[Chem. Pharm. Bull. 32(6)2316—2324(1984)]

Protective Effect of Molybdenum on the Acute Toxicity of Mercuric Chloride. III

TOSHIAKI KOIZUMI* and YASUHIRO YAMANE

Faculty of Pharmaceutical Sciences, Chiba University, 1–33, Yayoi-cho, Chiba 260, Japan

(Received September 3, 1983)

In order to gain a better understanding of the protective mechanism of Na_2MoO_4 against the acute toxicity of $HgCl_2$ in rats, turnover of ^{35}S-cysteine in the liver and kidney cytosols of rats given $HgCl_2$ (0.03 mmol/kg, once, *s.c.*) with or without Na_2MoO_4 pretreatment (1.24 mmol/kg, once a day for 3 days, *i.p.*) was investigated, together with lactic dehydrogenase (LDH) activity and mercury content in the cytosols. In the liver cytosol, there was no appreciable difference in the radioactivity of ^{35}S-cysteine incorporated into the high molecular weight (HM) fraction at various times between Hg-dosed and Mo–Hg-dosed groups, but that incorporated into the metallothionein-like (MT) fraction was higher in Hg-dosed rats than in Mo–Hg-dosed rats. In the kidney cytosol, the radioactivity of ^{35}S-cysteine was considerably higher in Mo–Hg-dosed rats than in Hg-dosed rats in all fractions including the MT fraction. On the other hand, the radioactivities of ^{35}S-cysteine incorporated into HM and MT fractions of liver and kidney cytosols decreased more rapidly in Mo–Hg-dosed rats than in Hg-dosed rats.

The rate in decrease of LDH activity observed in the liver and kidney cytosols after exposure to $HgCl_2$ was smaller in Mo–Hg-dosed rats than in Hg-dosed rats, although there was no difference in mercury content of HM fraction in the cytosols of these tissues at the same time point between the two groups.

These results suggest that Na_2MoO_4 may alleviate the acute $HgCl_2$ toxicity by affecting the metabolism of cysteine-containing proteins, including metallothionein-like protein, in the liver and kidney cytosols.

Keywords——acute toxicity; mercuric chloride; protective effect; sodium molybdate; lactic dehydrogenase

We recently reported that the acute toxicity of $HgCl_2$ in rats was alleviated by pretreatment with Na_2MoO_4 and that the protective mechanism of Na_2MoO_4 was related to the ability of this metal to decrease mercury content in the liver, kidney and spleen by affecting urinary excretion of mercury.[1] On the other hand, our subsequent study on the protective mechanism of Na_2MoO_4 against the acute toxicity of $HgCl_2$ demonstrated that the pretreatment of rats with Na_2MoO_4 increased mercury content in their renal metallothionein-like fraction.[2] This suggests that metallothionein may be involved in the protective mechanism of Na_2MoO_4 against the acute toxicity of $HgCl_2$. However, the role of molybdenum in the detoxification process of mercury that is related to metallothionein-like protein is not clear at present.

Metallothionein is a low molecular weight cytoplasmic protein which possesses high affinity for heavy metals, such as zinc, cadmium and mercury, owing to its cysteine-rich amino acid composition, and it can bind 6 to 7 mol of such metals per mol of the protein.[3] Thus, an increase of mercury content in the renal metallothionein-like fraction of rats given $HgCl_2$ after pretreatment with Na_2MoO_4 suggests that molybdenum may affect the biosynthesis or degradation of metallothionein-like protein, directly or indirectly. If this is true, molybdenum may also affect the metabolism of other cysteine-containing proteins and substances in the cytosol.

In the present study, we investigated whether or not the protective action of Na_2MoO_4 against acute $HgCl_2$ toxicity is related to metabolic alteration of cysteine-containing proteins and substances in the liver and kidney cytosols by measuring the turnover of ^{35}S-cysteine in the cytosols of those tissues of rats given $HgCl_2$ with or without Na_2MoO_4 pretreatment, together with activity of lactic dehydrogenase (a mercury-sensitive sulfhydryl-containing enzyme) and mercury content in the cytosols.

It is known that the toxic action of mercury within cells is closely connected with the interaction of the metal and protein-bound sulfhydryl groups,[4] and is influenced by non-protein sulfhydryl groups.[5]

Materials and Methods

Male Wistar strain rats (Matsumoto Labo-Animals Laboratory), each weighing 160—180 g, were used in all experiments. Animals were fed a commercial solid diet (CE-2, Clea Japan) *ad libitum* and were allowed free access to tap water.

[L] ^{35}S-Cysteine (1160 Ci/mmol, 11.13 mCi/ml in 20 mM potassium acetate containing 5 mM dithiothreitol) was obtained from Amersham. The labeled cysteine was diluted in 0.9% NaCl to give a fanal concentration of 40 μCi/ml. Sephadex G-75 was purchased from Pharmacia Fine Chemicals. All the other chemicals used were of reagent grade.

Turnover of ^{35}S-Cysteine in the Liver and Kidney Cytosols——A total of 24 rats was randomly divided into four treatment groups of 6 rats each. The rats in group 1 received 0.9% NaCl until the labeled cysteine was given. The rats in group 2 received Na_2MoO_4 (*i.p.*) dissolved in saline at a dose of 1.24 mmol/kg once a day for 3 days. At 24 h after the final *i.p.* injection of Na_2MoO_4, 0.9% NaCl was (*s.c.*) given once. The rats in group 3 were treated with 0.9% NaCl once a day for 3 days and were given one *s.c.* injection of $HgCl_2$ dissolved in saline at a dose of 0.03 mmol/kg at 24 after the final *i.p.* injection of saline. The rats in group 4 received the same dose of $HgCl_2$ as group 3 after the final *i.p.* injection of Na_2MoO_4 as in group 2. At 4 h after $HgCl_2$ or saline administration, 10 μCi/kg of ^{35}S-cysteine was given (*i.p.*) to all the animals in all experimental groups. Three rats from each group were sacrificed by cervical dislocation 12 and 40 h after injection of the labeled cysteine. Livers and kidneys were perfused with iced 1.15% KCl. Then the tissues were rapidly removed, rinsed with the same solution and blotted. The tissues were weighed and homogenized immediately with 5 vol. of iced 1.15% KCl in a Waring blender. The soluble fraction was prepared according to the method of Schneider and Hogeboom.[6] In a separate experiment, the rats were treated with ^{35}S-cysteine 38 h after exposure to $HgCl_2$ and then killed 4 h after the labeled cysteine injection, to assess the difference between Hg-dosed and Mo–Hg-dosed rats as regards incorporation rate of the a labeled cysteine into the liver and kidney cytosols at later time after exposure to $HgCl_2$. The experimental conditions were the same as in the above turnover study.

A 5 ml portion of liver and kidney soluble fraction was applied to a Sephadex G-75 column (2 × 70 cm) equilibrated with 0.02 M Tris-HCl buffer, pH 8.6, at 4 °C. The cytosol was eluted with the same buffer at a flow rate of 0.23 ml/min and 4 ml of column effluent per tube was collected. Absorption at 280 nm was monitored to determine relative protein concentration in the column effluent. A 1.5 ml portion of each fraction was dissolved in 10 ml of Triton X-100/toluene scintillant (about 3 : 7) and ^{35}S content was measured with a Beckman LS-100C liquidscintillation spectrometer. After counting of the radioactivity, liver and kidney cytosols were classified into three fractions according to the apparent molecular size of the labeled cysteine incorporated, as shown in Fig. 1. The fractions were as follows: high molecular weight (HM) fraction, tube numbers 11—25, metallothionein-like (MT) fraction, tube

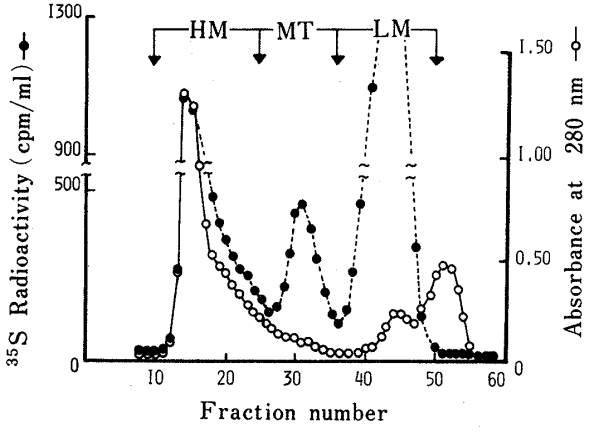

Fig. 1. Sephadex G-75 Gel Filtration Profile of ^{35}S-Cysteine Incorporated into the Liver Cytosol of Rats Given $HgCl_2$ Alone

A 5 ml portion of the liver cytosol was applied on a Sephadex G-75 column (2 × 70 cm) equilibrated with 0.02 M Tris-HCl, pH 8.6, at 4 °C. The cytosol was eluted with the same buffer at a flow rate of 0.23 ml/min. The ^{35}S radioactivity was counted in a collection tube, and the cytosol was classified according to the apparent molecular weight of the labeled cysteine incorporated. HM, high molecular weight fraction (tube numbers 11—25); MT, metallothionein-like fraction (tube numbers 26—35); LM, low molecular weight fraction (tube numbers 36—50).

numbers 26—35, low molecular weight (LM) fraction, tube numbers 36—50. ^{35}S radioactivities within these fractions were summed to assess the rate of incorporation or elimination of the labeled cysteine.

Mercury Content and Lactic Dehydrogenase (LDH) Activity in the Liver and Kidney Cytosols after Exposure to $HgCl_2$——A total of 60 rats was divided into four groups of 15 rats each. The rats were treated with Na_2MoO_4 and $HgCl_2$ as in the above turnover experiment. Five rats from each group were killed at 8, 24 and 44 h after exposure to $HgCl_2$. The liver and kidney cytosols were prepared as in the above experiment. A 5 ml portion of liver or kidney cytosol was chromatographed on the same size Sephadex G-75 column as in the above experiment. After the determination of mercury content in the column effluent, the metal contents in the fractions classified as in the above experiment were summed. Mercury content in the liver cytosol 24 h after administration of $HgCl_2$ was not determined.

LDH activity was measured in the cytosol fraction (105000 *g* sup.) prepared from the livers and kidneys 44 h after exposure to $HgCl_2$, according to the method of Hill.[7]

Results and Discussion

In order to elucidate the protective mechanism of Na_2MoO_4 against the acute toxicity of $HgCl_2$, the turnover of ^{35}S-cysteine in the liver and kidney cytosols after exposure to $HgCl_2$ was investigated. For this purpose, the liver and kidney cytosols prepared from four experimental groups (control, Mo-dosed, Hg-dosed and Mo–Hg-dosed groups) 12 and 40 h after labeled cysteine injection were gel-filtered on a Sephadex G-75 column. ^{35}S-cysteine was given to rats 4 h after exposure to $HgCl_2$.

In the Hg-dosed and Mo–Hg-dosed groups, three different ^{35}S radioactive peaks that correspond to HM, MT and LM fractions in Fig. 1 were observed in both tissues throughout this experiment. In the control and Mo-dosed groups ^{35}S radioactivity was observed only in the HM and LM fractions of both tissues.

^{35}S radioactivity within corresponding fractions was summed as described in Materials and Methods. Figure 2 shows the sum total of ^{35}S-cysteine radioactivity found in the respective fractions of the liver cytosols 12 and 40 h after the labeled cysteine injection. At 12 h, ^{35}S radioactivity was increased in all fractions of the Hg-dosed and in HM and MT fractions of the Mo–Hg-dosed group. There was no appreciable difference in ^{35}S content of the HM fraction between Hg-dosed and Mo–Hg-dosed groups, but that in the MT fraction was higher in the former than in the latter. The decreases of ^{35}S content in HM and MT fractions between 12 and 40 h were both larger in the Mo–Hg-dosed group than in the Hg-dosed group, as is evident from the differences in the slopes of the lines linking ^{35}S radioactivity values at the two time points.

On the other hand, there was no appreciable difference in ^{35}S radioactivity of HM and LM fractions at 12 h after the labeled cysteine injection between the control and Mo-dosed groups, but the decreases of ^{35}S radioactivity in both fractions between 12 and 40 h were both smaller in the Mo-dosed group than in the control group, and those of the Mo-dosed group were also smaller than those of the Mo–Hg-dosed group.

Figure 3 shows the sum total of ^{35}S radioactivity found in the respective fractions of kidney cytosol 12 and 40 h after the labeled cysteine injection. At 12 h, ^{35}S radioactivity was increased in the HM and LM fractions of the Mo-dosed group and in the HM and MT fractions of the Mo–Hg-dosed group, compared with the control group. The ^{35}S content in the HM and LM fractions of the Hg-dosed group was decreased, compared with those of the other three groups. On the other hand, in the Hg-dosed group, the decrease of ^{35}S radioactivity in the two fractions between 12 and 40 h was also reduced, compared with those of the other three groups.

There was a remarkable difference in the pattern of change in ^{35}S radioactivity of MT fraction from 12 to 40 h between the Hg-dosed and Mo–Hg-dosed groups. Namely, in the Hg-dosed group, ^{35}S radioactivity increased from 12 to 40 h, whereas that in the Mo–Hg-dosed group reached a peak at 12 h and was considerably decreased by 40 h.

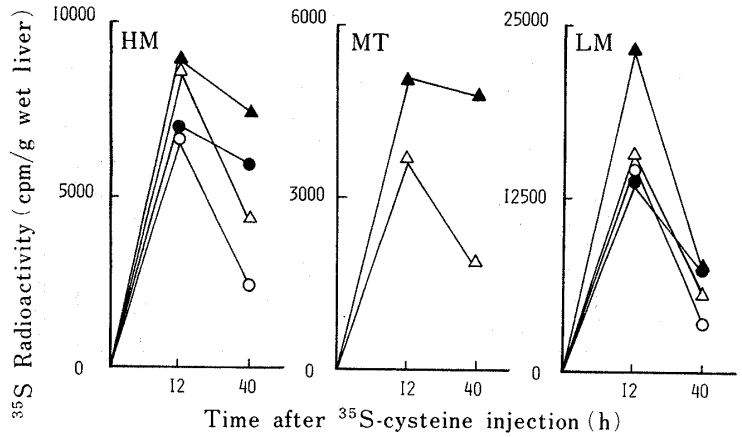

Fig. 2. Apparent ^{35}S-Cysteine Turnover in the Liver Cytosol after Treatment with NaCl or $HgCl_2$

The rats were treated with NaCl or Na_2MoO_4 or $HgCl_2$ or Na_2MoO_4 and $HgCl_2$ as described in Materials and Methods and were given ^{35}S-cysteine (10 μCi/kg, *i.p.*) 4 h after treatment with NaCl or $HgCl_2$. Three rats from each group were sacrificed 12 and 40 h after the labeled cysteine injection. The livers from the three rats were pooled for examination. The liver cytosol was classified into three fractions as in Fig. 1. Each value represents the sum total of ^{35}S radioactivity found in the respective fractions at each time point. HM, MT and LM are as in the legend to Fig. 1. ○—○, control group; ●—●, Mo-dosed group; ▲—▲, Hg-dosed group; △—△, Mo–Hg-dosed group.

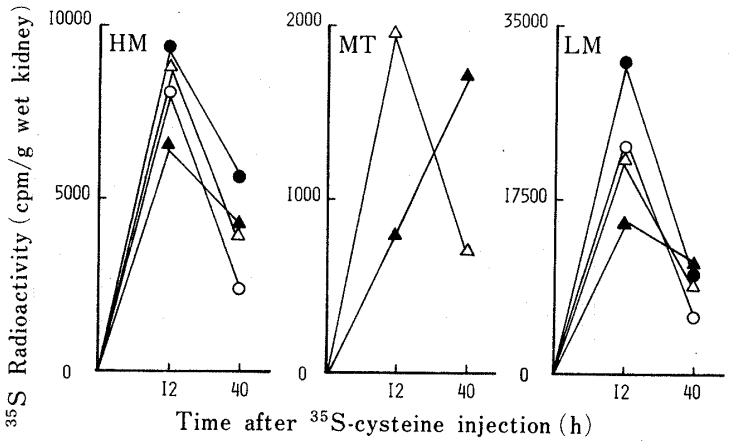

Fig. 3. Apparent Turnover of ^{35}S-Cysteine in the Kidney Cytosol after Treatment with NaCl or $HgCl_2$

This shows the results in the kidney cytosol corresponding to the liver cytosol described in Fig. 2. The kidney cytosol was processed and classified as described in Fig. 2. Each value represents the sum total of ^{35}S radioactivity found in the respective fractions at each time point. HM, MT and LM are as in the legend to Fig. 1. ○—○, control group; ●—●, Mo-dosed group; ▲—▲, Hg-dosed group; △—△, Mo–Hg-dosed group.

These data suggest that, in the liver, turnover of cytoplasmic cysteine-containing proteins including metallothionein-like protein was enhanced in the Mo–Hg-dosed group compared with the Hg-dosed group. In the kidney, the same phenomenon as in the liver was observed in high molecular weight proteins but there was a difference concerning the metabolism of metallothionein-like protein between the Hg-dosed and Mo–Hg-dosed groups.

We next examined the incorporation of ^{35}S-cysteine into liver and kidney cytosols at later time after exposure to $HgCl_2$. Rats were injected with ^{35}S-cysteine 38 h after the saline or $HgCl_2$ administration and were sacrificed 4 h later. The results are shown in Figs. 4 and 5. In

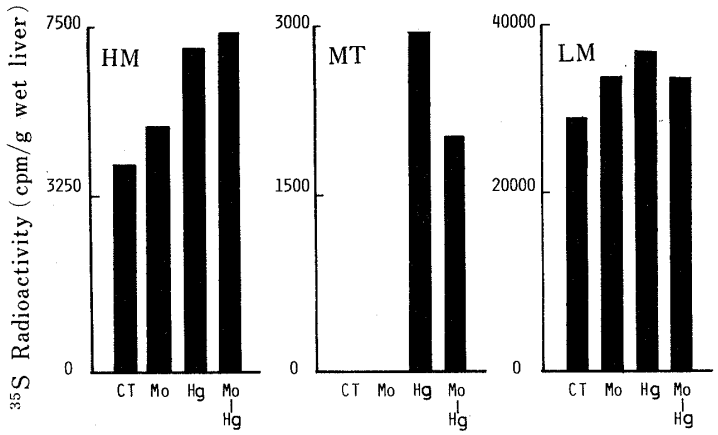

Fig. 4. Incorporation of ^{35}S-Cysteine into the Liver Cytosol after Administration of NaCl or $HgCl_2$

The rats were treated with NaCl or Na_2MoO_4 or $HgCl_2$ or Na_2MoO_4 and $HgCl_2$ as described in Materials and Methods and were given ^{35}S-cysteine (10 μCi/kg, *i.p.*) 38 h after treatment with NaCl or $HgCl_2$. The rats were sacrificed 4 h after the labeled cysteine injection. The liver cytosol was processed and classified as in Fig. 2. Each value represents the sum total of ^{35}S radioactivity found in the respective fractions. HM, MT and LM are as in the legend to Fig. 1. CT, control group; Mo, Mo-dosed group; Hg, Hg-dosed group; Mo–Hg, Mo–Hg-dosed group.

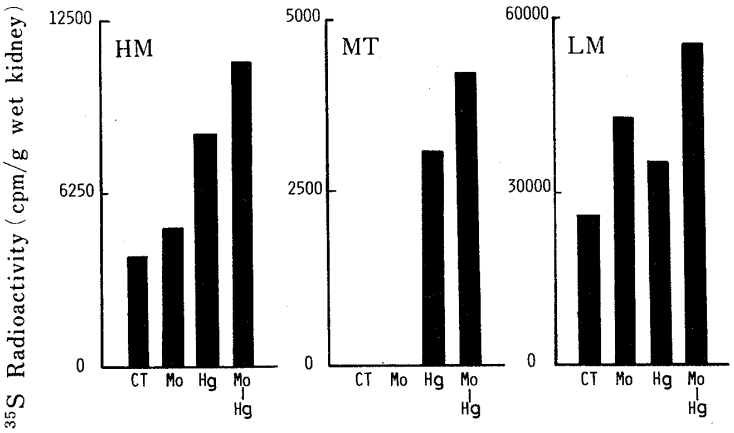

Fig. 5. Incorporation of ^{35}S-Cysteine into the Kidney Cytosol after Administration of NaCl or $HgCl_2$

This shows the results in the kidney cytosol corresponding to the liver cytosol described in Fig. 4. The kidney cytosol was processed and classified as in Fig. 2. Each value represents the sum total of ^{35}S radioactivity found in the respective fractions. HM, MT, LM, CT, Mo, Hg and Mo–Hg are as in the legend to Fig. 4.

the liver cytosol, incorporation of ^{35}S-cysteine was increased in the HM and LM fractions of the Mo-dosed, Hg-dosed and Mo–Hg-dosed groups, compared with that of the control group. Incorporation of the labeled cysteine into MT fraction was greater in the Hg-dosed group than in the Mo–Hg-dosed group.

In the kidney cytosol, incorporation of ^{35}S-cysteine was increased in the HM and LM fractions of the Mo-dosed, Hg-dosed and Mo–Hg-dosed groups, as was the case for the liver cytosol. Incorporation of the labeled cysteine into the MT fraction was greater in the Mo–Hg-dosed group than in the Hg-dosed group.

Several conclusions were drawn from the above results. Firstly, the ability to synthesize

cytoplasmic high molecular weight proteins which contain cysteine was enhanced in the livers of the Hg-dosed and Mo–Hg-dosed groups after exposure to $HgCl_2$, and the inducibility of metallothionein-like protein was larger in the Hg-dosed group than in the Mo–Hg-dosed group. Secondly, in the kidney, the ability to synthesize cytoplasmic high molecular weight proteins which contain cysteine was consistently stimulated in the Mo–Hg-dosed group, and this ability was also enhanced in the kidney of the Mo-dosed group at a relatively early time after exposure to $HgCl_2$ but then decreased. Thirdly, in the kidney of the Hg-dosed group, *de novo* synthesis of cytoplasmic high molecular weight proteins which contain cysteine was retarded at a relatively early time after exposure to $HgCl_2$ and then recovered gradually. Fourthly, the inducibility of metallothionein-like protein in the kidney after exposure to $HgCl_2$ was consistently larger in the Mo–Hg-dosed group than in the Hg-dosed group. Fifthly, the turnover of cysteine-containing proteins in the liver and kidney cytosols, including metallothionein-like protein was more rapid in the Mo–Hg-dosed group than in the Hg-dosed group.

In a separate experiment, we determined the mercury content in the liver and kidney cytosols prepared from the Hg-dosed and Mo–Hg-dosed groups, to assess the relationship between the metabolism of cysteine-containing proteins and accumulation of mercury. The cytosol was classified as shown in Fig. 1 after gel filtration on a Sephadex G-75 column. The results are shown in Fig. 6. In both tissues, mercury was found only in the HM and MT fractions. Mercury content in the HM and MT fractions of the liver increased with time in both groups. There was no appreciable difference in the mercury content of the HM fraction at 44 h between the two groups, but that in the MT fraction was greater in the Hg-dosed group than in the Mo–Hg-dosed group. These data suggest that there was a parallelism between accumulation of mercury in the HM and MT fractions of liver and incorporation of ^{35}S-cysteine into those fractions of the tissues, since there was no appreciable difference in ^{35}S radioactivity of the HM fraction at 16 and 42 h after exposure to $HgCl_2$ between the two groups and that in the MT fraction was greater in the Hg-dosed group than in the Mo–Hg-dosed group at both time points.

In the kidney cytosol, mercury content in the HM fraction of the Hg-dosed group decreased from 8 to 44 h, in contrast with the increase of the metal content in the MT fraction, but the mercury content in the HM fraction of this group was greater than that of the HM fraction of the Mo–Hg-dosed group up to 24 h. In the Hg-dosed group, incorporation of ^{35}S-cysteine into the HM fraction 16 h after exposure to $HgCl_2$ was less than those of the other groups and that into MT fraction increased from 16 to 44 h, so these data suggest that mercury may interact with high molecular weight proteins which do not contain cysteine as well as with cysteine-containing proteins including metallothionein-like protein at a relatively early time after exposure to $HgCl_2$. However, as metallothionein-like protein is biosynthesized, the metal preferentially interacts with this protein. On the other hand, in the Mo–Hg-dosed group, after 8 h exposure to mercury, about 55% of total mercury in the cytosol was associated with the MT fraction, and the metal content in this fraction increased linearly up to 44 h. However, mercury content in the HM fraction of this group did not decrease with time as in the Hg-dosed group. Mercury content in this fraction was unchanged up to 24 h and then increased to some extent. In this group, incorporation of ^{35}S-cysteine into HM and MT fractions 16 and 42 h after exposure to $HgCl_2$ was greater than in the other groups.

Alterations in the metabolism of cysteine-containing proteins in the cytoplasma may be important in relation to cellular injury by mercury. Our present results suggest that in the Mo–Hg-dosed group, turnover of cysteine-containing proteins, including metallothionein-like protein, in the liver and kidney cytosols was enhanced, compared with the Hg-dosed group. Thus, these groups may differ in mercury metabolism within the cells. However, there is no direct evidence supporting this hypothesis. We previously found that urinary excretion of

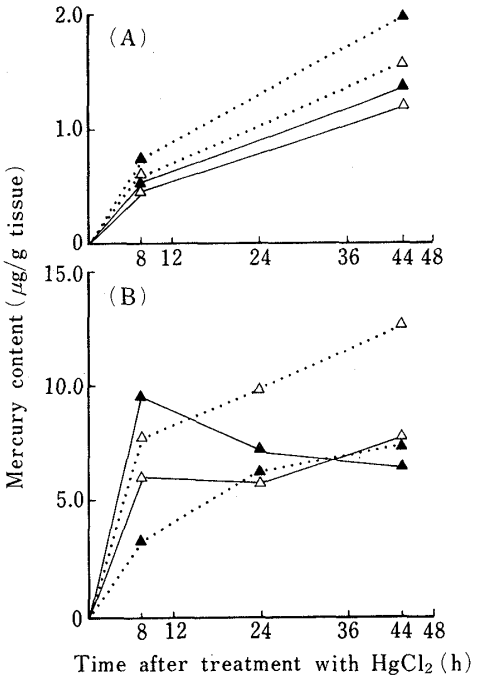

Fig. 6. Accumulation of Mercury in the Liver (A) and Kidney (B) Cytosol of Hg-Dosed (▲—▲) and Mo–Hg-Dosed (△—△) Groups

The rats were injected with Na_2MoO_4 and $HgCl_2$ as described in Materials and Methods and were sacrificed 8, 24 and 44 h after exposure to $HgCl_2$. A 5 ml portion of liver or kidney cytosol was gel-filtered on a Sephadex G-75 column (2 × 70 cm) and the mercury content in the column effluent fraction was determined. Then the liver or kidney cytosol was classified as in Fig. 1 and mercury contents within the respective fractions were summed. Solid and broken lines show the time course of accumulation of mercury in the high molecular weight and metallothionein-like fractions, respectively.

mercury was enhanced by Na_2MoO_4 pretreatment[1] and the pretreatment also decreased mercury content in some subcellular components in the liver and kidney;[2] this may be associated with such an alteration of the metabolism of cysteine-containing proteins. It is known that mercury is bound to sulfhydryl groups of cysteine and glutathion[8] and that complexes of sulfhydryl compound and mercury are present in the urine of rats treated with mercury.[8c] Moreover, it is known that the main excretory route of mercury is in the urine.[9]

We measured the activity of lactic dehydrogenase (LDH) in the liver and kidney cytosols prepared 44 h after exposure to $HgCl_2$. The results are shown in Tables I and II, respectively. In the Hg-dosed group, the enzyme activity was 77 and 66% of the corresponding control values. On the other hand, in the Mo–Hg-dosed group, no appreciable inhibition of the enzyme activity was observed in the liver cytosol, and in the case of kidney cytosol, the activity was decreased in both groups, compared with control, though the decrease was smaller in the Mo–Hg-dosed group.

These results are very interesting, since there were no appreciable differences in mercury content to the HM fraction in the liver and kidney cytosols 44 h after exposure to $HgCl_2$ between the Hg-dosed and Mo–Hg-dosed groups. It is known that this enzyme is inhibited in a dose-dependent manner by mercury.[7a] Again, the results may be connected with same alteration in the metabolism of cysteine-containing proteins in the liver and kidney cytosols.

As mentioned above, our present study suggests that pretreatment with Na_2MoO_4 may affect the metabolism of cysteine-containing proteins in the cytosols of liver and kidney of rats exposed to $HgCl_2$. This may be a combined effect of Na_2MoO_4 and $HgCl_2$, since either of these metals could stimulate the biosynthesis of cytoplasmic high molecular weight proteins which contain cysteine, but neither of them could enhance their degradation. Such an alteration in the metabolism of cysteine-containing proteins may imply that tissue regeneration was enhanced in the liver and kidney after treatment with Na_2MoO_4 and $HgCl_2$. Molybdenum may serve as a prehepatotoxic or prenephrotoxic agent to enhance the regeneration of tissue that might be damaged by subsequent administration of $HgCl_2$. It is known that synthesis and breakdown of proteins are enhanced before cell death.[10] It is also known that nephrotoxic agents which can promote regeneration of the tissue can reduce mercury toxicity.[11]

TABLE I. Effect of Molybdenum on the Inhibition of Rat Liver Lactic Dehydrogenase Activity by Mercury

Treatment and dose (mmol/kg)	Specific activity[a] mg of NADH oxidized /mg prot./min	Relative activity % of control
Control	6.56±0.20	100
Na_2MoO_4 (1.24)	6.52±0.34	99
$HgCl_2$ (0.03)	5.07±0.14	77
$Na_2MoO_4+HgCl_2$ (1.24) (0.03)	6.81±0.74[b]	104

a) Each value represents the mean±S.E. from 5 rats.
b) $p<0.10$. Significance of difference from Hg-dosed group.

TABLE II. Effect of Molybdenum on the Inhibition of Rat Kidney Lactic Dehydrogenase Activity by Mercury

Treatment and dose (mmol/kg)	Specific activity[a] mg of NADH oxidized /mg prot./min	Relative activity % of control
Control	4.53±0.03	100
Na_2MoO_4 (1.24)	4.42±0.03	98
$HgCl_2$ (0.03)	2.97±0.02	66
$Na_2MoO_4+HgCl_2$ (1.24) (0.03)	3.81±0.07[b]	84

a) Each value represents the mean±S.E. from 5 rats.
b) $p<0.01$. Significance of difference from Hg-dosed group.

On the other hand, an alteration of the metabolism of cysteine-containing proteins in the liver and kidney cytosols of the Mo–Hg-dosed group may imply catabolism of cytoplasmic proteins in the liver and kidney as a result of nonspecific stress. Molybdenum and mercury cause cooperatively cause stress. It is generally known that administration of chemical toxicants and changes in physiological conditions, such as cold and heat, induce stress.[12] Stress can elevate the blood levels of adrenocortical hormones[13] and adrenocortical hormones enhance protein breakdown by affecting the cellular content of lysosomes.[14,15] Although we previously found that Na_2MoO_4 pretreatment increased mercury content in the renal metallothionein-like protein fraction,[2] the present results suggest that this increase may be associated with biosynthesis of the protein. This may also be connected with stress. It is known that adrenocortical hormone and various types of stress induce metallothionein.[12,16] It is also noteworthy that molybdenum alters stress response in the rats.[17]

At present, we cannot adequately explain why the metabolism of cysteine-containing proteins should be altered in the liver and kidney cytosols of the Mo–Hg-dosed group. However, such changes might be significant for the protection of cells from mercury toxicity, in view of the interactions of the metal with sulfhydryl groups of high molecular weight proteins which contain cysteine[4] and with metallothionein.[18] Therefore, molybdenum may alleviate acute mercury toxicity by affecting the metabolism of cysteine-containing proteins in the cytoplasm of liver and kidney.

Acknowledgement The authors wish to thank Miss A. Matsuhisa for her skillful technical assistance throughout this study.

References

1) Y. Yamane and T. Koizumi, *Toxicol. Appl. Pharmacol.*, **65**, 212 (1982).
2) T. Koizumi and Y. Yamane, *Chem. Pharm. Bull.*, **32**, 631 (1984).
3) *a*) L. Magos and B. L. Vallee, *J. Am. Chem. Soc.*, **79**, 4813 (1957); *b*) J. H. R. Kagi and B. L. Vallee, *J. Biol. Chem.*, **236**, 2435 (1961); *c*) E. Sabbioni, *Environ. Physiol. Biochem.*, **5**, 132 (1957); *d*) J. H. R. Kagi and M. Nordberg, eds, "Metallothionein (Experientia supplementum 34)," Birkhauser Verlag, Basel, 1979, p. 46.
4) *a*) J. H. Stein, M. D. Lifschitz and L. D. Barnes, *Am. J. Physiol.*, **234**, F 171 (1978); *b*) R. C. Zalme, E. M. McDowell, R. S. Nagle, J. S. McNeil, W. Flamenbaum and B. F. Trump, *Virchows Arch. B. Cell Pathol.*, **22**, 197 (1976); *c*) W. W. Cleland, *Biochemistry*, **3**, 480 (1964).
5) *a*) R. J. Richardson and S. D. Murphy, *Toxicol. Appl. Pharmacol.*, **31**, 505 (1975); *b*) D. R. Johnson, *J. Toxicol. Environ. Health*, **9**, 119 (1982).
6) W. C. Schneider and G. H. Hogeboom, *J. Biol. Chem.*, **183**, 183 (1950).
7) *a*) B. R. Hill, *Cancer Res.*, **16**, 460 (1956); *b*) B. R. Hill, *Cancer Res.*, **31**, 272 (1961).
8) *a*) N. J. Taylor and A. J. Carty, *J. Am. Chem. Soc.*, **99**, 6144 (1977); *b*) B. J. Fuhr and D. L. Rabenstein, *J. Am. Chem. Soc.*, **95**, 6944 (1973); *c*) M. Jakubowski, J. Piotrowski and B. Trojanowska, *Toxicol. Appl. Pharmacol.*, **16**, 743 (1970).
9) A. Rothstein and A. D. Hayes, *J. Pharmacol. Exp. Ther.*, **130**, 166 (1960).
10) *a*) J. A. Popp, H. Shinozuka and E. Farber, *Am. J. Physiol.*, **74**, 58A (1974); *b*) I. D. Bowen, "Techniques for demonstrating cell death. Cell Death in Biology and Pathophysiology," ed. by I. D. Bowen and R. A. Lockshin, Chapman and Hall Ltd., New York, 1981, p. 379.
11) S. K. Tandon, L. Magos and J. R. P. Cabral, *Toxicol. Appl. Pharmacol.*, **52**, 227 (1980).
12) S. H. Oh, J. T. Deagen, P. D. Whanger and P. H. Weswig, *Am. J. Physiol.*, **234**, E282 (1978).
13) H. R. Hodges, M. T. Jones and M. A. Stockham, *Nature* (London), **193**, 1187 (1962).
14) *a*) B. S. Chertow, W. E. Buchanan, M. S. Mayron and T. B. Schwartz, *Endocrinology*, **92**, 722 (1973); *b*) M. F. Hopgood, M. G. Clark and F. J. Ballard, *Biochem. J.*, **196**, 33 (1981).
15) F. J. Ballard and J. M. Gunn, *Nutr. Rev.*, **40**, 33 (1982).
16) *a*) M. Karin and H. R. Herschman, *Science*, **204**, 176 (1979); *b*) M. L. Failla and R. J. Cousins, *Biochim. Biophys. Acta*, **543**, 293 (1978); *c*) C. D. Klaassen, *Toxicology*, **20**, 275 (1981); *d*) P. Z. Sobocinski, W. J. Canterbury, C. A. Mapes and R. E. Dinterman, *Am. J. Physiol.*, **234**, E399 (1978).
17) *a*) P. W. Winston, L. Hoffman and W. Smith, *Trace Substance Environ. Health*, **7**, 241 (1973); *b*) P. W. Winston and W. P. Trainor, *Trace Substance Environ. Health*, **12**, 254 (1979).
18) *a*) L. Magos, M. Webb and W. H. Butler, *Br. J. Exp. Pathol.*, **55**, 589 (1974); *b*) H. Takahashi and Y. Shibuya, *Toxicol. Appl. Pharmacol.*, **47**, 209 (1979).