[Chem. Pharm. Bull. 23(8)1726—1732(1975)]

UDC 547.25.09 : 546.69.08 : 615.31.033.034

Toxicity of Organic Mercury Compounds. IV.[1] Metabolism and Excretion of Alkoxyethylmercury Compounds in Mice

Masanori Yonaha, Shunji Ishikura[2a] and Mitsuru Uchiyama[2b]

Faculty of Pharmaceutical Sciences, Tokyo University of Science[2a] and National Institute of Hygienic Sciences[2b]

(Received November 13, 1974)

Metabolism and excretion of alkoxyethylmercury compounds in mice were studied, and their gastrointestinal absorption was discussed.

The total mercury and the organic mercury in blood, liver, kidney, and excreta at time intervals after a single subcutaneous injection of methoxyethylmercury chloride (MEMC) and *n*-propoxyethylmercury chloride (*n*-PEMC) were determined. Initially, the greater part of mercury in the blood, liver, and kidney was found as organic forms, but thereafter the organic mercury rapidly decreased with decrease of the total mercury. During 120 hr, the ratio of excretion of mercury in urine/feces was 2/1 after injection of MEMC and 1/2 after injection of *n*-PEMC, and more than half of the mercury in urine were in organic forms. Mercury in the feces was not determined as the organic form and was a chemically stable compound. It is most likely that the fecal mercury is mercuric sulfide, since it was formed from inorganic mercury ion in the cecum.

Inorganic mercury was found in the gastric contents initially after oral administration of MEMC. The release of inorganic mercury in the stomach indicates a reason for the poorer gastrointestinal absorption of alkoxyethylmercury compounds.

Introduction

Short-chained alkylmercury compounds are known to produce the characteristic neurotoxicity and to act on the central nervous system and on sensory peripheral nerves. The nervous symptoms are also caused by continuous administration of a higher dose of methoxyethylmercury chloride to rats[3] or mice,[4] and the animals show simultaneous renal damage,[1,3] but the symptoms are not exerted by *n*-propoxyethylmercury chloride.[5]

The distribution and elimination of mercury after administration of methoxyethylmercury compounds to rats are different from those observed after the administration of methylmercury compounds, and are rather similar to those after the administration of inorganic mercury.[6] With a single subcutaneous injection of methoxyethylmercury chloride to rats, Daniel, *et al.*[7] reported that a greater part of the dose is rapidly broken down in the tissue with the half-life of about 1 day to yield ethylene and inorganic mercury, probably by non-enzymatic reaction.

There is no information on metabolism and elimination of longer chain alkoxyethylmercury compounds. It is important to investigate the metabolism and the elimination of alkoxyethylmercury compounds in relation to the toxicity.

The experiments described below deal with the study on absorption, metabolism, and excretion of alkoxyethylmercury compounds in mice.

---

1) Part III: M. Yonaha, S. Ishikura, and M. Uchiyama, *Chem. Pharm. Bull.* (Tokyo), **23**, 1718 (1975).
2) Location: *a) 12, Funagawara, Shinjuku, Tokyo; b) Kamiyoga-1-chome, Setagaya, Tokyo.*
3) K. Lehotzky and S. Bordas, *Med. Lavoro*, **59**, 241 (1968).
4) S. Ishikura, N. Inoue, and M. Yonaha, *Eisei Kagaku*, **17**, 33 (1971).
5) M. Yonaha, T. Nakamura, and S. Ishikura, *Eisei Kagaku*, **18**, 248 (1972).
6) U. Ulfvarson, *Int. Arch. Gewerbepath.*, **19**, 412 (1962).
7) J.W. Daniel, J.C. Gage, and P.A. Lefevre, *Biochem. J.*, **121**, 411 (1971).

Experimental

Animal Experiments——The following mercury compounds were used in the experiments; ethylmercury chloride (EMC), methoxyethylmercury chloride (MEMC), *n*-propoxyethylmercury chloride (*n*-PEMC), and mercury chloride (MC). Aqueous solutions of mercury compounds were administered to dd strain male mice (20—24 g) with dose described below. These compounds were obtained as described previously.[5] To study metabolism and excretion of alkoxyethylmercury compounds, a dose of 10 mg Hg/kg (as a solution of 400 μg Hg/ml) was administered subcutaneously or orally. To determine mercury content in blood after oral administration were given 5 mg Hg/kg to mice. Animals were fed Oriental diet MF (Oriental Yeast Co.) When urine and feces were needed to cellect separately, animals were transferred to individual metabolic cages.

Reaction with SH Compounds *in Vitro*——Phosphate buffers, pH 4.0, 5.0, 6.0, and 7.2 were prepared by the method of Daniel *et al.*[7] The reaction mixtures contained 1 mM cysteine or glutathione and 9.375 μM alkoxyethylmercury chlorides in 40 ml of 0.2 M phosphate buffer, were bubbled with nitrogen gas to expel oxygen, and then incubated at 37°.

Determination of Mercury——Total mercury was determined as preceding experiments,[5] and when the oxidative digestion of feces was insufficient, they were completely digested by several additions of 5 ml of hydrogen peroxide to the reaction mixture. Alkoxyethylmercury in organs and excreta was determined by the method previously reported.[1] The total amount of blood was taken as 8.3% of the body weight, and mercury contents were calculated as percent of dose in whole tissue and excreta. Organic and inorganic mercury in the gastrointestinal contents and in the reaction mixture *in vitro* were estimated by the Ishikura's method,[8] except that hydrochloric acid was omitted from the extraction procedure.

Results

Mercury Concentration in Blood after Oral Administration of Various Mercury Compounds

Concentration of mercury in the blood after oral administration of EMC, *n*-PEMC, MEMC, and MC are shown in Fig. 1. The peak of the concentration of mercury in the blood appeared 9 hr after administration of EMC, and 3 (MEMC) or 6 (*n*-PEMC) hr after administration of alkoxyethylmercury compounds, which showed a rapid decrease during 24 hr thereafter. The peak due to EMC was much higher than that due to alkoxyethylmercury compounds. The concentration of mercury in the blood after administration of MC was much lower than that brought by other mercury compounds, and fairly constant up to 24 hr. Such differences in concentrations of mercury in blood during the initial period may reveal some difference in gastrointestinal absorption rates of these mercury compounds.

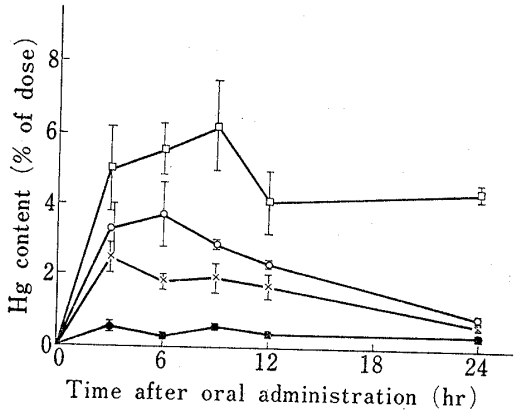

Fig. 1. Total Mercury Content in the Blood of Mice orally Administered with EMC, MEMC, *n*-PEMC and MC (5 mg Hg/kg)

Data for each point represent the mean ± SE of samples from three mice.
—□— :EMC —○— :MEMC, —×— :*n*-PEMC, —●— :MC

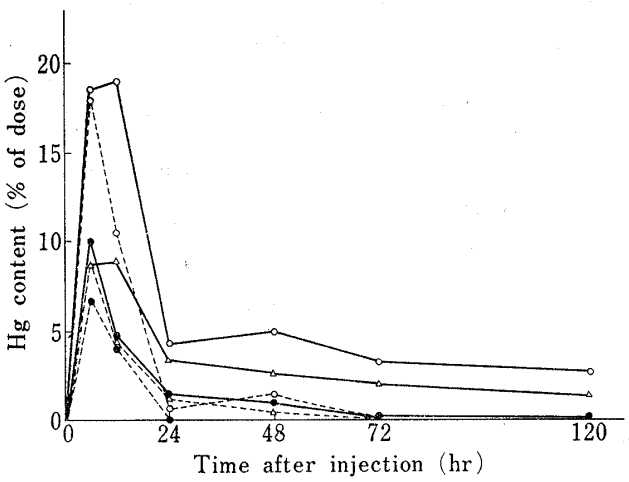

Fig. 2. Total and Organic Mercury Content in Organs of Mice Injected with MEMC (10 mgHg/kg)

△—△ :liver (total), △·····△ :liver (organic),
○—○ :kidney (total), ○·····○ :kidney (organic),
●—● :blood (total), ●·····● :blood (organic).

8) S. Ishikura and K. Yokota, *Chem. Pharm. Bull.* (Tokyo), **11**, 939 (1963).

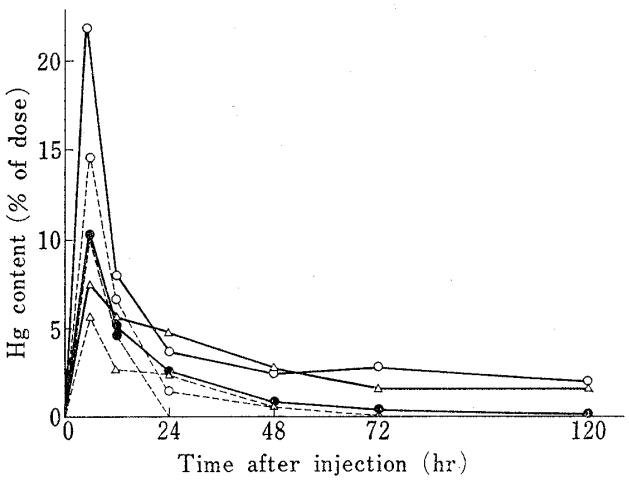

Fig. 3. Total and Organic Mercury Content in Organs of Mice Injected with *n*-PEMC (10 mg Hg/kg)

△—△ :liver (total), △·····△ :liver (organic),
○—○ :kidney (total), ○·····○ :kidney (organic),
●—● :blood (total). ●·····● :blood (organic)

Metabolism and Excretion of MEMC and *n*-PEMC

Time courses of total and organic mercury contents in liver, kidney, and blood after subcutaneous injection of MEMC and *n*-PEMC are given in Fig. 2, 3. The total mercury content was initially the highest in the kidney followed by blood, and liver in the decreasing order. The peaks of the total and organic mercury contents in blood after injection of MEMC and *n*-PEMC appeared at 6 hr, almost at the same time in the liver and kidney. The total mercury content in the liver and kidney had been high up to 12 hr after injection of MEMC, while low 12 hr after injection of *n*-PEMC. And the total mercury had appreciably decreased after 24 hr, and remained only 1—3% of the injected dose after 120 hr. High amounts of mercury in all the tissues analyzed were found initially as organic forms. The organic mercury content decreased with a decrease of the total mercury content. After 24 hr the organic mercury had not been determined in the blood, and in the liver and kidney after 72 hr.

The blood samples from mice injected subcutaneously with MEMC and *n*-PEMC were fractionated into plasma, stroma-free hemolyzate, and stroma, and the time course of mercury distribution in these fractions was investigated (Table I). There were no differences in distribution pattern of mercury between both compounds. Mercury in the blood distributed mainly in stroma-free hemolyzate, and the distribution in this fraction after a single injection is higher than that obtained after a long term exposure reported previously.[1)]

Total and organic mercury contents in urine and feces after a single subcutaneous injection and oral administration of MEMC and *n*-PEMC are listed in Tables II, III. During 120 hr after injection of MEMC, about 54% of mercury dosed appeared in urine and 27% in feces, while after injection of *n*-PEMC about 29% appeared in urine and 59% in feces. More than

Table I. Distribution of Mercury in the Blood Components of Mice Injected with MEMC and *n*-PEMC (10 mgHg/kg)

Time after injection (hr)	Distribution of mercury (%)		
	Plasma	Stroma free hemolyzate	Stroma
6	20.3 (20.9—19.7)	77.1 (76.7—77.4)	2.6 (2.4—2.9)
24	19.9 (16.8—22.9)	77.0 (79.0—75.0)	3.1 (4.2—2.1)
48	17.0 (14.4—19.6)	79.6 (80.8—78.5)	3.3 (4.8—1.9)
6	19.2 (20.4—17.9)	76.5 (75.1—77.6)	4.3 (4.3—4.3)
24	22.7 (24.7—20.6)	72.0 (69.3—74.7)	5.3 (5.9—4.7)
48	15.9 (18.6—13.3)	81.4 (77.1—85.7)	2.7 (4.3—1.0)

Each value represents the mean of samples from two mice and figures in parentheses indicate individual results.

80% of mercury excreted within 72 hr in urine after the injection of MEMC were determined as an organic form. That is also the case after the injection of *n*-PEMC, namely, about 65% of excreted mercury were in organic mercury. On the contrary, alkoxyethylmercury was not detected in feces throughout the experimental period.

Major portions of mercury excreted after oral administration of MEMC and *n*-PEMC were found in feces. However, the amount of mercury excreted in urine was significant, and more than half of it was in organic mercury. In feces the organic mercury was not determined. This shows that alkoxyethylmercury compounds may be degraded in the gastrointestinal tract to inorganic mercury which is known to be absorbed to a limited extent.

TABLE II. Excretion of Total and Organic Mercury *via* Feces and Urine of Mice Injected with MEMC and *n*-PEMC (10 mg Hg/kg)

		Time after subcutaneous injection (hr)						
		12	24	48	72	96	120	Total
(MEMC) Feces:	total	3.5 (5.3—1.7)	6.7 (5.7—7.7)	8.8 (7.3—10.3)	2.9 (2.4—3.5)	3.9 (5.5—2.3)	1.7 (1.7—1.6)	27.5 (27.9—27.1)
	organic	ND	ND	ND	ND	ND	ND	—
(MEMC) Urine:	total	21.7 (23.3—20.1)	17.6 (13.3—21.9)	5.1 (6.9—3.3)	6.2 (7.4—4.9)	2.1 (1.7—2.5)	1.7 (2.1—1.2)	54.4 (54.7—53.9)
	organic	22.4 (19.9—24.8)	19.2 (14.8—23.6)	2.8 (3.0—2.7)	1.6 (1.9—1.3)	ND	ND	46.0 (39.6—52.4)
(*n*-PEMC) Feces:	total	2.1 (3.8—0.3)	39.4 (37.7—41.1)	10.8 (8.8—12.7)	3.4 (3.2—3.5)	2.1 (2.0—2.2)	1.1 (1.1—1.1)	58.9 (56.9—60.9)
	organic	ND	ND	ND	ND	ND	ND	—
(*n*-PEMC) Urine:	total	4.0 (4.6—3.3)	14.0 (11.0—18.7)	6.0 (4.6—7.4)	1.8 (1.8—1.7)	1.5 (1.2—1.9)	0.8 (0.8—0.8)	29.0 (24.0—33.8)
	organic	3.7 (4.7—2.7)	10.2 (10.8—9.6)	5.5 (4.6—6.4)	0.7 (0.7—0.7)	ND	ND	20.1 (20.8—19.4)

ND: not detected. Each value represents the mean of percentage of dose of samples from two mice, and figures in parentheses indicate percentage of dose of samples from each mouse.

TABLE III. Excretion of Total and Organic Mercury *via* Feces and Urine of Mice orally Administered with MEMC and *n*-PEMC (10 mgHg/kg)

			Time after oral administration (hr)						
			12	24	48	72	96	120	Total
(MEMC)	Feces	total	20.7 (3.1— 38.1)	34.1 (44.9— 23.3)	4.6 (2.3— 6.9)	0.7 (7.7— 0.7)	0.8 (0.4— 1.2)	0.3 (0.3— 0.3)	61.2 (52.0— 70.5)
		organic	ND	ND	ND	ND	ND	ND	—
	Urine	total	6.3 (3.7— 8.9)	16.8 (17.4— 16.8)	7.6 (8.2— 7.1)	1.5 (1.5— 1.5)	0.8 (0.7— 0.9)	0.3 (0.4— 0.3)	33.3 (31.9— 35.0)
		organic	3.2 (1.8— 4.5)	14.1 (11.6— 16.6)	1.9 (1.7— 2.1)	ND	ND	ND	19.2 (15.1— 23.2)
(*n*-PEMC)	Feces	total	27.2 (22.3— 32.1)	48.1 (46.1— 50.1)	7.2 (11.0— 3.3)	0.5 (0.5— 0.5)	1.9 (2.6— 1.2)	0.1 (0.1— 0.1)	85.0 (82.6— 87.3)
		organic	ND	ND	ND	ND	ND	ND	—
	Urine	total	6.8 (5.3— 8.2)	9.0 (9.3— 8.6)	1.8 (1.5— 2.1)	0.2 (0.2— 0.2)	0.4 (0.4— 0.4)	0.1 (0.1— 0.1)	18.3 (16.8— 19.6)
		organic	5.9 (2.8— 9.0)	5.2 (6.8— 3.5)	0.4 (0.5— 0.3)	ND	ND	ND	11.5 (10.1— 12.8)

ND: not detected. Each value represents the mean of percentage of dose of samples from two mice, and figures in parentheses indicate percentage of dose of samples from each mouse.

The mercury detected in the feces after dosing alkoxyethylmercury had the following chemical characteristics: Firstly, the mercury could be determined by atomic absorption spectrophotometry after the oxidative digestion of feces followed by reduction with Sn^{2+} to metallic mercury. Secondly, the mercury could be reduced with Sn^{2+} to metallic mercury in strong alkaline medium, but not in acidic medium. And thirdly, the mercury could not be extracted with dithizone-carbon tetrachloride. From the facts mentioned above, it is considered that the mercury which was produced in the gastrointestinal tract from alkoxyethylmercury formed itself into chemically stable mercury compound there. Table IV indicates the mercury in the gastrointestinal tract after oral administration of MEMC. After 0.5 hr, a large amount of the total mercury remained in gastric contents, in which appreciable amounts of inorganic mercury (II) were already formed. A relative amount of inorganic mercury

Table IV. Total Mercury and Relative amounts of Inorganic and Organic Mercury in the Gastrointestinal Tract of Mice orally Administered with MEMC (10 mgHg/kg)

Gastrointestinal contents	Time after administration (hr)								
	0.5			3.5			7		
	Total Hg (% of dose)	Hg^{++} (% ratio)	RHg^{+} (% ratio)	Total Hg (% of dose)	Hg^{++} (% ratio)	RHg^{+} (% ratio)	Total Hg (% of dose)	Hg^{++} (% ratio)	RHg^{+} (% ratio)
Stomach	62.8 (2.7)	7.6 (1.1)	92.4	40.2 (2.7)	8.5 (1.8)	91.5	1.8 (0.3)	—	—
Small intestine (upper)	3.0 (0.6)	22.0 (7.2)	78.0	1.7 (0.7)	—	—	3.9 (0.7)	18.7 (4.1)	81.3
Small intestine (lower)	3.0 (1.9)	26.2 (13.1)	73.8	1.9 (0.8)	—	—	5.1 (0.6)	27.3 (3.2)	72.7
Large intestine	0.2 (0.1)	0	0	6.0 (0.3)	0	0	15.2 (4.9)	0	0

Each value represents the mean (SE) of samples from three mice.

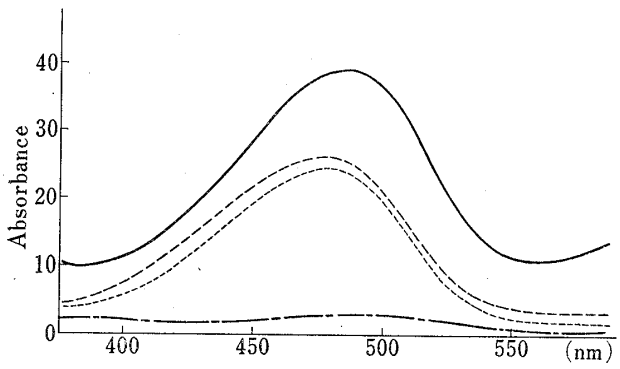

Fig. 4. Absorption Spectrum of Mercury Dithizonate Obtained from the Contents after Injection of MEMC and MC to the Cecum

Five ml of dithizone-carbon tetrachloride were added to the contents from the cecum, to which mercury compounds were injected or not (refference), followed by extraction, and 4 ml of organic layer was applied for column chromatography on alumina. The eluates obtained were made 10 ml with carbon tetrachloride and estimated by Hitachi recording spectrophotometer-323.
--------: methoxyethylmercury dithizonate 20 μgHg (standard),
———: mercury dithizonate 20 μgHg (standard),
·····: MEMC25 μgHg was injected,
·—·—·: MC 25 μgHg was injected.

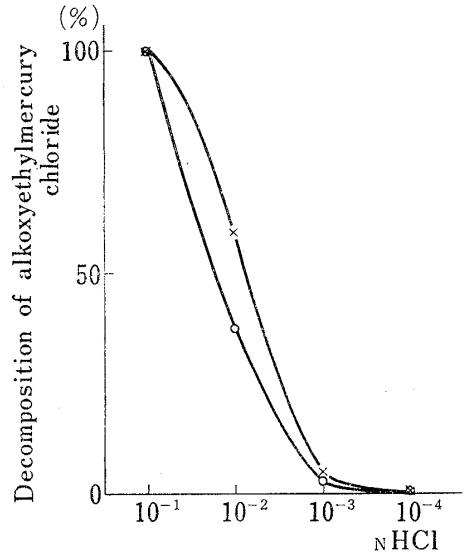

Fig. 5. Decomposition Curves of MEMC and *n*-PEMC in Hydrochloride Solution

The reaction solutions were incubated at 37° for 1.5 hr.
—○—: MEMC, —×—: *n*-PEMC

increased at lower part of the small intestine. On the other hand, no mercury extractable with dithizone-carbon tetrachloride was found in the large intestinal contents, where about 15% of the administered dose were determined as the total mercury after 7 hr. Because a major part of the large intestinal contents is occupied by the cecum ones, transformation into the dithizone-unextractable mercury in the cecum is considered. Therefore, extraction with dithizone-carbon tetrachloride from the contents was carried out 10 min after the direct injection of MEMC and MC into the cecum (Fig. 4). The results show that methoxyethylmercury dithizonate was detected from the contents, but after injection of MC mercury dithizonate was not almost detected, nevertheless mercury was determined after the oxidative digestion. This indicates that the dithizone-unextractable mercury is formed in the cecum from inorganic mercury.

Decomposition of Alkoxyethylmercury Compounds in Acidic Medium

Decomposition of MEMC and *n*-PEMC in diluted acqueous hydrochloric acid solutions was shown in Fig. 5. At the acidic media over 10^{-3}N hydrochloric acid, both compounds were decomposed to inorganic mercury, and *n*-PEMC was slightly more labile than MEMC, but no inorganic mercury was formed at 10^{-4}N.

Decomposition of MEMC and *n*-PEMC in the presence of cysteine or glutathione was compared at pH 4.0—7.2 (Fig. 6). No breakdown occurred, when MEMC or *n*-PEMC was incubated for 5 hr at pH 4.0, but complete decomposition of MEMC was brought about by the addition of cysteine as well as glutathione. MEMC was decomposed to a smaller extent than *n*-PEMC in the presence of SH compounds. Decomposition of both MEMC and *n*-PEMC decreased at higher pH areas, and increased with incubation time.

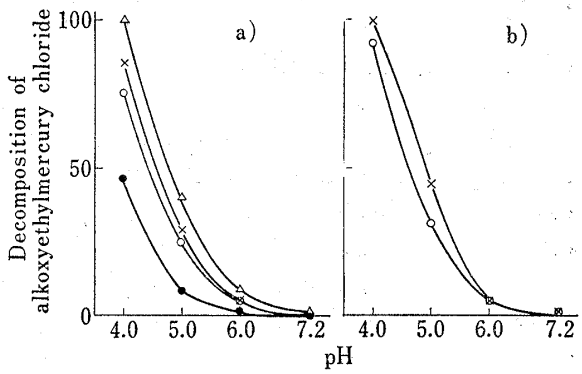

Fig. 6. Decomposition Curves of MEMC and *n*-PEMC in the Presence of 1 mM Cysteine (a) or Glutathione (b)

The reaction solutions were incubated at 37°.
incubation time:
MEMC (9.375 μM): 1 hr (●——●), 3 hr (○——○), 5 hr (△——△) *n*-PEMC (9.375 μM): 3 hr (×——×)

Discussion

The urinary excretion in organic forms and the rapid degradation of organic mercury to inorganic mercury in the body after administration of alkoxyethylmercury compounds should be allowed to expect the fast elimination of mercury from the animal body. Actually, after the single subcutaneous injection of MEMC or *n*-PEMC the greater part of the mercury is found as the organic mercury initially, but disappeared within 24 hr from the blood and within 72 hr from the liver.

Weiner, *et al.*[9] observed that all organomercury diuretics were acid labile and the degradation was accelerated in the presence of SH compounds. Daniel, *et al.*[7] indicated that the degradation of methoxyethylmercury chloride in rats is not mediated by an enzyme, since the degradation at pH 6 is about 1%/hr in the presence of cysteine *in vitro*, a rate similar to that was also observed *in vivo*.

In the present investigation, MEMC and *n*-PEMC were degraded in the presence of cysteine or glutathione in acidic media *in vitro*, and *n*-PEMC was slightly more labile than MEMC.

The mercury in the kidney, where the distribution of mercury is practically high, presents almost as organic mercury up to 6 hr, and thereafter MEMC decreased faster than *n*-PEMC.

9) I.M. Weiner, R.I. Levy, and G.H. Mudge, *J. Pharmacol.*, **138**, 96 (1962).

In urine, the organic mercury had been excreted up to 72 hr after injection of MEMC and *n*-PEMC. The amount of urinary excretion of the organic mercury after injection of MEMC was greater than that observed after the injection of *n*-PEMC. Thus, the decreasing rate of organic mercury in the kidney after injection of MEMC is seemed to be due to the effective urinary elimination of organic mercury. On the other hand, after injection of *n*-PEMC the mercury was mainly eliminated through the feces, being in accordance with the results after oral administration.

From whole-body counting studies on mice, Clarkson, *et al.*[10] evaluated the net absorption of mercuric chloride through the gastrointestinal tract is small, averaging less than 2% of the intake, while gastrointestinal absorption of methylmercury chloride is practically complete. From the Ulfvarson's study[6] on rats it seems that ethylmercury absorption rates are comparable to those of methylmercury salts.

The concentration of mercury in blood during the initial period after oral administration of MEMC and *n*-PEMC were much lower than after administration of MC. This is thought to reflect the degree of the gastrointestinal absorption of alkoxyethylmercury compounds.

During 120 hr after a single subcutaneous injection of MEMC, the ratio of excretion of mercury in urine/feces was 2/1, whereas on oral administration about 61% of the dose were excreted in the feces and about 33% in the urine. Consequently, about 44% of the dose excreted in the feces after oral administration are calculated to have not been absorbed, based on the result obtained from a single subcutaneous injection. The ratio of excretion in urine/feces was 1/2 during 120 hr after a single subcutaneous injection of *n*-PEMC. About 85% of the dose were excreted 120 hr in the feces and about 18% in the urine after oral administration of *n*-PEMC. Therefore, about 49% of the dose excreted in the feces are calculated to have not been absorbed by the same assumption as above. It could be presumed that the gastrointestinal absorption of MEMC and *n*-PEMC are approximately 55% and 50%, respectively.

Inorganic mercury was found in the gastric contents initially after oral administration of MEMC. Alkoxyethylmercury compounds were easily degraded to inorganic mercury in acidic medium. These facts reveal that the poorer gastrointestinal absorption of alkoxyethylmercury compounds in comparison with alkylmercury compounds is due to their partial degradation by the action of hydrochloric acid in the stomach to inorganic mercury, which is known to be poorly absorbed.

The mercury excreted *via* feces was not found as an organic mercury but chemically stable mercury compound. In the present experiments, the degradation of alkoxyethylmercury compounds to inorganic mercury is obvious in the stomach and in the body, but not clear whether the degradation occurred in the cecum. As for the fecal mercury, it is most likely that inorganic mercury ion (II) in the cecum reacted with hydrogen sulfide produced by micro-organisms to form mercuric sulfide, which is not extractable with dithizone-carbon tetrachloride.

10) T.W. Clarkson and R.E. Shapiro, "Mercury in the Environment," ed. by L. Friberg and L. Vostal, Chemical Rubber Co., Press, Ohio, 1972, p. 31, 34.