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Carbon–Mercury Bond Breakage in Milk, Cerebrum, Liver, and Kidney of Rats Fed Methyl Mercuric Chloride[1] (38067)

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Transfer of radioactivities from lactating rats to their nursing pups was demonstrated by Yang *et al.* (1) when the lactating rats were force-fed ^{203}Hg-labeled methyl mercuric chloride. Similarly, Baltrukiewicz (2) has found that mercury is transmitted to neonatal rats after an intravenous injection of neohydrin to lactating mothers. Studies in lactating women (3, 4), goats (5), cows (6) and guinea pigs (7) also indicated a mammary transmission of mercury and in mice (8), an appreciable quantity of mercury was detected in mammary tissues after exposure to mercuric chloride.

Whether the transfer in milk is the original compound given to the mother or its biotransformed products is not known. The degree of biotransformation is to a large extent dependent upon the breakage of the carbon to mercury bond in the case of the alkyl mercurial compounds. This investigation was initiated to study carbon-mercury bond breakages in the milk, milk fractions and several tissues of rats after force-feeding methyl mercuric chloride. Furthermore, since no report could be found on the distribution of mercury in milk components after alkyl mercurial intoxications, this was also studied.

Procedures. Lactating Sprague-Dawley rats each nursing 10 pups were divided into three groups of 3 each during the 16th day of lactation. The lactating rats in each group were force-fed a dose of differently labeled methyl mercuric chloride[2] via stomach tube. The different radioactive tracer compounds and dosages used were 30 μCi $^{14}CH_3HgCl$, 30 μCi $CH_3{}^{203}HgCl$ or 30 μCi $^{14}CH_3{}^{203}HgCl$ for the first, second and third group, respectively.

After force-feeding the different radioactive tracers, the dams were returned to their own pups. On the 17th day, a day after force-feeding, mothers were separated from their pups for 5 hr in order to allow milk to accumulate in the gland. At this time, milk was obtained using a rat milking machine adapted from Feller and Boretos (9) 1 min after an intramuscular injection of 1 unit oxytocin.

Part of the milk obtained from the rat was fractionated into fat, whey and casein according to the method of Bohren and Wenner (10). The milk fat obtained from the fractionation was further purified using the Folch *et al.* (11) method which was repeated 3 times in order to remove traces of contamination. Casein pellets from the fractionation were purified by washing the pellet three times with alcohol-carbon tetrachloride mixture (4:1) and then 3 times with ether.

After milking, the dam was killed and cerebrum, liver and kidney were saved for radioactive determination. For this purpose, each tissue was first homogenized with deionized water and representative samples digested with hyamine hydroxide. ^{14}C radioactivities of the digest were counted in a Nuclear Chicago Scintillation Counter and

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[2] $^{14}CH_3HgCl$ and $CH_3{}^{203}HgCl$ were purchased from New England Nuclear, Boston, Massachusetts.

TABLE I. The Magnitude of Carbon–Mercury Bond Breakage in Milk and Milk Fractions and Tissues of Rat One Day After Force-Feeding $^{14}CH_3$ $^{203}HgCl$. Table Proportional Values Should be Compared to the Proportional Values of 65.4 and 34.6 for ^{14}C and ^{203}Hg of $^{14}CH_3$ $^{203}HgCl$ Fed to the Rat.

Samples	Proportion		Breakage (%)
	^{14}C (%)	^{203}Hg (%)	
Whole milk	69.9 ± 18.3[a]	30.1 ± 7.9	4.5 ± 1.2
Milk fractions			
Whey	64.6 ± 6.8	35.4 ± 3.7	0.8 ± 0.1
Fat	97.8 ± 26.7	2.2 ± 0.6	32.4 ± 8.8
Casein	76.4 ± 12.6	23.6 ± 3.9	11.0 ± 1.8
Cerebrum	59.2 ± 0.7	40.8 ± 0.5	6.2 ± 0.1
Liver	59.2 ± 1.7	40.8 ± 1.2	6.2 ± 0.2
Kidney	57.4 ± 0.7	42.6 ± 0.5	8.0 ± 0.1

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

^{203}Hg radioactivities were counted in a Nuclear Chicago Gamma Counter. The magnitude of the carbon–mercury bond breakage was determined by the change in the proportion of ^{14}C to ^{203}Hg radioactivities in the milk, milk fractions and tissues from the proportion of the ^{14}C and ^{203}Hg radioactivities of the original compound. The principles of double-label technique and measurement of the magnitude of carbon to mercury bond split have been used successfully in previous studies (12).

Results and Discussion. Based on the change in proportion of ^{14}C to ^{203}Hg radioactivities in milk from that in the double-labeled methyl mercuric chloride, there was an average of 4.5% bond breakage (Table I). The bond breakage occurred mainly in the fat and casein fractions wherein there is a disproportionately greater ^{14}C radioactivity than in the original compound. Breakages of the carbon to mercury bond also occurred in cerebrum, 6.2%; liver, 6.2%; and kidney, 8.0% (Table I).

Disproportionate concentrations of ^{14}C and ^{203}Hg in the milk and tissues studied, may also indicate breakage of the bond. In particular, concentrations of the ^{14}C and ^{203}Hg were statistically different in cerebrum, liver and kidney (Table II). The breakage

TABLE II. Concentrations of ^{14}C and ^{203}Hg in Milk and Milk Fractions and Tissues of Rat One Day After Force-Feeding Single- or Double-Labeled Methyl Mercuric Chloride.

Samples	Percent of dose × 10^{-1} per gram			
	Double labeled CH_3HgCl		Single labeled CH_3HgCl	
	^{14}C	^{203}Hg	^{14}C	^{203}Hg
Whole milk	0.22 ± 0.04[a]	0.18 ± 0.04	0.33 ± 0.17	0.15 ± 0.03
Milk fractions				
Whey	0.06 ± 0.00	0.06 ± 0.01	0.08 ± 0.03	0.11 ± 0.03
Fat	0.56 ± 0.24	0.02 ± 0.00	0.98 ± 0.30	0.02 ± 0.01
Casein	0.64 ± 0.13	0.35 ± 0.04	0.81 ± 0.06	0.42 ± 0.11
Cerebrum	0.73 ± 0.06[b]	0.95 ± 0.07	0.83 ± 0.09	0.58 ± 0.03
Liver	5.25 ± 0.35[b]	6.82 ± 0.19	4.84 ± 0.33	4.72 ± 0.97
Kidney	11.26 ± 1.05[b]	15.91 ± 1.84	11.59 ± 1.71	9.39 ± 0.81

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

[b] Significant mean difference between ^{14}C and ^{203}Hg within group at $p < 0.05$.

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

	Double labeled CH_3HgCl		Single labeled CH_3HgCl	
Milk fractions	^{14}C	^{203}Hg	^{14}C	^{203}Hg
Whey	21.5 ± 3.5[a]	55.1 ± 1.2	23.0 ± 7.4	88.7 ± 0.2
Fat	46.2 ± 12.0	3.9 ± 0.4	40.5 ± 12.4	2.4 ± 1.1
Casein	32.3 ± 9.5	41.0 ± 1.5	36.5 ± 6.2	8.9 ± 1.0
Total	100.0	100.0	100.0	100.0

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

of the bond based on these indirect evidences agrees with that found in previous studies of blood and blood fractions of rats fed the same compound (12).

In milk, the fat contained 46.2% of the ^{14}C radioactivity while casein and whey contained 32.3% and 21.5%, respectively (Table III). On the other hand, 55.1% of ^{203}Hg radioactivities was deposited in the whey fraction. Only 3.9% ^{203}Hg activities was in fat and 41.0% in casein. The data presented in Table III thus not only support the fact that carbon–mercury bond breaks but also a differential deposition of ^{14}C and ^{203}Hg after breakage in milk and milk fractions.

The concentration of radioactivities expressed as a percentage of the dose/g sample was highest in the kidney followed by liver and cerebrum (Table II). Other investigators have also found that the concentration of mercurial compounds is greater in the kidney than most other tissues (13, 14). In the milk, the predominant concentration of radioactivities was either in the casein or in the fat (Table II). This is not surprising since it is known that many mercurial compounds including methyl mercuric chloride will attach to proteins especially those containing thiols and cysteine (15–17). The high radioactivity in the milk fat is also expected since methyl mercuric chloride is fat soluble (Table II).

The radioactivities found in the milk and milk fractions were several magnitudes lower than those found in blood and blood fractions of previous studies (12). For example, the percent of dose of $^{14}CH_3HgCl$ in one gram of blood was 1.76 compared to 0.02 in one gram milk. These findings, thus further substantiate our contention that the mammary gland is an effective barrier to transmission of methyl mercury chloride from mother to their nursing pups (1). Deshimaru (18) however, has observed neurological lesions in pup brains when milk was the only source of mercurial intoxication. The dose given to the mother was large in this case. The toxicity produced in the nursing pups was likely caused by the high affinity of the immature brain of the pups for mercurial compounds.

Summary. A double-label technique was used to determine the carbon–mercury bond breakages in milk and milk fractions, cerebrum, liver and kidneys of rats one day after force-feeding double-labeled methyl mercuric chloride. Breakages of the carbon–mercury bond occurred mainly in the fat and casein fractions and not in the whey of milk. In the cerebrum, liver and kidney, the breakages of the bond were respectively 6.2%, 6.2%, and 8.0%. The ^{14}C and ^{203}Hg concentrations (percent of dose/g) were high in fat and casein fractions and low in whey fraction of milk.

The ^{14}C radioactivities in milk were distributed 46.2% in fat, 32.3% in casein and 21.5% in whey. For ^{203}Hg radioactivities in milk, distribution was 55.1% in whey, 41.0% in casein and 3.9% in fat. Of the three organs studied, the kidney contained the highest percent of dose/gram tissue whereas the cerebrum contained the lowest.

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