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Suppressive Effect of Zinc on the Toxicity of Mercury

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Male rats were given mercuric chloride (0.018 mmol/kg/day) by subcutaneous injection and zinc acetate (3.0 mmol/kg/day) by oral administration. Mercury and zinc were administered at the same time, once every 24 hr for 5 days. Only 1 of 10 rats given mercury alone survived for 3 days, and this one rat died on the 4th day. In the group given mercury and zinc at the same time, all the 10 animals were alive on the 5th day, indicating the marked effect of zinc in suppressing the toxicity of mercury.

Based on such a marked effect of zinc, examinations were made to see whether biosynthesis of metallothionein would occur in the presence of zinc or mercury by the incorporation of ^{14}C-cysteine into the metallothionein fraction. High rate of incorporation of radioactivity into the metallothionein in the rat liver was observed by the administration of zinc but the incorporation was not so marked by the administration of mercury. This fact suggested that the metallothionein induced by zinc might have some effect on lowering of mercury toxicity and then, distribution of mercury and zinc in the soluble fraction was examined.

It was found that, in the liver, mercury bound in larger amount to high molecular fraction during single administration of mercury transited to the metallothionein fraction when zinc was administered concurrently. The amount of mercury bound as Hg-thionein in the kidney after the concurrent administration of mercury and zinc is about 4 times that after single administration of mercury.

Keywords——mercury; zinc; metallothionein; suppressive effect; incorporation; ^{14}C-cysteine; biosynthesis

Introduction

Mercury compounds have the strongest toxicity among metals and because of the high affinity of mercury with thiol groups, they are known to affect living organisms by damaging proteins and thiol enzymes. Even among mercury compounds, mercuric chloride gives the strongest acute toxicity and is said to produce nephritis, neuritis, and impairment of walking, besides gastrointestinal dysfunction.

Suppressive effect of zinc on cadmium toxicity has been reported by Parizek[2)] and by Webb.[3)] As a mechanism for such antagonistic action of zinc and cadmium, participation of metallothionein and substitution competition have been suggested. Since zinc, cadmium, and mercury are homologous elements, we anticipated that zinc might also act suppressively against the toxicity of mercury. Therefore, effect of zinc on the toxicity of mercury was examined. It was found that mercury toxicity was decreased remarkably and metallothionein was induced by administration of zinc.[4)]

Based on these results, we considered that biosynthesis of metallothionein induced by zinc plays a great part in the mechanism of this suppression and that binding of mercury with metallothionein, resulting in the maintenance of mercury in inactive form *in vivo*, is responsible for lowering of mercury toxicity.

1) Location: *1–33, Yayoi-cho, Chiba.*
2) J. Parizek, *J. Endocr.*, **15**, 56 (1957).
3) M. Webb, *Biochem. Pharmacol.*, **21**, 2767 (1972).
4) Y. Yamane, H. Fukino, and M. Imagawa, *Chem. Pharm. Bull.* (Tokyo), **24**, 836 (1976).

Materials and Methods

1. **Chemicals**——L-[U-^{14}C]-cysteine was obtained from Radiochemical Centre, Amersham, Bucks, and other reagents were analytical grade products.

2. **Animals**——Male rats of the Wistar strain aged 5 weeks, were purchased and fed solid diets (CE-2) which were obtained from CLEA Japan Inc., Tokyo. The animals aged 7 weeks (140—160 g body wt) were used for studying the effect of zinc on the toxicity of mercury and those aged 9—11 weeks (200—300 g body wt) were employed throughout the other experiment.

3. **Effect of Zinc on the Toxicity of Mercury**——Male rats were divided into 4 groups, which were administered with 0.9% sodium chloride (control), mercuric chloride, mercuric chloride-zinc acetate, and zinc acetate, respectively. Mercuric chloride and mercuric chloride-zinc acetate group consisted of 10 animals, and control and zinc acetate group, of 5 animals. Mercuric chloride and zinc acetate were dissolved in 0.9% sodium chloride solution. The animals of control and mercuric chloride group, were given subcutaneous injection of 0.9% sodium chloride solution and mercuric chloride (0.018 mmol/kg/day), once every 24 hr for 5 days, respectively. The animals of mercuric chloride-zinc acetate group, were administered with mercuric chloride (0.018 mmol/kg/day) subcutaneously and zinc acetate (3.0 mmol/kg/day) orally, at the same time once every 24 hr for 5 days, respectively. The animals of zinc acetate group, were given oral administration of zinc acetate (3.0 mmol/kg/day) once every 24 hr for 5 days. Zinc acetate was administered orally once 24 hours before the concurrent administration of mercury and zinc. Rats had free access to food and water. Effect of zinc on the toxicity of mercury, was examined from the change in body weight and survival of rats.

4. **Determination of Mercury and Zinc in the Liver and the Kidney**——Male rats were divided into 4 groups, which were administered with 0.9% sodium chloride (control), mercuric chloride, mercuric chloride-zinc acetate, and zinc acetate, respectively. Each group consisted of 3 animals. Mercuric chloride and zinc acetate were dissolved in 0.9% sodium chloride solution. The animals of control, mercuric chloride, mercuric chloride–zinc acetate, and zinc acetate groups, were given subcutaneous injection of 0.9% sodium chloride solution, mercuric chloride (0.010 mmol kg/day), mercuric chloride (0.010 mmol kg/day)–zinc acetate (0.75 mmol kg/day), and zinc acetate (0.75 mmol/kg/day), once every 24 hr for 3 days, respectively. They were decapitated 24 hours after the last treatment. Total mercury in the liver and the kidney, were analysed by flameless atomic absorption spectrophotometry[5] (Hiranuma HG-1) of vaporized mercury after wet digestion of the organs. Zinc was extracted from the digested solution by DDTC-MIBK method[6] and determined by atomic absorption spectrophotometry (Hitachi 208).

5. **Subcellular Distribution of Mercury and Zinc in the Liver and the Kidney**——The livers and kidneys which were obtained from 3 rats treated with mercury and zinc, were put together, respectively. Then, they were homogenized in an acid-washed all glass homogenizer. The homogenate was made 20% (W/V) with 1.15% KCl, and cell nuclei and debris, mitochondria, microsomes, and soluble fraction were fractionated by the method of Hogeboom.[7] Mercury and zinc in each fraction were determined.

6. **Sephadex G-75 Chromatography of the Soluble Fraction from the Liver**——Soluble fraction was filtered through Sephadex G-75 column (1.8 × 36 cm) and the column was eluted with 0.01 M Tris-HCl buffer (pH 7.4) at 4°. Eluates (4 ml fractions) were analysed for zinc and absorbance at 280 nm.

7. **Biosynthesis of Metallothionein by Mercury and Zinc in the Liver and the Kidney**——Male rats treated with mercury or zinc, were administered with 5 μCi of L-[U-^{14}C]-cysteine intraperitoneally and decapitated 24 hours after the last treatment. Soluble fraction from the liver and the kidney was filtered through Sephadex G-75 column (2.5 × 36 cm), and the incorporation of L-[U-^{14}C]-cysteine into metallothionein fraction was examined. The radioactivity was determined by a liquid scintillation counter (Aloka LSC-502), using a Triton X-100-toluene (1 : 2) scintillator.

8. **Distribution of Mercury and Zinc in the Soluble Fraction from the Liver and the Kidney**——Soluble fraction was separated into three major peaks by gel filtration, the same method as shown in **6**. The first, the second, and the third major peaks represented High Molecular fraction (HM-fraction), Metallothionein fraction (MT-fraction), and Low Molecular fraction (LM-fraction), respectively. Amount of mercury and zinc in each fraction was determined by the same method as shown in **4**.

9. **Degree of Affinity of Mercury with Zn-thionein *in Vitro***——Soluble fraction was obtained from the liver of the rat treated with zinc acetate (0.75 mmol/kg) subcutaneously once. Mercury (0, 10, 20, 40, 80 μg as Hg^{2+}) was added to 2 ml of the soluble fraction and the mixture was incubated at 37° 30 min. Thereafter, it was filtered by the same method as shown in **6**, and amount of mercury bound to thionein was measured in order to examine degree of affinity of mercury with Zn-thionein *in vitro*.

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5) "Standard Methods of Analysis for Hygienic Chemists with Commentary," ed. by the Pharmaceutical Society of Japan, Kanehara Press Inc., Tokyo, 1973, pp. 303—304.
6) "Standard Methods of Analysis for Hygienic Chemists with Commentary," ed. by the Phamaceutical Society of Japan, Kanehara Press Inc., Tokyo, 1973, pp. 293—294.
7) G.H. Hogeboom, "Method in Enzymology," Vol. 1, Academic Press Inc., New York, 1955, p. 16.

10. Effect of Mercury and Zinc on Fructose-1,6-diphosphate Aldolase (FDP-Aldolase) and Lactate Dehydrogenase (LDH) Activity——Male rats were divided into 4 groups, which were administered with 0.9% sodium chloride (control), mercuric chloride, mercuric chloride–zinc acetate, and zinc acetate, respectively. Each group consisted of 3 animals. The animals of control and mercuric chloride group, were injected subcutaneously with 0.9% sodium chloride solution and mercuric chloride (0.014 mmol/kg/day) once every 24 hr for 2 days, respectively. The animals of mercuric chloride–zinc acetate group, were administered with mercuric chloride (0.014 mmol/kg/day) subcutaneously and zinc acetate (1.50 mmol/kg/day) orally at the same time once every 24 hr for 2 days, respectively after pre-treatment with zinc once 24 hours before the concurrent administration. The animals of zinc acetate group, were given orally zinc acetate (1.50 mmol/kg/day) once every 24 hr for 3 days. They were decapitated 24 hours after the last treatment. The liver was removed, and 10% (w/v) homogenate in 1.15% KCl was prepared. The homogenate was centrifuged at 105000 *g* for 60 min, and supernatant was used as enzyme source. FDP-Aldolase activity was assayed by the method of Ludvigsen[8] and LDH activity, by Hill's modification.[9]

Results

Effect of Zinc on the Toxicity of Mercury

Suppressive effect of zinc on the toxicity of mercuric chloride was very marked. As shown in Table I, all the animals given mercury alone died on the 4th day. When mercury and zinc were given concurrently, all the animals were alive on the 5th day, and suppressive rate was 100% in mortality.

Table I. Effect of Successive Administrations of Zinc Acetate on Survival of Rats Injected with Mercuric Chloride

Treatment	No. of rats	No. of surviving rats on day				
		1	2	3	4	5
Control (0.9%NaCl)	5	5	5	5	5	5
$HgCl_2$	10	10	6	1	0	0
$HgCl_2+Zn(AcO)_2$	10	10	10	10	10	10
$Zn(AcO)_2$	5	5	5	5	5	5

Body weight change was shown in Fig. 1. By the concurrent administration of mercury and zinc, increase of body weight was smaller than that of control and zinc group, but decrease of body weight by the administration of mercury, was suppressed by that of zinc.

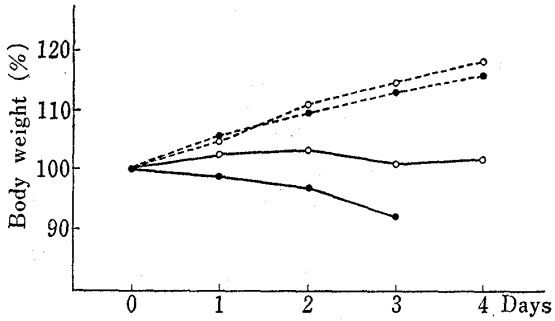

Fig. 1. Changes in Body Weight of Rats treated with Mercuric Chloride and Zinc Acetate

○----○, control 0.9% NaCl (*s.c.*).
○——○, $HgCl_2$–$Zn(AcO)_2$.
●——●, $HgCl_2$ 0.018 mmol/kg/day (*s.c.*).
●----●, $Zn(AcO)_2$ 3.0 mmol/kg/day (*p.o.*).

Determination of Mercury and Zinc in the Liver and the Kidney

Because of remarkable decrease of mercury toxicity by zinc, amount of mercury and zinc in the liver and the kidney which were known as target organs of mercury, was examined. As shown in Table II, amount of mercury in the liver decreased slightly by the concurrent administration of mercury and zinc. But in the kidney, a large amount of mercury was accumulated by the concurrent administration of mercury and zinc. There was no great difference in the amount of zinc between the group administered with zinc alone, and that administered with mercury and zinc both in the liver and the kidney.

8) B. Ludvigsen, *J. Lab. and Clin. Med.*, **61**, 329 (1963).
9) B.R. Hill, *Cancer Res.*, **21**, 271 (1961).

Table II. Amount of Mercury and Zinc in the Liver and Kidney

Treatment	Hg μg/g tissue		Zn μg/g tissue	
	Liver	Kidney	Liver	Kidney
Control	—	—	32.4±1.0	27.2±1.8
Hg	9.09±0.41	50.9±0.4	24.0±0.7	33.4±0.7
Hg--Zn	7.58±0.10[a)]	77.0±3.0[b)]	83.3±6.3	49.2±2.6
Zn	—	—	103.3±8.0	45.3±0.7

Treatment: Male rats of the Wistar strain, control; 0.9% NaCl *s.c.*, Hg; $HgCl_2$ (0.010 mmol/kg/day) *s.c.*, Zn; $Zn(AcO)_2$ (0.75 mmol/kg/day) *s.c.*, Hg–Zn; $HgCl_2$ (0.010 mmol/kg/day) and $Zn(AcO)_2$(0.75 mmol/kg/day), *s.c.* simultaneously, The animals were administered at the same time for 3 days. Each value represents the mean±S.E. of 3 rats in each group. Significance of difference from the group treated with mercury alone, *a*) $p<0.05$, *b*) $p<0.01$.

Subcellular Distribution of Mercury and Zinc in the Liver and the Kidney

Subcellular distribution of mercury was shown in Table III. There was no great difference in the distribution pattern of mercury both in the liver and the kidney. But it was accumulated mostly in the soluble fraction.

Subcellular distribution of zinc was shown in Table IV. There was no great difference in the distribution pattern of zinc both in the liver and the kidney. But it was accumulated mostly in the soluble fraction as well as mercury.

Table III. Subcellular Distribution of Mercury in the Liver and the Kidney

	Treatment	Nuclei and debris %	Mitochondria %	Microsomes %	Soluble fraction %
Liver	Hg	30.8	18.2	4.5	46.5
	Hg-Zn	23.9	12.5	3.9	59.8
Kidney	Hg	28.2	11.2	10.4	50.2
	Hg-Zn	29.4	10.2	7.0	53.4

Treatment: Experimental conditions are as given in Table II.
Samples were prepared from 3 rats. Each value represents percentage of mercury distribution in each fraction.

Table IV. Subcellular Distribution of Zinc in the Liver and the Kidney

	Treatment	Nuclei and debris %	Mitochondria %	Microsomes %	Soluble fraction %
Liver	Control	35.2	12.4	10.5	42.0
	Hg-Zn	23.9	12.4	5.2	58.6
	Zn	28.5	13.0	4.7	53.8
Kidney	Control	25.9	24.7	13.8	35.6
	Hg-Zn	33.7	9.0	9.6	47.8
	Zn	20.8	18.7	12.2	48.3

Treatment: Experimental conditions are as given in Table II.
Samples were prepared from 3 rats. Each value represents percentage of zinc distribution in each fraction.

Percentage of zinc distribution in the soluble fraction from the rats administered with mercury and zinc concurrently and zinc alone, was greater than that from control rats. Namely, it was found that majority of zinc increased by zinc administration accumulated in the soluble fraction.

Sephadex G-75 Chromatography of Soluble Fraction from the Liver

Both mercury and zinc were accumulated mostly in the soluble fraction from the liver and the kidney, and majority of zinc increased by zinc administration was found to accumulate in the soluble fraction. Judging from these facts, it was considered possible that the behavior of mercury and zinc in the soluble fraction is closely related to the appearance of mercury toxicity. Therefore, as shown in Fig. 2, the soluble fraction from the liver was filtered through Sephadex G-75 and distribution of zinc in these fractions was examined.

The absorption at 280 nm of protein obtained from the rat administered with zinc showed the same pattern as that from control rat. When zinc was administered, amount of zinc in HM-fraction was equal to that of control rat. But a large peak was found to be eluted at 60 ml of elution volume. Zinc binding protein does not absorption at 280 nm and was eluted at the same position as cytochrome C (Mol. Wt. 12400) and then, it seemed to be metallothionein(mol.wt. 10000) found by Vallee.[10,11]

Based on the fact that majority of zinc increased by zinc administration was present in metallothionein fraction, effect of zinc and mercury on biosynthesis of metallothionein was examined.

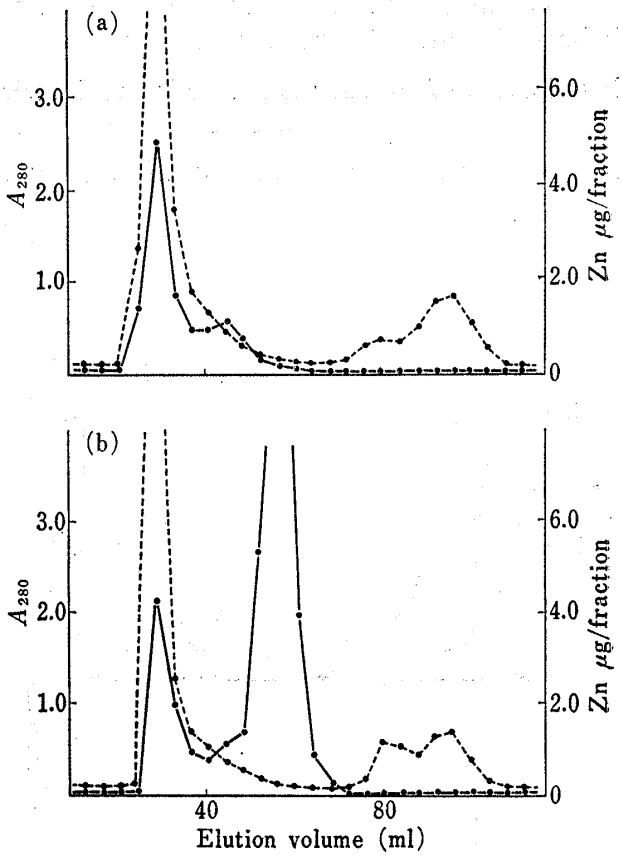

Fig. 2. Sephadex G-75 Chromatogram of Soluble Fraction from the Liver

(a) control, (b) Zn-treated
Treatment: control; 0.9%NaCl *s.c.*, Zn-treated; $Zn(AcO)_2$ 0.75 mmol/kg/day *s.c.* for 3 days.
Soluble fraction from the liver was filtered through Sephadex G-75 column (1.8×36 cm) with 0.01M Tris-HCl buffer, at pH 7.4, and 4°. Fraction of 4 ml was collected for analysis of zinc and then, amount of zinc was determined. The absorption at 280 nm was also measured.
●-----●, A_{280}; ●——●, Zn μg/fraction.

Biosynthesis of Metallothionein by Mercury and Zinc in the Liver and the Kidney

Metallothionein is occupied about 30% of its constituent amino acid by cysteine.[12] Therefore, incorporation of ^{14}C-cysteine into MT-fraction was examined and biosynthesis of metallothionein was determined. As shown in Fig. 3, high rate of incorporation of ^{14}C-cysteine into HM-fraction and LM-fraction in the liver of control rat, was observed. By the administration of zinc, high rate of incorporation of ^{14}C-cysteine into MT-fraction in the liver, was found very remarkably, but by the administration of mercury, the incorporation into MT-fraction was not so marked.

In the kidney, as shown in Fig. 4, high rate of incorporation of ^{14}C-cysteine into HM-fraction and LM-fraction was observed in the control rat. By the administration of zinc, some incorporation of ^{14}C-cysteine into MT-fraction was observed, but was not so greatly

10) J.H.R. Kägi and B.L. Vallee, *J. Biol. Chem.*, **235**, 3460 (1960).
11) J.H.R. Kägi and B.L. Vallee, *J. Biol. Chem.*, **236**, 2435 (1961).
12) G.F. Nordberg, M. Nordberg, M. Piscator, and O. Vesterberg, *Biochem. J.*, **126**, 491 (1972).

as compared with the liver. By the administration of mercury some incorporation of ^{14}C-cysteine into MT-fraction was observed, too.

Distribution of Mercury and Zinc in the Soluble Fraction from the Liver and the Kidney

The distribution of mercury was shown in Table V. In the liver, the amount of mercury in the soluble fraction was not affected by the concurrent administration of mercury and

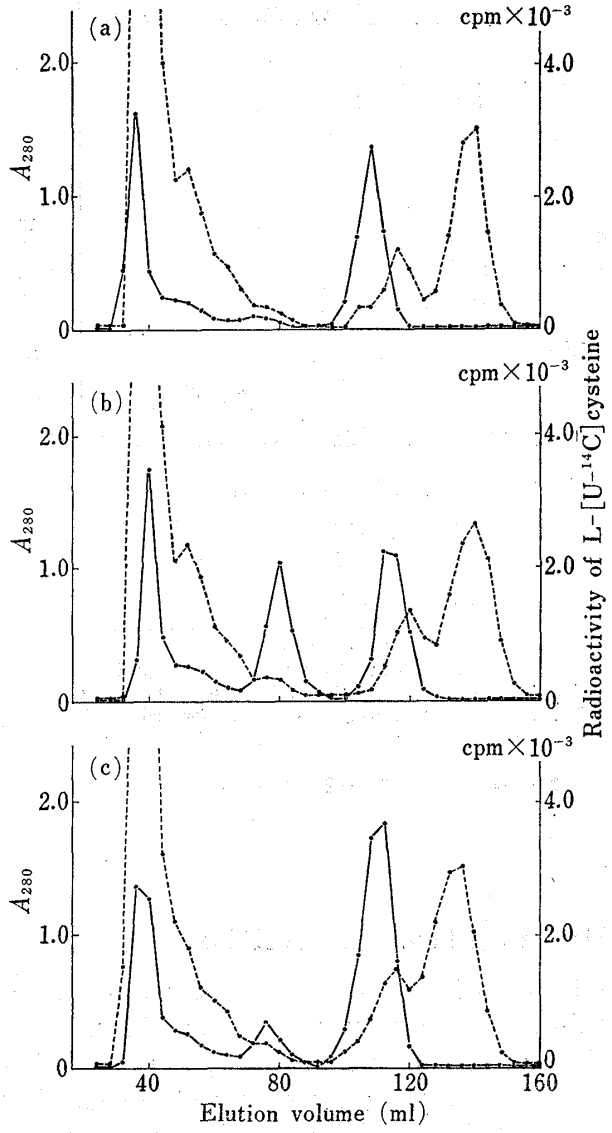

Fig. 3. Chromatogram of Liver Soluble Fraction of Rats Treated with L-[U-^{14}C]cysteine

Liver soluble fraction was fractionated by gel filtration on the column (2.5×36 cm) of Sephadex G-75 with 0.01M Tis-HCl buffer, at pH 7.4 and 4°. Eluates (4 ml fractions) were analysed for radioactivity (●——●) and protein (A_{280}) (●------●).
Treatment: (a): control, (b): $Zn(AcO)_2$ 0.75 mmol/kg/day (*s.c.*), (c): $HgCl_2$ 0.01 mmol/kg/day once every 24 hr for 2 days (*s.c.*), (a) (b) (c): 5 μCi L-[U-^{14}C]cysteine/rat treated 24 hrs before killing (*i.p.*).

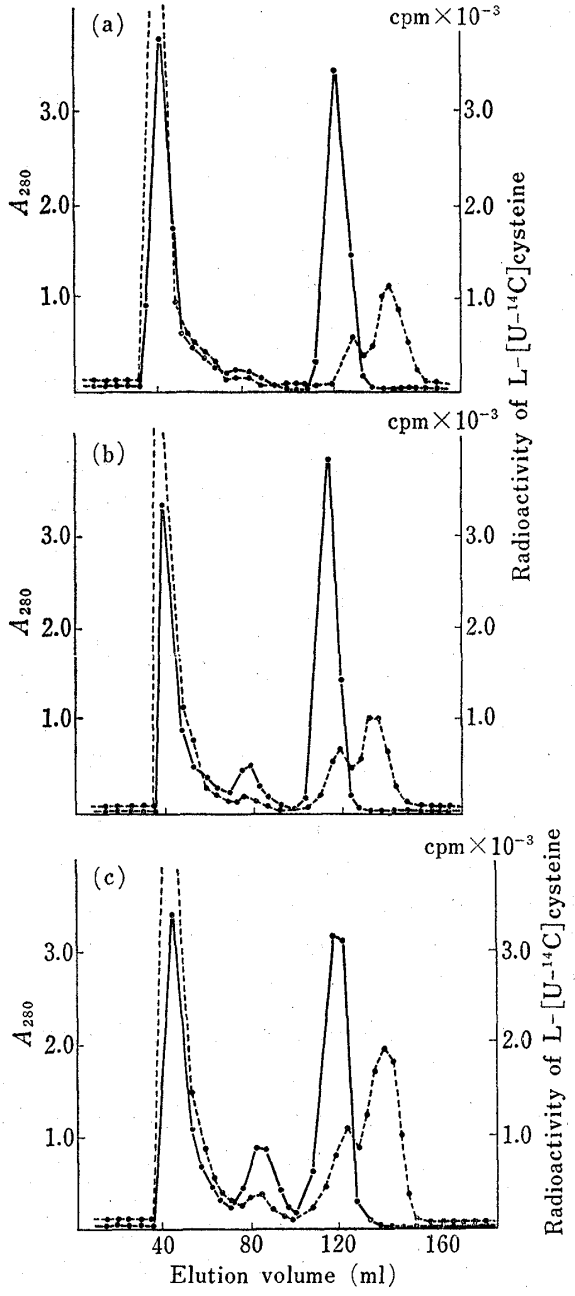

Fig. 4. Chromatogram of Kidney Soluble Fraction of Rats Treated with L-[U-^{14}C]cysteine

Kidney soluble fraction was fractionated by gel filtration on the column (2.5×36 cm) of Sephadex G-75 with 0.01M Tris-HCl buffer, at pH 7.4 and 4°. Eluates (4 ml fractions) were analysed for radioactivity (●——●) and protein (A_{280}) (●------●).
Treatment: (a): control, (b): $Zn(AcO)_2$ 0.75 mmol/kg/day (*s.c.*), (c): $HgCl_2$ 0.01 mmol/kg/day once every 24 hr for 2 days (*s.c.*), (a) (b) (c): 5 μCi L-[U-^{14}C]cysteine/rat treated 24 hr before killing (*i.p.*).

zinc, but there was great change in the distribution of mercury. Mercury was bound in 59% to HM-fraction and in 40% to MT-fraction when mercury alone was given, but it was bound in 24% to HM-fraction and in 75% to MT-fraction when mercury and zinc were given concurrently.

Table V. Distribution of Mercury in the Soluble Fraction from the Liver and the Kidney

	Treatment	Soluble fraction Hg μg /0.5gtiss.	HM-fraction Hg μg (%)	MT-fraction Hg μg (%)	LM-fraction Hg μg (%)	MT/HM
Liver	Hg	1.95	1.11±0.03(59)	0.81±0.08(40)	0.03±0.00(1)	0.72
	Hg-Zn	1.89	0.45±0.02(24)	1.42±0.01(75)	0.02±0.00(1)	3.20
Kidney	Hg	6.96	2.83±0.33(41)	3.90±1.07(56)	0.23±0.04(3)	1.39
	Hg-Zn	14.29	2.26±0.05(16)	11.80±0.60(83)	0.23±0.03(2)	5.23
	Δ[(Hg-Zn)-(Hg)]μg	+7.33	−0.57	+7.88	±0.00	

Treatment: Experimental conditions are as given in Table II.
MT/HM; the ratio of mercury contents of MT to HM, Δ[(Hg-Zn)−(Hg)]; amount of mercury increased.
Each value represents the mean ± S.E. of 3 rats.

The ratio of mercury contents of MT-fraction to HM-fraction in the liver, was 0.72 and 3.20 when mercury was injected without or with zinc, respectively. In the kidney, a large amount of mercury was bound to MT-fraction by concurrent administration of mercury and zinc. The ratio of MT-fraction to HM-fraction in the kidney, was 1.39 and 5.23 when mercury was injected without or with zinc, respectively. The ratio in the concurrent administration of mercury and zinc, was about 4 times greater than that in the administration of mercury alone. Total mercury content in the soluble fraction of the concurrent administration of mercury and zinc, was about twice that of mercury alone. All amount of mercury increased was accumulated in MT-fraction.

As shown in Table VI, amount of zinc accumulated in MT-fraction by the concurrent administration of mercury and zinc, was lower than that by the administration of zinc alone in the liver.

Table VI. Distribution of Zinc in the Soluble Fraction from the Liver and the Kidney

	Treatment	Soluble fraction Zn μg	HM-fraction Zn μg (%)	MT-fraction Zn μg (%)	MT/HM
Liver	Hg-Zn	33.0	10.9±0.8(33)	22.1±1.2(67)	2.03
	Zn	40.1	10.7±0.6(27)	29.4±1.5(73)	2.75
Kidney	Hg-Zn	13.4	6.7±1.0(50)	6.7±0.8(50)	1.00
	Zn	10.8	4.6±0.4(42)	6.2±0.5(58)	1.35

Treatment: Experimental conditions are as given in Table II.
MT/HM; the ratio of zinc contents of MT to HM. Each value represents the mean ± S.E. of 3 rats.

Degree of Affinity of Mercury with Zn-thionein *in Vitro*

Amount of mercury bound to metallothionein tended to be larger when mercury was given with zinc than when mercury alone was given. Therefore, degree of affinity of mercury with Zn-thionein was examined *in vitro*. As shown in Fig. 5, molar ratio of mercury to zinc was examined and then, total amount of mercury plus zinc in MT-fraction was not affected by the addition of mercury. The results showed that amount of mercury bound to thionein

increased and that of zinc decreased according to increase of the mercury concentration. It was found that degree of affinity of mercury with Zn-thionein was high. When 10 and 20 μg of mercury was added, 85 percent of added mercury was found in MT-fraction.

Effect of Mercury and Zinc on Fructose-1,6-diphosphate Aldolase(FDP-Aldolase) and Lactate Dehydrogenase(LDH) Activity

As shown in Fig. 6, it was found that FDP-Aldolase activity lowered remarkably by the administration of mercury was restored by the concurrent administration of mercury and zinc and then, LDH activity showed the same tendency as FDP-Aldolase activity.

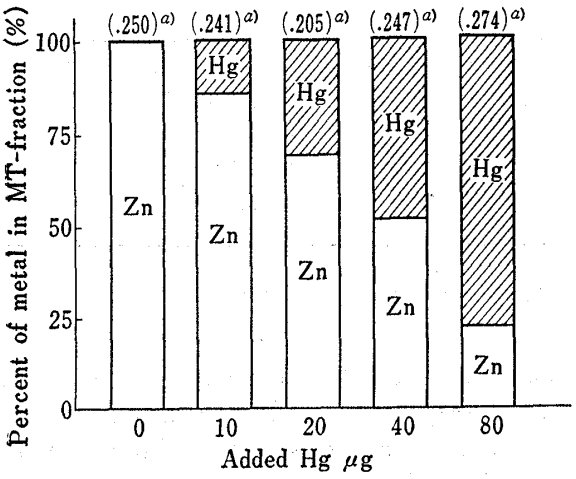

Fig. 5. Degree of Affinity of Mercury with Zn-thionein *in Vitro*

Soluble fraction was obtained from the liver of the rat treated with zinc acetate (0.75 mmol/kg) subcutaneously once.

Mercury (0, 10, 20, 40, 80, μg as Hg^{2+}) was added to 2 ml of the soluble fraction and the specimen was incubated at 37° for 30 min. Thereafter, it was filtered through Sephadex G-75 column and the amount of mercury and zinc bound to thionein, was measured.

a) Total(Zn+Hg) μmol concentration in MT-fraction.

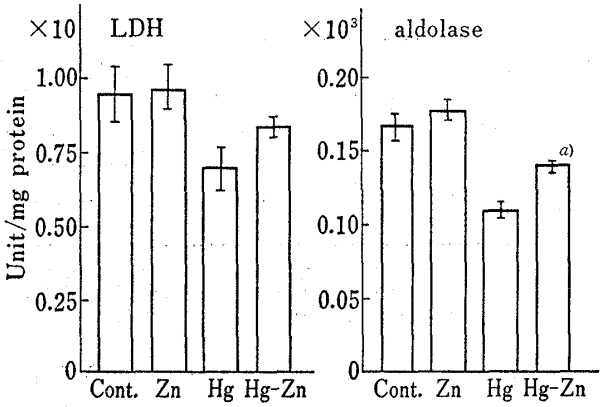

Fig. 6. Effect of Mercury and Zinc on Fructose-1,6-diphosphate Aldolase (FDP-Aldolase) and Lactate Dehydrogenase(LDH) Activity

Treatment: control; 0.9% NaCl *s.c.* once a day, Zn; $Zn(AcO)_2$ (1.50 mmol/kg) *p.o.* once a day, Hg; $HgCl_2$ (0.014 mmol/kg) *s.c.*, once a day, Hg-Zn; $HgCl_2$ (0.014 mmol/kg) *s.c.* and Zn $(AcO)_2$ (1.50 mmol/kg) *p.o.*, once a day simultaneously. The animals were administered with 0.9% NaCl, mercury and zinc for 2 days, respectively, and decapitated 24 hours after the last administration. They were preinjected with zinc once before the beginning of the concurrent administration of mercury and zinc. Each value represented the mean ± S.E. of 3 rats. Significance of difference from the group treated with mercury alone,[a)] $p<0.05$.

Discussion

Effect of zinc on the toxicity of mercuric chloride is very marked and the suppressive rate was 100% in mortality. Since the target organs of mercury were the liver and the kidney, amount of mercury in the liver and the kidney was examined to elucidate the action mechanism of zinc in lowering the toxicity of mercury. Amount of mercury in the liver decreased slightly by the concurrent administration of mercury and zinc as compared with the administration of mercury alone, but a large amount of mercury was accumulated in the kidney by the concurrent administration of mercury and zinc. This very interesting fact that lowering of mercury toxicity is accompanied by a large accumulation of mercury in the kidney suggests that the lowering of toxicity is not due to lowering of absorption or accelerated excretion of mercury. Consequently, cellular distribution of mercury and zinc in the liver and the kidney was examined, but there was no great difference in their distribution pattern, and both metals were accumulated mostly in the soluble fraction. A fairly large amount of zinc was also present in the control rat and majority of zinc increased by zinc administration was found to be accumulated in the soluble fraction. It was therefore considered possible that the behavior of mercury and zinc in the soluble fraction is closely related to the appearance of toxicity of mercury and its suppression.

Therefore, the soluble fraction from the liver and the kidney were filtered through Sephadex G-75 and distribution of zinc in these fractions after single administration of zinc was examined. Majority of zinc was found to be bound to metallothionein with a molecular weight of around 10000 and which does not have a large absorption at 280 nm. This metallothionein[10,11,13–16] has a small molecular weight as a protein, does not show absorption at 280 nm due to the absence of aromatic amino acid, and about 30% of its constituent amino acid is occupied by cysteine whose thiol group binds to Zn^{2+} and Cu^{2+}, as well as poisonous metals like Cd^{2+} and Hg^{2+}, and this is considered to be responsible for its detoxication of heavy metals.

Based on these evidences, we considered that metallothionein was taking part in the lowering of mercury toxicity by zinc and, therefore, biosynthesis of metallothionein during the concurrent administration of mercury and zinc was examined. Incorporation of ^{14}C-cysteine into metallothionein induced by mercury and zinc was observed both in the liver and the kidney, but that of ^{14}C-cysteine into metallothionein by zinc, was highly marked in the liver, and that of ^{14}C-cysteine by mercury, being very slight. Incorporation of ^{14}C-cysteine into metallothionein induced by mercury, was slightly larger than that of ^{14}C-cysteine by zinc, in the kidney.

Biosynthesis of metallothionein induced by zinc has been suggested by many workers; Webb and others[17] by working with female rat liver, Kimura and others[18] with rabbit liver and kidney, and Bremner and others[19–21] with rat liver and pancreas. On the other hand, Lucis and others[22] reported that administration of zinc chloride did not increase the formation of cadmium-bound protein.

Many workers have reported the biosynthesis of metallothionein in the kidney by mercury.[23–26] Piotrowski and others[25] reported that the amount of protein in metallothionein fraction did not increase in the liver after long exposure to mercury and that there was no incorporation of ^{14}C-cysteine. However, this is a result obtained after administration of a small amount of mercury over a long period and leaves the possibility that administration of a large amount of mercury might induce metallothionein formation. Our experimental result also indicated that there is little incorporation of ^{14}C-cysteine into metallothionein fraction in the liver as compared with that in the kidney, and biosynthesis of metallothionein maintained mercury would be small in the liver. This may have a great effect on the appearance of toxicity of mercury.

A considerable amount of zinc is present in the usual diet given to rats and we have found 30—40 ppm of zinc in the diet used in our experiments. Since zinc was not found to be bound to metallothionein in control rats, this amount of zinc might not be sufficient to induce metallothionein formation. In this respect, it is interesting that Bremner[19] stated that zinc in metallothionein fraction began to increase when zinc in the liver increased to above 30 μg/g tissue.

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Examination of the distribution of mercury and zinc in the soluble fraction from the liver and the kidney showed that, in the liver, mercury that was bound in larger amount to HM-fraction during single administration of mercury transited to metallothionein when zinc was administered concurrently. This fact suggests that single administration of mercury does not induce metallothionein greatly, and mercury is bound to HM-fraction to show its toxicity but the concurrent administration of zinc and mercury results in binding of mercury with metallothionein induced by zinc and the amount of mercury bound to HM-fraction including enzyme protein like thiol enzymes decreases, resulting in the suppression of its toxicity.

The fact that mercury is separated in the form bound to metallothionein in the kidney, where mercury is accumulated in the largest quantity, suggests decrease of mercury in other tissues of the rat. The amount of mercury bound as Hg-thionein in the kidney after the concurrent administration of mercury and zinc is about 4 times that after single administration of mercury, and this binding is considered to play a large part in lowering the toxicity of mercury.

Examination of distribution of zinc in the organs showed that the amount of zinc bound to metallothionein, both in the liver and in the kidney, tended to be smaller when zinc was given with mercury than when zinc alone was given, suggesting that mercury might replace zinc in Zn-thionein. Therefore, degree of affinity of mercury with Zn-thionein was examined *in vitro*, and the result showed that the addition of mercury resulted in the release of zinc and binding of mercury to thionein. There is also a possibility that mercury not only replace zinc in Zn-thionein but also binds to excess of free thionein synthesized by zinc, and it seems rational to consider biosynthesis of excess of free thionein by zinc for detoxication of heavy metals but this point needs further examinations.

On the other hand, amount of mercury increased markedly in the kidney with the concurrent administration of mercury and zinc. Incorporation of ^{14}C-cysteine into MT-fraction induced by the concurrent administration of mercury and zinc was nearly equal to that by the administration of mercury alone and zinc alone. Judging from these facts, zinc might have accelerating effect on the biosynthesis of metallothionein induced by mercury in the kidney and mercury bound to metallothionein induced by zinc in the liver might have transited to the kidney.[27]

Examinations on representative thiol enzymes present in HM-fraction of the soluble fraction from the liver showed that the enzyme activity lowered by mercury was found to be restored by the concurrent administration of mercury and zinc, supporting our assumed mechanism that mercury binds to the metallothionein synthesized by zinc, resulting in the isolation of mercury which loses affinity with other physiologically active sites and in the suppression of the toxicity. These examinations have suggested the important part played by metallothionein in the mechanism for the suppression of mercury toxicity by zinc.

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