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Studies on the Mechanism of Protective Effects of Selenium against the Toxicity of Methylmercury

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Interaction of methylmercury and selenium was studied in order to examine the mechanism of protective effects of selenium against the toxicity of methylmercury, mainly using methylmercuric chloride labeled with carbon-14. It was found that discharge of ^{14}C into respiratory excretion was markedly accelerated by the concurrent administration of sodium selenite and ^{14}C-labeled methylmercuric chloride, as compared with control of a single administration of ^{14}C-labeled methylmercuric chloride. That is, the total amount of ^{14}C discharged during 48 hr after the concurrent administration, was about 5.5 times as much as that in a single administration of ^{14}C-labeled methylmercury. The acceleration of demethylation of methylmercury seemed to be more responsible for the action of selenium.

Secondly, the uptake of methylmercury into erythrocytes, was examined and then, the amount of mercury in erythrocytes showed a tendency to become lower in the group given selenium. It was found that the uptake of methylmercury into erythrocytes was inhibited by about 30% with the administration of selenite.

Keywords——methylmercury; selenium; demethylation; interaction; respiratory; protective

Introduction

Our preceding report[2] suggested the acceleration of demethylation as one of the mechanism of protective effects of selenium against the toxicity of methylmercury. In the present work interaction of methylmercury and selenium was studied in order to further examine the mechanism of protective effects of selenium against the toxicity of methylmercury, mainly by using methylmercuric chloride labeled with carbon-14.

Materials and Methods

1. Chemicals——^{14}C-labeled methylmercuric chloride (specific activity: 4.17 mCi/mmol, radiochemical purity: greater than 98%) was obtained from New England Nuclear and other reagents were analytical grade products. Sample oxidizer's absorption solutions consist of two mixture (C_1: C_2=1: 2).

C_1....monoethanolamine+methanol (1: 1, v/v)
C_2....C_3+methanol (7: 5; v/v)
C_3....toluene (1000 ml)+PPO (2,5-diphenyloxazole 10 g)+POPOP (1,4-bis-2-[5-phenyloxazolyl]-benzene 0.5 g)

2. Animals——Male rats of the Wistar strain aged 5 weeks, were purchased from Nihon Rat Co., Urawa, and fed solid diets (CE-2) which were obtained from CLEA Japan Inc., Tokyo. The animals aged 11 weeks (250—300 g body wt) were employed throughout the present experiment.

3. Effect of Sodium Selenite on Respiratory Excretion of ^{14}C of ^{14}C-labeled Methylmercuric Chloride——Rats were devided into 2 groups. Each group consisted of 2 animals. Rats in group I, were administered with methylmercuric chloride (8 mg/kg/day) and ^{14}C-labeled methylmercuric chloride (8 μCi/rat), intraperitoneally at the same time once. The animals in group II, were administered with sodium selenite (3 mg/kg/day) subcutaneously, methylmercuric chloride (8 mg/kg/day) and ^{14}C-labeled methylmercuric chloride

1) Location: *1–33, Yayoi-cho, Chiba.*
2) Y. Yamane, H. Fukino, Y. Aida, and M. Imagawa, *Yakugaku Zasshi*, **97**, 667 (1977).

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(8 μCi/rat) intraperitoneally and simultaneously once, and administered with sodium selenite (3 mg/kg/day) subcutaneously once 24 hr before the concurrent administration of methylmercury and selenium.

Two rats in groups I and II were put in a desiccator where the animals were able to breathe freely. The exhaust pipe was connected to the absorption bottle in which were put the solutions saturated with mercuric chloride and it's outlet was connected to a handy aspirator. The respiratory excretions of rats were collected in it by sucking operation of handy aspirator. Furthermore, respiratory excretions of ^{14}C-labeled carbon dioxide, were collected into the absorption bottle in which were put monoethanolamine solutions. At regular intervals, the absorption solutions were changed and these solutions were employed as analytical samples. The radioactivities in them were measured with a liquid scintillation counter (Aloka LSC-502) by using Bray's scintillator.[3] A counting efficiency was corrected by external standard ratio. Eath value was shown as total radioactivity of respiratory excretion of two rats. The animal experiments were repeated 3 times.

4. Distribution of ^{14}C in Tissues of Rats and Excretion into Feces and Urine——Rats were devided into 2 groups. Each group consisted of 3 rats. The animals in group III, were administered with ^{14}C-labeled methylmercuric chloride (3 μCi/rat) orally once. The animals in group IV, were administered with sodium selenite (1.5 mg/kg/day) subcutaneously and ^{14}C-labeled methylmercuric chloride (3 μCi/rat) orally, at the same time once, and administered with sodium selenite (1.5 mg/kg/day) subcutaneously once 24 hrs before the concurrent administration of selenium and ^{14}C-labeled methylmercury. Each rat was put in the metabolic cage which was able to separate feces and urine. Tissues, feces and urine were put on filter paper, weighed, dried, and burned by using a sample oxidizer (Aloka-ASC-112). Then, ^{14}C-labeled carbon dioxides which were obtained from them were sucked in vial bottles by using sample oxidizer's absorption solutions (C_1: 6 ml, C_2: 12 ml). The radioactivities of them were measured with the same liquid scintillation counter as shown in 3.

5. Distribution of ^{14}C of Brain Subcellular Fractions in Rats——Rats were devided into 2 groups. Each group consisted of 3 animals. Rats in group V, were administered with ^{14}C-labeled methylmercuric chloride (5 μCi/rat) orally once. The animals in group VI, were administered with sodium selenite (1.5 mg/kg/day) subcutaneously and ^{14}C-labeled methylmercuric chloride (5 μCi/rat) orally, at the same time once, and administered with sodium selenite (1.5 mg/kg/day) subcutaneously once 24 hr before the concurrent administration of selenium and ^{14}C-labeled methylmercury. The brains from each group were removed and weighed. The homogenate was made 10% (w/v) with 0.32 M sucrose solution. The 10% homogenate was centrifuged, in order, at 700 ***g*** for 10 min, at 15000 ***g*** for 30 min, and 100000 ***g*** for 60 min, to obtain nuclei and debris, mitochondria, microsomes, and soluble fractions. Acid soluble fraction, phospholipid fraction, nucleic acid fraction and protein, were obtained respectively by using the Schmidt-Thannhauser-Schneider method.[4]

6. Effect of Sodium Selenite on the Uptake of Methylmercuric Chloride into Erythrocytes——Rats were devided into 2 groups. Each group consisted of 5 animals. Rats in group VII, were administered with methylmercuric chloride (10 mg/kg/day) orally once. The animals in group VIII, were administered with sodium selenite (1 mg/kg/day) subcutaneously and methylmercuric chloride (10 mg/kg/day) orally, at the same time once, but were preinjected with sodium selenite (1 mg/kg/day) subcutaneously once a day for 3 days. The animals were killed by bleeding from the carotid 24 hr after the last administration. The heparinized blood was separated into plasma and erythrocytes. Total mercury in erythrocytes was analyzed by a flameless atomic absorption spectrophotometry (Hiranuma HG-1) of vaporized mercury after the wet digestion.[5]

Results

Effect of Sodium Selenite on Respiratory Excretion of ^{14}C of ^{14}C-Labeled Methylmercuric Chloride

As shown in Fig. 1, it was found that discharge of ^{14}C into respiratory excretion was markedly accelerated by the concurrent administration of sodium selenite and ^{14}C-labeled methylmercuric chloride (group II), as compared with control of a single administration of ^{14}C-labeled methylmercuric chloride (group I). That is, the total ^{14}C discharged during 48 hr after the concurrent administration of sodium selenite and ^{14}C-labeled methylmercuric chloride, was about 5.5 times as much as that in a single administration group of ^{14}C-labeled methylmercuric chloride.

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4) W.C. Schneider, *J. Biol. Chem.*, **164**, 747 (1946).
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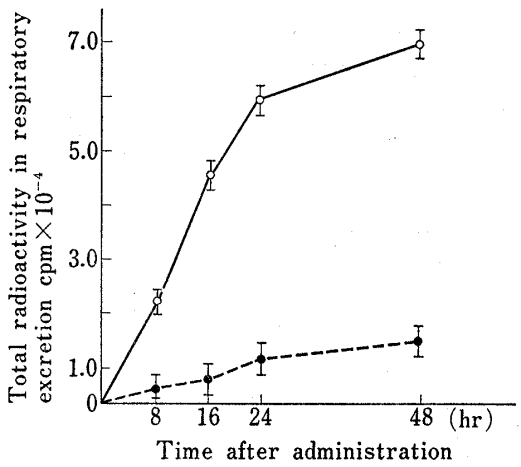

Fig. 1. Effect of Sodium Selenite on Respiratory Excretion of ^{14}C of ^{14}C-labeled Methylmercuric Chloride

●----● $^{14}CH_3HgCl$, ○——○ $^{14}CH_3HgCl$-Na_2SeO_3.
$^{14}CH_3HgCl$: The rats were administered with methylmercuric chloride (8 mg/kg/day) and ^{14}C-labeled methylmercuric chloride (8 μCi/rat), intraperitoneally at the same time once.
$^{14}CH_3HgCl$-Na_2SeO_3: The rats were administered with sodium selenite (3 mg/kg/day) subcutaneously once 24 hr before the concurrent administration of selenium and methylmercury. The animals were administered with sodium selenite (3 mg/kg/day) subcutaneously, methylmercuric chloride (8 mg/kg/day) and ^{14}C-labeled methylmercuric chloride (8 μCi/rat), intraperitoneally at the same time once. Each value represents total radioactivity of respiratory excretion of two rats in the solutions saturated with mercuric chloride and the mean (n=3). Vertical bars represent standard errors.

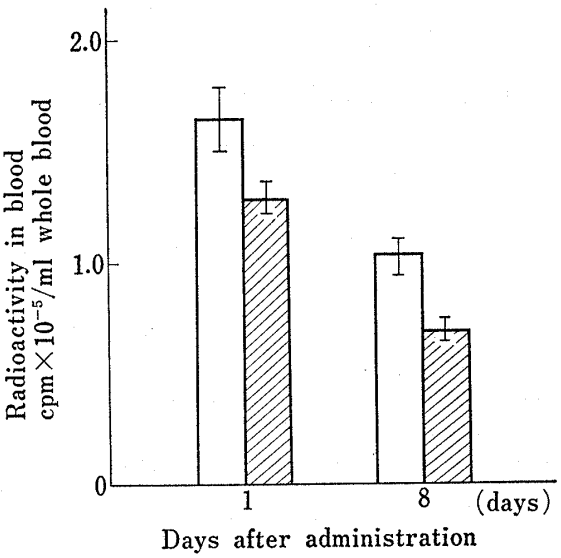

Fig. 2. Effect of Sodium Selenite on the Distribution of ^{14}C in Blood of Rats administered with ^{14}C-labeled Methylmerculic Chloride

□ $^{14}CH_3HgCl$, ▨ $^{14}CH_3HgCl$-Na_2SeO_3.
$^{14}CH_3HgCl$: The rats were administered with ^{14}C-labeled methylmercuric chloride (3 μCi/rat *p.o.*) (group III).
$^{14}CH_3HgCl$-Na_2SeO_3: The rats were administered with sodium selenite (1.5 mg/kg *s.c.*) once 24 hr before the concurrent administration of selenium and ^{14}C-labeled methylmercury. The animals were administered with sodium selenite (1.5 mg/kg *s.c.*) and ^{14}C-labeled methylmercuric chloride (3 μCi/rat *p.o.*) simultaneously (group IV). Each value represents the mean of 3 rats. Vertical bars represent standard errors.

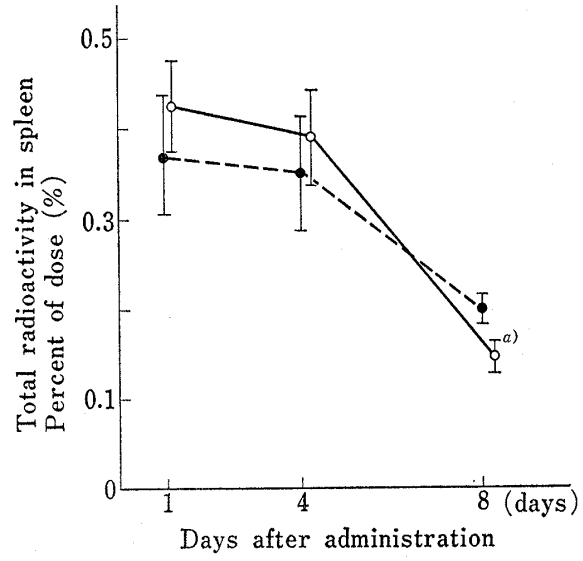

Fig. 3. Effect of Sodium Selenite on the Distribution of ^{14}C in Spleen of Rats administered with ^{14}C-labeled Methylmercuric Chloride

●——● $^{14}CH_3HgCl$, ○——○ $^{14}CH_3HgCl$-Na_2SeO_3.
Experimental conditions are the same as described in Fig. 2.
Significance of difference from the group administered with methylmercuric chloride alone, *a*) $p<(0.05)$.

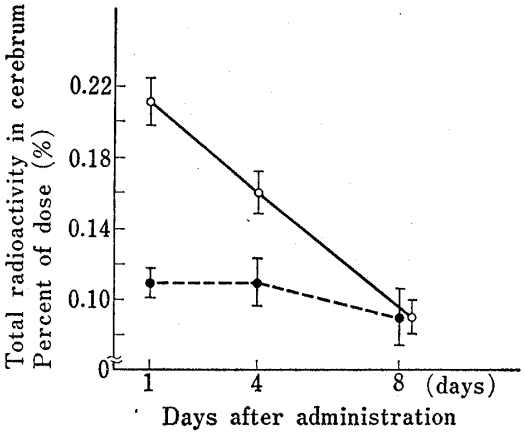

Fig. 4. Effect of Sodium Selenite on the Distribution of ^{14}C in Cerebrum of Rats administered with ^{14}C-labeled Methylmercuric Chloride

●——● $^{14}CH_3HgCl$,
○——○ $^{14}CH_3HgCl$-Na_2SeO_3.
Experimental conditions are the same as described in Fig. 2.

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Besides, monoethanolamine was used as the absorption solution that was able to trap carbon dioxide, but the activities of ^{14}C were not at all counted in it.

Distribution of ^{14}C in Tissues of Rats and Excretion into Feces and Urine

As shown in Fig. 2, the amount of ^{14}C in blood of a single administration (group III), were already larger than that of the concurrent administration (group IV) a day after the administration.

On the other hand, as shown in Fig. 3, in spleen, there was not significant difference of the amount of ^{14}C between group III and group IV a day and 4 days after the administration On the contrary, the amount of ^{14}C in group IV tended to decrease 8 days after the administration ($p<0.05$).

As shown in Fig. 4, in cerebrum, the amount of ^{14}C in group IV showed a marked increase a day after the administration, but it decreased remarkably during 8 days after the administration. In a single administration (group III), there was no great variation in the amount of ^{14}C, between a day and 8 days after the administration.

In cerebellum, the tendency of distribution of ^{14}C, was the same as the result in cerebrum.

In liver, the amount of ^{14}C both in group III and in group IV, represented the same tendency of decrease, respectively a day, 4 days and 8 days after the administration. There was not significant difference between group III and group IV.

In kidney, there was no variation in the amount of accumulation of ^{14}C between group III and group IV a day and 8 days after the administration.

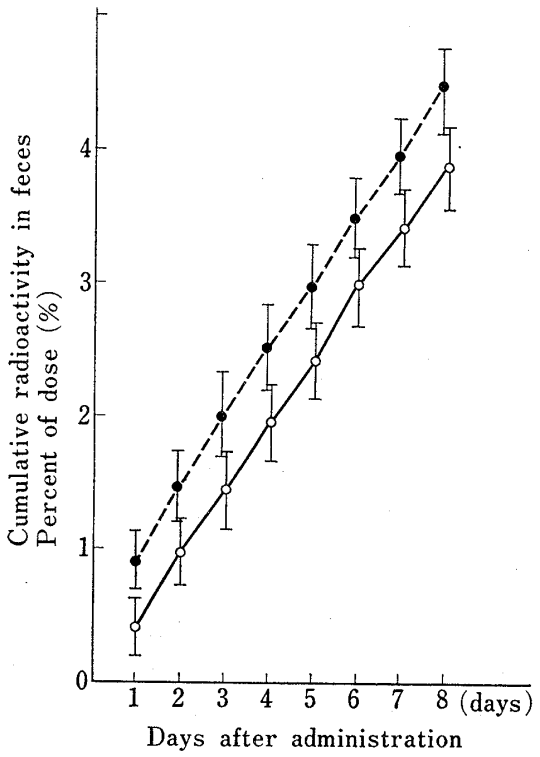

Fig. 5. Effect of Sodium Selenite on ^{14}C in Feces of Rats administered with ^{14}C-labeled Methylmercuric Chloride

●----● $^{14}CH_3HgCl$, ○——○ $^{14}CH_3HgCl$-Na_2SeO_3.
Experimental conditions are the same as described in Fig. 2.

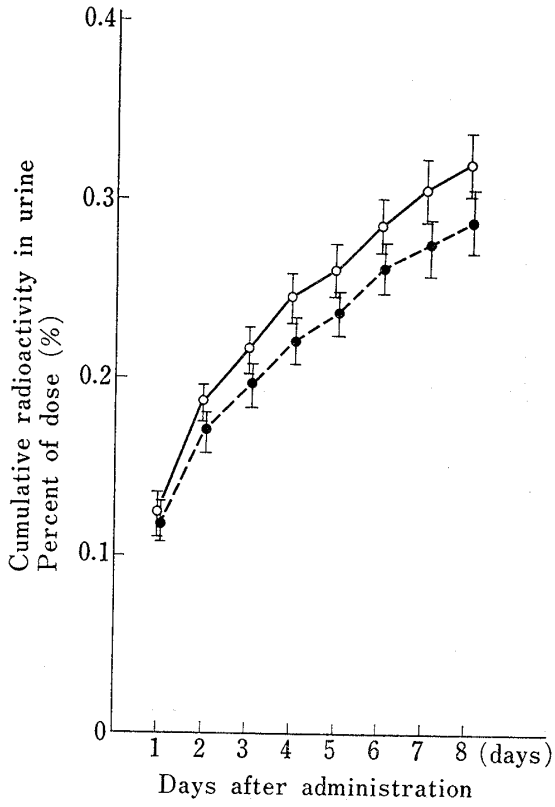

Fig. 6. Effect of Sodium Selenite on ^{14}C in Urine of Rats administered with ^{14}C-labeled Methylmercuric Chloride

●----● $^{14}CH_3HgCl$, ○——○ $^{14}CH_3HgCl$-Na_2SeO_3.
Experimental conditions are the same as described in Fig. 2.

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As shown in Fig. 5, the amount of ^{14}C in feces from group IV, was lower than that from group III a day after the administration, but there was no difference between group III and group IV in total amount of excretion of ^{14}C into feces for 8 days.

Furthermore, as shown in Fig. 6, there was no difference between group III and group IV in total amount of excretion of ^{14}C into urine for 8 days.

Distribution of ^{14}C of Brain Subcellular Fractions in Rats

As shown in Table I, subcellular fractions, nuclei and debris, mitochondria, microsomes, and soluble fraction from brain of rats were obtained 24 hr after the administration. The distribution rate of ^{14}C into each fraction from group V, was all the same tendency as that from group VI. The same tendency was seen in each fraction 8 days after the administration.

Table I. Effect of Sodium Selenite on the Distribution of ^{14}C in Subcellular Fractions of Rat Brain administered with ^{14}C-labeled Methylmercuric Chloride

Days after treatment	Fraction	CH_3HgCl cpm $\times 10^{-3}$/organ	%	CH_3HgCl–Na_2SeO_3 cpm $\times 10^{-3}$/organ	%
1 day	Nuclei and Debris	2.42	22	5.54	16
	Mitochondira	4.36	40	15.03	43
	Microsomes	0.94	9	2.97	9
	Soluble	3.24	29	11.23	32
8 days	Nuclei and Debris	1.46	10	2.23	13
	Mitochondria	6.51	45	7.90	48
	Microsomes	1.04	7	1.00	6
	Soluble	5.45	38	5.45	33

CH_3HgCl: The rats were administered with ^{14}C-labeled methylmercuric chloride (5 μCi/rat *p.o.*) (group V).
CH_3HgCl–Na_2SeO_3: The rats were administered with sodium selenite (1.5 mg/kg *s.c.*) once 24 hr before the concurrent administration of selenium and ^{14}C-labeled methylmercury. The animals were administered with sodium selenite (1.5 mg/kg *s.c.*) and ^{14}C-labeled methylmercuric chloride (5 μCi/rat *p.o.*) simultaneously (group VI). Each value represents the mean of 3 rats in each group.

Secondly, as shown in Table II, acid soluble fraction, phospholipid fraction, nucleic acid fraction and protein fraction, were obtained from the brain of rats 24 hr after the administration. The distribution rate of ^{14}C into each fraction from group V, was all the same tendency as that from group VI. The same tendency was seen in each fraction 8 days after the administration.

Table II. Effect of Sodium Selenite on the Distribution of ^{14}C in Subcellular Constituents of Rat Brain administered with ^{14}C-labeled Methylmercuric Chloride

Days after treatment	Fraction	CH_3HgCl cpm $\times 10^{-3}$/organ	%	CH_3HgCl–Na_2SeO_3 cpm $\times 10^{-3}$/organ	%
1 day	Acid soluble	1.11	13	4.07	16
	Phospholipid	1.68	19	4.38	17
	Nucleic acid	0.27	3	0.94	3
	Protein	5.70	65	16.32	64
8 days	Acid soluble	1.34	13	0.96	8
	Phospholipid	2.04	19	1.50	12
	Nucleic acid	0.83	8	0.66	5
	Protein	6.46	61	9.01	74

The treatment was the same as described in Table I.
Each value represents the mean of 3 rats in each group.

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Effect of Sodium Selenite on the Uptake of Methylmercuric Chloride into Erythrocytes

As shown in Table III, the amount of mercury of rats in group VIII, was two thirds of that in group VII and then, it was found that the uptake of methylmercuric chloride into erythrocytes was inhibited by about 30% with the administration of sodium selenite.

TABLE III. Effect of Sodium Selenite on the Uptake of Methylmercuric Chloride into Erythrocytes

Treatment	Hg μg/g cell	Relative value
CH_3HgCl	111.6±4.6	1.00
CH_3HgCl–Na_2SeO_3	75.0±8.5	0.67

CH_3HgCl: The rats were administered with methylmercuric chloride (10 mg/kg *p.o.*) once. CH_3HgCl-Na_2SeO_3: The rats were preinjected with sodium selenite (1 mg/kg *s.c.*) once a day for 3 days. The animals were administered with sodium selenite (1 mg/kg *s.c.*) and methylmercuric chloride (10 mg/kg *p.o.*) simultaneously. The animals were killed 24 hr after the treatment. Each value represents the mean ±S.E. of 5 rats in each group.

Discussion

We suggested, from the results of the preceding report, that selenium accelerated demethylation of methylmercury. In general, organomercury compounds are known to be changed to inorganic mercury *in vivo*. Miller and others[6] found this process in the kidney. Gage and others[7] found it not only in the kidney but also in the liver and serum, while Norseth and others[8] found conversion of organic to inorganic in the brain, though to a lesser extent, besides the above organs. Fang[9] reported that the activity of phenylmercuric acetate cleavage enzyme in the liver was increased from rats supplemented with sodium selenite in the drinking water, but such influence was not seen for the cleavage of methylmercuric and ethylmercuric chloride. Stillings and others[10] presumed that protective effect of selenium against methylmercury toxicity is alleviation of kidney dysfunction by decreased accumulation of mercury in the kidney and acceleration of demethylation of methylmercury by selenium. On the other hand, selenium ingested *in vivo* is known to be discharged as dimethylselenium in respiratory excretion.[11,12]

Based on these informations, we considered that selenium administered might sever the C–Hg bond in methylmercury and form dimethylselenium to be discharged into respiratory excretion, thereby, accelerating demethylation of methylmercury *in vivo*. Therefore, using ^{14}C-labeled methylmercury, we examined the behavior of ^{14}C periodically after its administration with and without selenium (Fig. 1).

It was thereby found that discharge of ^{14}C into respiratory excretion was markedly accelerated by selenium administration and the total amount discharged during 48 hr after its administration was about 5.5 times as much as that in the non-administration group (Fig. 1), presuming that demethylation of methylmercury was accelerated by selenium. But we could not show the dimethylselenium directly and it was also considered that the radioactivity of respiratory excretion might be dimethylmercury and/or methylmercury. Then, as our experiment was carried out according to the method used in such reports that selenium ingested *in vivo* was discharged as dimethylselenium in respiratory excretion,[11,12] we con-

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sidered that the radioactivity of respiratory excretion might be dimethylselenium and the acceleration of demethylation of methylmercury, might be more responsible for the action of selenium.

Examination of the distribution of ^{14}C in various tissues has shown that the amount of ^{14}C tended to decrease slightly in blood after the administration of selenium but the amount of ^{14}C showed a marked increase (2—3 times) in the brain after 24 hr. The amount of ^{14}C in the brain decreased remarkably for the next 8 days and the tendency was the same as analytical results reported by Iwata and others[13] who assumed that this decrease is due to accelerated discharge of methylmercury by selenium. However, Stillings and others[10] denied this acceleration from the result of analysis of mercury in urine and feces. Judging from the absence of difference in the excretion of ^{14}C in urine and feces by selenium administration, this decrease of ^{14}C in the brain does not seem to be due to acceleration of discharge of methylmercury by selenium (Fig. 5).

Protective effect of selenium against mercury toxicity, in spite of a marked increase of ^{14}C in the brain, was considered to be due to the difference in the distribution of methylmercury in cerebral fraction and, therefore, examinations were made on the distribution of ^{14}C in cerebral fractions and on incorporation of methylmercury into erythrocytes which would affect invasion of ^{14}C into the brain. As shown in Table I and II, there was no effect of selenium administration on the distribution rate of ^{14}C into various fractions but the invasion of methylmercury into erythrocytes was inhibited by about 30%.

In general, methylmercury invades more into erythrocytes, differing from inorganic mercury, and the ratio of mercury in erythrocytes and plasma is reported to be 9:1 or more.[14-16] Swennson[15] and Lundgren[17] stated that the erythrocyte : plasma ratio of methylmercury is proportional to the dose of methylmercury and the ratio increase with increasing dosage.

Therefore, we presumed that selenium is playing some role in the detoxication of methylmercury by the acceleration of demethylation and also, by the suppression of the uptake of methylmercury into erythrocytes with the concurrent administration of methylmercury and selenium.

Acknowledgement The author thanks are due to Miss K. Iwanaga of this faculty for her excellent technical assistance.

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