing procedure was repeated three times and the hemoglobin fractions were pooled after each of the centrifugation procedures.

Total plasma fat was obtained according to the method of Folch *et al.* (14). Plasma protein fractions were isolated by means of centrifuging plasma at 1000*g* for 30 min in membrane ultrafilters.[4] The fraction retained in the filter has mol wt of 50,000 and above and was considered the protein fraction. The ultrafiltrate was considered to be the nonprotein fraction. The ultrafiltration method was chosen because we anticipate other methods to change the physical properties of the protein and thus may alter their original affinity for mercury.

^{14}C radioactivities in the fractions were counted by a Nuclear Chicago Scintillation Counter whereas ^{203}Hg radioactivities were counted by a Nuclear Chicago Gamma Counter. Samples containing both ^{14}C and ^{203}Hg were first counted in the scintillation counter to determine the total activities from both sources and then for ^{203}Hg activities with the gamma counter. The difference in activity between the scintillation and gamma counts was considered to be ^{14}C activity. Standards prepared with either $^{14}CH_3HgCl$ or $CH_3{}^{203}HgCl$ or $^{14}CH_3{}^{203}HgCl$ were used to calculate the radioactivities of the samples. Sample radioactivities were also corrected for background and physical decay. A statistical procedure was followed so that sample activities were determined to an accuracy of plus or minus 2% (15).

Results and Discussion. Cleavage of the carbon–mercury bond of methyl mercuric chloride for different blood fractions was proven with the use of the double-labeled methyl mercuric chloride (Table I). In blood and its fractions, 5.1–10.6% of the carbon to mercury bond breakage occurred. The extent of carbon–mercury bond breakage for the different blood fractions in increasing order were as follows: nonprotein fraction, trace; plasma fat, 5.1%; hemoglobin, 5.8%; red blood cell ghost, 6.1%; plasma, 7.3%; whole blood, 8.6% and red blood cells, 10.6% (Table I). These data indicate that the extent of the carbon–mercury bond breakage was quantitatively small and is in agreement with that reported by others (5, 6). Greater magnitude of split was reported for aryl (4, 9, 10, 11, 16, 17), and alkoxyalkyl (7, 18) mercurial compounds.

Further evidence to support conclusion of a carbon to mercury bond cleavage in rats came from comparisons of the uptake of ^{14}C and ^{203}Hg by blood and blood fractions (Table II). In the double-label experiment, the mean percent of dose/g of whole blood, red blood cells, hemoglobin or red blood cell ghost was statistically different between ^{14}C and ^{203}Hg. These data also suggest that the deposition in tissues was disproportional for ^{14}C and ^{203}Hg after breakage. Blood fractions that contained lower concentrations of ^{14}C or ^{203}Hg were not statistically different from each other for their respective fraction. Thus, the distribution was not statistically different for ^{14}C and ^{203}Hg in plasma and plasma fractions (Table II). In the single-label experiment wherein the ^{14}C labeled methyl mercury was fed to one group of rats and the ^{203}Hg compound was fed to another group, the only statistical difference occurred in the percent of dose per gram of hemoglobin.

[4] Membrane ultrafilters were purchased from Amicon Corporation, Lexington, Massachusetts.

TABLE I. Proportion of ^{14}C and ^{203}Hg Radioactivities and the Percentage of Carbon–Mercury Bond Breakage in Blood and Blood Fractions. The Proportional Values Presented in This Table Should Be Compared to the Proportions of ^{14}C and ^{203}Hg Radioactivities Which Were 65.4% and 34.6% Respectively of the Double-Labeled Methyl Mercuric Chloride Fed to the Rat.

Blood and blood fractions	Proportion of ^{14}C and ^{203}Hg radioactivities[a]		C–Hg bond split
	^{14}C (%)	^{203}Hg (%)	(%)
Whole blood	56.8 ± 1.5	43.2 ± 1.1	8.6 ± 0.2
Red blood cells	54.8 ± 3.2	45.2 ± 2.6	10.6 ± 0.6
Hemoglobin	59.6 ± 0.4	40.4 ± 0.3	5.8 ± 0.1
Red blood cell ghost	59.3 ± 4.5	40.7 ± 3.7	6.1 ± 0.6
Plasma	58.1 ± 0.8	41.9 ± 0.6	7.3 ± 0.1
Plasma fat	70.5 ± 12.2	29.5 ± 5.1	5.1 ± 0.9
Plasma protein fraction	57.4 ± 1.5	42.6 ± 1.1	8.0 ± 0.2
Nonprotein fraction	Trace[b]	Trace	—

[a] Each value represents a mean of three observations ± SE.
[b] Almost equal to background count.

Other blood fractions were not significantly different for the two groups of rats. Nevertheless, the differential deposition of ^{14}C and ^{203}Hg in the hemoglobin confirmed the cleavage of the carbon–mercury bond.

The studies also confirmed early findings that mercurial compounds are largely bound to the hemoglobin (9). The affinity of mercury for the thiol groups of hemoglobin has already been documented. Our data further show that very little mercury was in the nonprotein fraction of plasma and that plasma fat contained a higher concentration of radioactivities than plasma protein. The higher concentration may be due to the lipid solubility of methyl mercury.

Generally, our data indicate that around 99% of the ^{14}C or ^{203}Hg radioactivities of

TABLE II. Percentage of Dose Per Gram of Blood or Blood Component of Rats One Day After Force-Feeding Single- or Double-Labeled Methyl Mercuric Chloride.

Blood and blood fractions	Percent of dose/gram[a]			
	Double-labeled CH_3HgCl		Single-labeled CH_3HgCl	
	^{14}C	^{203}Hg	^{14}C	^{203}Hg
Whole blood	1.76 ± 0.03	2.53 ± 0.08[b]	1.89 ± 0.09	1.50 ± 0.03
Red blood cells	3.75 ± 0.53	5.95 ± 0.12[b]	3.48 ± 0.36	2.37 ± 0.76
Hemoglobin	5.19 ± 0.32	6.71 ± 0.43[c]	5.09 ± 0.19	3.46 ± 0.02[b]
Red blood cell ghost	1.78 ± 0.29	2.86 ± 0.09[b]	2.11 ± 0.05	1.50 ± 0.06
Plasma	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Plasma fat	0.25 ± 0.02	0.20 ± 0.04	0.15 ± 0.08	0.05 ± 0.01
Plasma protein	0.06 ± 0.03	0.08 ± 0.02	0.06 ± 0.01	0.03 ± 0.00
Nonprotein fraction	Trace	Trace	Trace	Trace

[a] Each value represents a mean of three observations ± SE.
[b] Significant mean difference between ^{14}C and ^{203}Hg at $P < 0.05$.
[c] Significant mean difference between ^{14}C and ^{203}Hg at $P < 0.01$.

TABLE III. Percentage Distribution of ^{14}C and ^{203}Hg Radioactivities in Blood Fractions of Rat One Day After Force-Feeding Single- or Double-Labeled Methyl Mercuric Chloride.

Blood fractions	Double-labeled CH_3HgCl		Single-labeled CH_3HgCl	
	^{14}C	^{203}Hg	^{14}C	^{203}Hg
Hemoglobin	98.3 ± 0.5[a]	98.3 ± 0.5	99.0 ± 0.3	98.8 ± 0.1
Red blood cell ghost	0.9 ± 0.3	1.0 ± 0.3	0.4 ± 0.1	0.7 ± 0.1
Plasma fat	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Plasma protein fraction	0.7 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.4 ± 0.0
Plasma nonprotein fraction	Trace	Trace	Trace	Trace
Total	100.0	100.0	100.0	100.0

[a] Each value represents a mean of three observations ± SE.

the methyl mercuric chloride in the blood is deposited in red blood cells one day after the rats were force-fed the compound (Table III). In the red blood cells, nearly 99% of the radioactivities was found in the hemoglobin fraction. Among the plasma fractions, plasma fat had the highest concentration of radioactivities. However, the radioactivity of the plasma fat is only 0.1% of the whole blood radioactivities. On the other hand, plasma protein fraction constitutes around 92% of the plasma fraction radioactivity and 0.7% of the whole blood.

Summary. Methyl mercuric chloride labeled with ^{14}C or ^{203}Hg or both were force-fed to rats in order to quantitatively determine breakage of the carbon–mercury bond in different fractions of blood. Based on the hypothesis that a change in the ratio of ^{14}C to ^{203}Hg radioactivities in the tissue from that in the compound fed to rats would indicate breakage, values ranging from 5.1% to 10.6% were observed depending upon the blood fraction studied.

Since the site of deposition of mercurial compounds is important in determining toxicities, the concentrations of either ^{14}C or ^{203}Hg radioactivities in blood fractions were determined after force-feeding the single or double-labeled methyl mercuric chloride. One day after force-feeding, the highest concentration in the erythrocytes was in the hemoglobin. In the plasma, fat contained the highest concentration. However, plasma fat radioactivities constitute only 0.1% of the whole blood radioactivities whereas plasma protein radioactivities constitute 0.7%. Nearly 99% of blood radioactivities was in the hemoglobin.

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