Defense Mechanisms against Cadmium Toxicity
III. Effects of Pretreatment with a Small Oral Dose of Cadmium on Metallothionein Synthesis after a Large Oral Dose of Cadmium in Mice

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Abstract—Pretreatment of female mice with a small oral dose of Cd$^{2+}$ (15 mg Cd$^{2+}$/kg) decreased Cd$^{2+}$ uptake by the liver and kidney and increased that by the small intestinal mucosa at 4 or 24 hr after challenge with a large oral dose of Cd$^{2+}$ (100 mg Cd$^{2+}$/kg). By 4 hr after the challenge dose, more Cd$^{2+}$ taken up by the liver was bound to metallothionein (MT) in the Cd$^{2+}$-pretreated mice than the water-pretreated controls (10 ml H$_2$O/kg); but at 24 hr, the amount of Cd$^{2+}$ bound to MT in the liver and kidney were lower in the former than the latter. The amount of Cd$^{2+}$ not bound to MT in the liver at 4 and 24 hr after the challenge dose and that in the kidney at 24 hr were lower in the Cd$^{2+}$-pretreated mice than the water-pretreated controls. These results suggested that the factor directly related to the toxic action of Cd$^{2+}$ was the amount of Cd$^{2+}$ not associated with MT in the liver and other organs. More Cd$^{2+}$ taken up by the small intestinal mucosa at 24 hr after the challenge dose was associated with MT in the Cd$^{2+}$-pretreated mice than the water-pretreated controls. The present study indicates that MT induced in the small intestinal mucosa by pretreatment prevents Cd$^{2+}$ absorption by sequestering subsequently administered Cd$^{2+}$, and Cd$^{2+}$ taken up by the liver and kidney is bound to MT in an inert form, thus the decrease in the amount of Cd$^{2+}$ not bound to MT, giving protection from the acute oral toxicity of the cation. Pretreatment 24 hr prior to the challenge dose was found to be the most effective.

Cadmium uptake in the liver of rats after parenteral administration is increased by pretreatment with the metal (1, 2). Similarly, pretreatment of rats with a small oral dose of Cd$^{2+}$ enhances the Cd$^{2+}$ uptake by the liver, kidney and testis after a large oral dose of the cation (3). In such cases, the first administration of Cd$^{2+}$ induces metallothionein (MT) synthesis and the MT formed sequesters subsequently administered Cd$^{2+}$, thus reducing its acute toxicity (2–4).

In contrast, my previous study (5) demonstrated that the uptake of Cd$^{2+}$ by the liver and kidney of mice after a large oral dose of Cd$^{2+}$ is reduced by pretreatment with a small oral dose of the cation. These observations are due to decreased absorption and increased fecal excretion of Cd$^{2+}$ produced by pretreatment. It has been suggested that MT sequesters dietary Cd$^{2+}$ in the intestinal mucosal cells and prevents the transport of the cation to the circulatory system during chronic low level exposure in rats (6).

Therefore, the present study was undertaken to investigate the relationship between the reduced uptake of Cd$^{2+}$ in the liver and kidney caused by pretreatment and MT synthesis in the liver, kidney and small intestinal mucosa.

Materials and Methods

Animals and treatment: Female ICR mice (Clea Japan, Inc., Osaka), 5 to 6 weeks of age, were used. They were maintained in a room with constant temperature (23±2°C)
and relative humidity (60±10%). Standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo) and water were freely available throughout the study.

Cadmium chloride dissolved in deionized water was administered to mice by the oral route. The small dose of Cd²⁺ employed was 15 mg Cd²⁺/kg, while the large dose was 100 mg Cd²⁺/kg. At 6, 24, 48 or 72 hr after pretreatment with a small dose of Cd²⁺, mice were challenged with a large dose of the cation. Control animals were given the same volume of deionized water (10 ml/kg) instead of Cd²⁺ solution. Four or 24 hr after the Cd²⁺ challenge, the mice were sacrificed, and the liver, kidney and small intestine were immediately removed. The intestine was incised lengthwise, and its contents were removed by washing with deionized water. After blotting with filter paper, the intestinal mucosa were scraped with a glass slide.

**Gel filtration on Sephadex G-75:** The livers, kidneys or small intestinal mucosa obtained from 6 to 8 mice in each group were homogenized in 3 volumes of 10 mM Tris-HCl buffer (pH 8.6) with a Polytron homogenizer. Portions of the homogenate were centrifuged for 1 hr at 105,000 g. A 4 ml aliquot of the supernatant was loaded onto a 2.5×45 cm column of Sephadex G-75 equilibrated with 10 mM Tris-HCl buffer (pH 8.6) containing 0.02% sodium azide. Descending buffer flow was used at a flow rate of 20 ml/hr, and 6 ml fractions were collected. The other 4 ml aliquot, saturated with Cd²⁺ by a modified method of Probst et al. (7) (the “Cd²⁺-saturated” supernatant), was also fractionated on Sephadex G-75.

**Determination of Cd²⁺ and Zn²⁺ concentrations:** Portions of the homogenate were digested with nitric acid and perchloric acid (1:1), and the dry residues were dissolved in 1 N-hydrochloric acid for analysis. The acid-digested solution, the 105,000 g supernatant and the collected fractions were analyzed for Cd²⁺ and Zn²⁺ by atomic absorption spectrometry.

**Statistical analysis:** Statistical differences between the groups were examined by Student’s t-test.

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**Results**

**Sephadex G-75 chromatographic profile of the 105,000 g liver supernatant:** Typical elution patterns from the Sephadex G-75 column of the 105,000 g liver supernatant from mice challenged with a large dose of Cd²⁺ are shown in Fig. 1. In this system, the high molecular weight proteins (HMWP) fraction corresponded to fractions 13 to 17 (5 fractions with the highest Cd²⁺ concentrations in a peak) and the metallothionein (MT) fraction corresponded to fractions 24 to 29 (6 fractions with the highest Cd²⁺ concentrations in a peak) on the basis of measurement of the absorbance at 250 and 280 nm.

In mice pretreated with deionized water at 24 hr prior to challenge with a large dose of Cd²⁺, Cd²⁺ in the liver supernatant was much greater in the HMWP fraction than in the MT fraction at 4 hr after the challenge dose, but almost all of the Cd²⁺ in the supernatant was found in the MT fraction at 24 hr. On the other hand, in mice pretreated with a small dose of Cd²⁺ at 24 hr prior to the challenge dose, after the Cd²⁺ challenge, most of the Cd²⁺ in the supernatant at 4 hr and almost all that at 24 hr was present in the MT fraction (Fig. 1).

The Cd²⁺ content in the MT fraction of the liver supernatant was 2.2 times higher in the Cd²⁺-pretreated mice than the water-pretreated controls at 4 hr after the Cd²⁺ challenge, but was 1.5 times higher in the latter than the former at 24 hr (Fig. 1). The Zn²⁺ content in the liver supernatant was greater in the Cd²⁺-pretreated mice than in the water-pretreated controls. In both groups, the Zn²⁺ contents in the supernatant and the MT fraction after the Cd²⁺ challenge increased with time.

**Distribution of Cd²⁺ and Zn²⁺ in the liver and its supernatant proteins:** There were no significant differences in Cd²⁺ concentration in the total liver homogenate at 4 and 24 hr after challenge with a large dose of Cd²⁺ in each paired group (Table 1), suggesting that Cd²⁺ uptake by the liver increased rapidly. The Cd²⁺ concentration in the total liver homogenate at 4 and 24 hr after the Cd²⁺ challenge was decreased to the greatest

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extent by pretreatment with a small dose of Cd\(^{2+}\) at 24 hr prior to the challenge dose, to 69% and 64% of those in the water-pretreated controls, respectively, but was unaffected by pretreatment at 72 hr prior to the challenge dose. On the other hand, the Zn\(^{2+}\) concentration in the total liver homogenate was increased with time by the Cd\(^{2+}\) challenge and increased further by pretreatment.

The proportion of Cd\(^{2+}\) in the liver supernatant after the Cd\(^{2+}\) challenge was increased with time in each paired group. At 4 hr after the Cd\(^{2+}\) challenge, the proportion of Cd\(^{2+}\) in the supernatant was greater in the Cd\(^{2+}\)-pretreated mice than the water-pretreated controls; but at 24 hr, no significant differences were observed among the groups. There were no significant differences among the Cd\(^{2+}\)-pretreated mice and the water-pretreated controls in the proportion of Zn\(^{2+}\) in the supernatant (Table 1).

At 4 hr after the Cd\(^{2+}\) challenge, more Cd\(^{2+}\) in the liver supernatant was bound to MT than HMWP in the Cd\(^{2+}\)-pretreated mice and the reverse in the water-pretreated controls. In the former, the proportion of Cd\(^{2+}\) associated with MT in the supernatant was increased maximally by pretreatment 24 hr prior to the challenge dose. The Sephadex G-75 elution pattern for the supernatant in the Cd\(^{2+}\)-pretreated mice approached that of the water-pretreated controls as the time interval between pretreatment and challenge increased. However, at 24 hr, almost all of the Cd\(^{2+}\) in the supernatant was bound to MT in each group, with no significant differences in the Sephadex G-75 elution pattern for the supernatant. Meanwhile, more Zn\(^{2+}\) in the liver supernatant was bound to HMWP than MT in each group after the Cd\(^{2+}\) challenge. The proportion of Zn\(^{2+}\) associated with MT in the supernatant was greater at 24 hr after the Cd\(^{2+}\) challenge than at 4 hr in each paired group, although there was no significant difference among the Cd\(^{2+}\)-pretreated mice and the water-pretreated controls (Table 1).

The concentration of Cd\(^{2+}\) bound to MT
| Group        | Cd(II) in supernatant (µg/g wet liver) | Zn(II) in supernatant (µg/g wet liver) | % MT-fraction (Cd(II) of total Cd(II)) | % MT-fraction (Zn(II) of total Zn(II)) | % saturation with Cd(II) in MT* |
|--------------|--------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------|
| Aq           | 64.9                                  | 86.7                                   | 1.1                                    | 0.9                                    | 1.0                              |
| Aq–Cd(L)     | 64.9                                  | 86.7                                   | 1.1                                    | 0.9                                    | 1.0                              |
| Cd(S)–Cd(L)  | 64.9                                  | 86.7                                   | 1.1                                    | 0.9                                    | 1.0                              |
| Cd(II)–Cd(L) | 64.9                                  | 86.7                                   | 1.1                                    | 0.9                                    | 1.0                              |

The homogenate was obtained from the pooled livers of 6 to 8 mice. The 105,000 g supernatant was fractionated on Sephadex G-75. Each value represents the mean ± S.E. of 2 or 3 separate experiments. Aq–Cd(L), Cd(II)–Cd(L), Aq–Cd(L), Cd(II)–Cd(L) were preincubated with Cd(II) (100 µmol/liter) and sacrificed after further 4 and 24 hr. HMPF was measured using the fractionation of 24–29.

* Significantly different from the corresponding control group Aq–Cd(L) at P < 0.05 and P < 0.01, respectively.

** Significantly different from the corresponding control group Aq–Cd(L) at P < 0.05 and P < 0.01, respectively.
in the liver of the Cd\textsuperscript{2+}-pretreated mice was higher than that of the water-pretreated controls at 4 hr after the Cd\textsuperscript{2+} challenge, but was rather lower than that of the latter at 24 hr. However, the Cd\textsuperscript{2+} concentration not bound to MT in the liver at 4 and 24 hr after the Cd\textsuperscript{2+} challenge was reduced by pretreatment. The largest effect was observed for pretreatment at 24 hr prior to the challenge dose, but the effect decreased as the time interval between pretreatment and challenge increased (Table 1).

Hepatic MT concentration was measured indirectly as a function of the total Cd\textsuperscript{2+}-binding capacity of MT by gel filtration of the "Cd\textsuperscript{2+}-saturated" supernatant on Sephadex G-75. The saturation rate with Cd\textsuperscript{2+} in the hepatic MT of the Cd\textsuperscript{2+}-pretreated mice was lower than that of the water-pretreated controls at 4 and 24 hr after the Cd\textsuperscript{2+} challenge, suggesting the presence of more available Cd\textsuperscript{2+}-binding sites in MT of the Cd\textsuperscript{2+}-pretreated mice (Table 1).

In mice given a small dose of Cd\textsuperscript{2+}, the concentrations of Cd\textsuperscript{2+} and Zn\textsuperscript{2+} in the total liver homogenate and the proportion of Zn\textsuperscript{2+} bound to MT in the supernatant were greater than in the controls given deionized water (Table 1). The proportion of Cd\textsuperscript{2+} bound to HMWP in the supernatant decreased to a minimum by 24 to 48 hr after a small dose of Cd\textsuperscript{2+} and thereafter increased. Meanwhile, the proportion of Cd\textsuperscript{2+} associated with MT in the supernatant was maximal at 24 hr after a small dose and then decreased with time. However, the proportion of Cd\textsuperscript{2+} bound to HMWP in the supernatant was greater in mice with a small dose than mice with a large dose (the water-pretreated mice) at 24 hr after the Cd\textsuperscript{2+} administration, while that associated with MT was the reverse. The ability to induce MT synthesis may be weaker in mice with a small dose of Cd\textsuperscript{2+} than a large dose. No significant changes were observed in the concentrations of Cd\textsuperscript{2+} and Zn\textsuperscript{2+} in the total homogenate, the proportions of Cd\textsuperscript{2+} and Zn\textsuperscript{2+} in the supernatant, and the proportion of Zn\textsuperscript{2+} bound to HMWP and MT in the supernatant between 6 and 72 hr after a small dose of Cd\textsuperscript{2+}. The concentration of Cd\textsuperscript{2+} bound to MT in the liver was approximately constant between 6 and 48 hr after a small dose of Cd\textsuperscript{2+} and slightly decreased at 72 hr. However, the Cd\textsuperscript{2+} concentration not bound to MT in the liver was maximal at 6 hr and constant with slightly decreased levels thereafter.

**Distribution of Cd\textsuperscript{2+} and Zn\textsuperscript{2+} in the kidney and its supernatant proteins:** The concentration of Cd\textsuperscript{2+} in the total kidney homogenate 24 hr after challenge with a large dose of Cd\textsuperscript{2+} was reduced by pretreatment with a small dose of the cation (Table 2). The largest effect was found with pretreatment at 24 hr prior to the challenge dose, and the effect of pretreatment decreased as the time interval between pretreatment and challenge increased. There was no significant difference in Zn\textsuperscript{2+} concentration in the total homogenate of each group.

The proportions of Cd\textsuperscript{2+} and Zn\textsuperscript{2+} in the supernatant and the proportions of these cations bound to HMWP and MT in the supernatant 24 hr after the Cd\textsuperscript{2+} challenge were not significantly influenced by pretreatment (Table 2).

The concentration of Cd\textsuperscript{2+} bound to MT and not bound to MT in the kidney 24 hr after the Cd\textsuperscript{2+} challenge was decreased by pretreatment, which was most effective at 24 hr prior to the challenge dose (Table 2).

**Distribution of Cd\textsuperscript{2+} and Zn\textsuperscript{2+} in the small intestinal mucosa and its supernatant proteins:** The concentration of Cd\textsuperscript{2+} in the total homogenate of the small intestinal mucosa 24 hr after challenge with a large dose of Cd\textsuperscript{2+} was increased by pretreatment with a small dose of the cation (Table 3). Pretreatment at 24 hr prior to the challenge dose had the largest effect. However, no significant differences were observed in the Zn\textsuperscript{2+} concentration in the total homogenate of the Cd\textsuperscript{2+}-pretreated mice and the water-pretreated controls.

The proportions of Cd\textsuperscript{2+} and Zn\textsuperscript{2+} in the supernatant of the small intestinal mucosa 24 hr after the Cd\textsuperscript{2+} challenge were unaffected by pretreatment. More Cd\textsuperscript{2+} in the supernatant 24 hr after the Cd\textsuperscript{2+} challenge was bound to MT than HMWP in the Cd\textsuperscript{2+}-pretreated mice and the reverse in the water-pretreated controls. However, the proportion of Zn\textsuperscript{2+} associated with HMWP and MT in the supernatant were approximately the same in
Table 2. Distribution of Cd²⁺ and Zn²⁺ in the kidney supernatant proteins after a challenge dose of Cd²⁺ in mice pretreated with a small dose of Cd²⁺

| Group   | Interval (hr) | Cd²⁺ in total homogenate (µg/g kidney) | Zn²⁺ in total homogenate (µg/g kidney) | Cd²⁺ in supernatant (% of total homogenate) | Zn²⁺ in supernatant (% of total homogenate) | Cd²⁺ in HMWP-fraction (µg/g total supernatant) | Zn²⁺ in HMWP-fraction (µg/g total supernatant) | Cd²⁺ in MT-fraction (µg/g total supernatant) | Zn²⁺ in MT-fraction (µg/g total supernatant) | Cd²⁺ not bound to MT (µg/g kidney) | Zn²⁺ not bound to MT (µg/g kidney) |
|---------|---------------|----------------------------------------|----------------------------------------|---------------------------------------------|---------------------------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------|-----------------------------------------------|----------------------------------------|----------------------------------------|
| Aq–Cd(L)| 24            | 15.49 ± 1.61                           | 23.58 ± 2.15                           | 85.6 ± 4.3                                  | 71.9 ± 3.3                                  | 12.4 ± 2.1                                    | 36.5 ± 3.3                                    | 76.4 ± 1.6                                  | 23.4 ± 1.8                                     | 5.40                                   | 5.40                                    |
| Cd(S)–Cd(L)| 6  | 10.21 ± 2.14                           | 21.87 ± 1.97                           | 80.9 ± 2.6                                  | 63.6 ± 4.0                                  | 13.2 ± 1.7                                    | 40.4 ± 1.4                                    | 71.4 ± 1.1                                  | 19.5 ± 1.2                                     | 4.30                                   | 4.30                                    |
| Cd(S)–Cd(L)| 24 | 6.47 ± 0.80**                          | 21.61 ± 0.92                           | 86.4 ± 3.5                                  | 66.5 ± 3.6                                  | 10.3 ± 0.8                                    | 45.8 ± 2.1                                    | 77.7 ± 1.5                                  | 14.8 ± 1.8                                     | 2.12                                   | 2.12                                    |
| Cd(S)–Cd(L)| 48 | 8.74 ± 1.35*                           | 20.06 ± 1.72                           | 85.4 ± 2.4                                  | 66.1 ± 2.1                                  | 11.8 ± 1.5                                    | 39.0 ± 1.5                                    | 73.1 ± 1.6                                  | 22.4 ± 1.9                                     | 3.29                                   | 3.29                                    |
| Cd(S)–Cd(L)| 72 | 10.74 ± 1.29                           | 22.29 ± 2.72                           | 85.9 ± 1.3                                  | 75.8 ± 4.5                                  | 9.5 ± 0.6                                     | 44.4 ± 1.2                                    | 80.6 ± 1.4                                  | 16.6 ± 1.2                                     | 3.30                                   | 3.30                                    |

The homogenate was obtained from the pooled kidneys of 6 to 8 mice. The 105,000 g supernatant was fractionated on Sephadex G-75. Each value represents the mean±S.E. of 2 or 3 separate experiments. Aq–Cd(L), Cd(S)–Cd(L): 6, 24, 48 or 72 hr after pretreatment with either deionized water (10 ml/kg, p.o.) or Cd²⁺ (15 mg/kg, p.o.), mice were challenged with Cd²⁺ (100 mg/kg, p.o.) and sacrificed after a further 24 hr. HMWP-fraction: High molecular weight protein fraction (fractions 13–17). MT-fraction: Metallothionein fraction (fractions 24–29). *, **: Significantly different from group Aq–Cd(L) at P<0.05 and P<0.01, respectively.

Table 3. Distribution of Cd²⁺ and Zn²⁺ in the supernatant proteins of small intestinal mucosa after a challenge dose of Cd²⁺ in mice pretreated with a small dose of Cd²⁺

| Group   | Interval (hr) | Cd²⁺ in total homogenate (µg/g small intest. mucosa) | Zn²⁺ in total homogenate (µg/g small intest. mucosa) | Cd²⁺ in supernatant (% of total homogenate) | Zn²⁺ in supernatant (% of total homogenate) | Cd²⁺ in HMWP-fraction (µg/g total supernatant) | Zn²⁺ in HMWP-fraction (µg/g total supernatant) | Cd²⁺ in MT-fraction (µg/g total supernatant) | Zn²⁺ in MT-fraction (µg/g total supernatant) | Cd²⁺ not bound to MT (µg/g small intest. mucosa) | Zn²⁺ not bound to MT (µg/g small intest. mucosa) |
|---------|---------------|------------------------------------------------------|-----------------------------------------------------|---------------------------------------------|---------------------------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------|-----------------------------------------------|----------------------------------------|----------------------------------------|
| Aq–Cd(L)| 24            | 19.99 ± 2.15                                         | 12.97 ± 1.42                                         | 82.7 ± 4.8                                  | 58.0 ± 2.8                                  | 13.9 ± 2.3                                    | 32.8 ± 2.3                                    | 66.9 ± 2.3                                  | 22.8 ± 2.6                                     | 8.91                                   | 8.91                                    |
| Cd(S)–Cd(L)| 6  | 27.80 ± 2.74                                         | 13.84 ± 1.36                                         | 73.0 ± 6.7                                  | 55.4 ± 2.4                                  | 7.0 ± 2.4                                    | 32.8 ± 2.4                                    | 80.5 ± 2.3                                  | 27.6 ± 2.4                                     | 11.40                                  | 11.40                                  |
| Cd(S)–Cd(L)| 24 | 30.23 ± 1.56*                                        | 12.31 ± 1.79                                         | 77.5 ± 4.9                                  | 59.7 ± 2.7                                  | 7.0 ± 1.4                                    | 32.9 ± 2.7                                    | 80.9 ± 2.5                                  | 27.9 ± 2.5                                     | 11.29                                  | 11.29                                  |
| Cd(S)–Cd(L)| 48 | 25.41 ± 1.67                                         | 13.06 ± 1.61                                         | 76.0 ± 6.5                                  | 58.8 ± 2.1                                  | 6.9 ± 1.8                                    | 34.3 ± 2.1                                    | 81.0 ± 2.5                                  | 24.2 ± 2.5                                     | 9.73                                   | 9.73                                    |
| Cd(S)–Cd(L)| 72 | 26.78 ± 1.30                                         | 12.74 ± 1.61                                         | 78.9 ± 4.6                                  | 60.0 ± 2.1                                  | 6.3 ± 1.2                                    | 30.3 ± 2.1                                    | 77.8 ± 1.7                                  | 24.9 ± 1.7                                     | 10.34                                  | 10.34                                  |

The homogenate was obtained from the pooled small intestinal mucosa of 6 to 8 mice. The 105,000 g supernatant was fractionated on Sephadex G-75. Each value represents the mean±S.E. of 2 or 3 separate experiments. Aq–Cd(L), Cd(S)–Cd(L): 6, 24, 48 or 72 hr after pretreatment with either deionized water (10 ml/kg, p.o.) or Cd²⁺ (15 mg/kg, p.o.), mice were challenged with Cd²⁺ (100 mg/kg, p.o.) and sacrificed after a further 24 hr. HMWP-fraction: High molecular weight protein fraction (fractions 13–17). MT-fraction: Metallothionein fraction (fractions 24–29). *, **: Significantly different from group Aq–Cd(L) at P<0.05 and P<0.01, respectively.
Cadmium Toxicity and Metallothionein

The concentration of Cd2+ bound to MT in the small intestinal mucosa 24 hr after the Cd2+ challenge was greater in the Cd2+-pretreated mice than the water-pretreated controls. However, there were no significant differences among the groups in the concentration of Cd2+ not bound to MT in the small intestinal mucosa (Table 3).

Discussion

The previous study (8) has shown that pretreatment with small oral doses of Cd2+ protects mice against the acute oral toxicity of the cation as evidenced by an increased LD50. The most effective protection is observed for pretreatment with 1/7 of the challenge Cd2+ doses. The protection is maximal at 24 hr after pretreatment and is diminished when the Cd2+-pretreated mice are challenged 72 or more hr later.

It is well established that metallothionein (MT) provides a defense mechanism against Cd2+ (2, 4, 9–13). In the studies on the time course of hepatic MT synthesis after the parenteral administration of Cd2+ (4, 14–17), Cd2+ taken up by the liver is immediately bound to high molecular weight proteins (HMWP) eluted at or near the void volume of a Sephadex G-75 column. Cd2+ bound to HMWP is redistributed to MT with time. According to Cempel and Webb (15), there is a lag phase of 3 to 4 hr between the i.v. administration of Cd2+ and the onset of MT synthesis in the liver of male rats. In rats treated with Cd2+ at 24 hr prior to a subsequent dose of the cation, further synthesis of hepatic MT occurs without a lag phase in response to the second dose.

In the present study, the proportion of Cd2+ associated with MT in the liver after a small dose of Cd2+ was maximal at 24 hr and then decreased with time. Probst et al. (7) have also showed that hepatic MT-bound Cd2+ reaches its maximal level at 36 hr after an i.p. administration of Cd2+ and decreases with time. These observations suggest a degradation of MT, probably by lysosomal proteases (18). The half-life of cadmium-thionein in rat liver is found to be 3.5 to 4.2 days (19, 20). With regard to redistribution of Cd2+ from the liver to other organs, Cousins (21) denies the transport of the cation from the liver to the kidney as MT, but hepatic MT can be released into the blood and transferred to the kidney in some types of hepatic disorders (22).

On the other hand, at 4 hr after challenge with a large dose of Cd2+, the cation taken up by the liver of the water-pretreated controls was mainly bound to HMWP, while most of the cation in the liver of the Cd2+-pretreated mice was already bound to MT. The proportion of Cd2+ associated with the hepatic MT was the greatest in those mice which were Cd2+-pretreated at 24 hr prior to the challenge dose. The Sephadex G-75 elution pattern for the liver supernatant of the Cd2+-pretreated mice approached that of the water-pretreated controls as the time interval between pretreatment and challenge increased. The reduction of the acute oral toxicity of Cd2+ produced by pretreatment (8) is due, in part, to this fact that Cd2+ taken up by the liver was more abundantly and more rapidly bound to MT in the Cd2+-pretreated mice than the water-pretreated controls at earlier times after the Cd2+ challenge. Further, 24 hr after the Cd2+ challenge, 76 to 81% and 74% of the Cd2+ in the liver were associated with MT in the Cd2+-pretreated mice and the water-pretreated controls, respectively. At that time there was no significant difference in the Sephadex G-75 elution pattern for the liver supernatant of the Cd2+-pretreated mice and the water-pretreated controls. However, the amount of Cd2+ bound to MT in the liver and the hepatic MT concentration, indirectly determined by a modified method of Probst et al. (7), were rather lower in the Cd2+-pretreated mice, the lowest in mice pretreated with Cd2+ at 24 hr prior to the challenge dose, than the water-pretreated controls 24 hr after the Cd2+ challenge. Metallothionein concentration in the liver increases in proportion to the parenteral dose of Cd2+ at all except the earliest times (23). In the present study, Cd2+ uptake by the liver and kidney 24 hr after the Cd2+ challenge was greater in the water-pretreated controls than the Cd2+-pretreated mice, so that the amount of Cd2+ associated with MT in these organs was higher in the former than the latter at
that time. At 4 and 24 hr after the Cd\(^{2+}\) challenge, the amount of Cd\(^{2+}\) not bound to MT in the liver was reduced the most by pretreatment at 24 hr prior to the challenge dose, but reached the level of the water-pretreated controls as the time interval between pretreatment and challenge increased. Similar phenomena were also observed in the kidney 24 hr after the Cd\(^{2+}\) challenge. These observations suggest that the factor directly related to the toxic action of Cd\(^{2+}\) is the amount of Cd\(^{2+}\) not bound to MT rather than the amount of the cation bound to MT or the MT concentration in the liver and other organs. Thus, there was a reciprocal relationship between the amount of Cd\(^{2+}\) not associated with MT in the hepatic MT, the presence of more available binding sites of Cd\(^{2+}\) in MT, in the Cd\(^{2+}\)-pretreated mice than the water-pretreated controls may influence the reduced acute toxicity of the cation by pretreatment.

Webb and Verschoyle (2) have suggested that the sequestration of Cd\(^{2+}\) by MT does not play a significant role in protection against the acute toxicity of the cation. Their results indicate that protection of rats against the acute i.v. toxicity of Cd\(^{2+}\) by pretreatment with a small dose of the cation is maximal 1 to 3 days after pretreatment and then decreases, although the increased capacity to incorporate Cd\(^{2+}\) into hepatic MT is maintained for 10 days.

Chen et al. (19) have demonstrated that the maximal hepatic MT synthesis occurs between 5 and 24 hr after a s.c. administration of Cd\(^{2+}\). From the present data that the proportion of Cd\(^{2+}\) bound to MT in the liver supernatant at 4 hr after the Cd\(^{2+}\) challenge was the largest in mice pretreated with a small dose of Cd\(^{2+}\) at 24 hr prior to the challenge dose, the capacity to incorporate the subsequently administered Cd\(^{2+}\) into the hepatic MT may be markedly increased during the active duration of the MT synthesis induced by pretreatment.

The marked reduction of Cd\(^{2+}\) uptake by the liver and kidney at 4 or 24 hr after the Cd\(^{2+}\) challenge produced by pretreatment at 24 hr prior to the challenge dose involved the increased uptake of Cd\(^{2+}\) by the small intestinal mucosa, with the increased Cd\(^{2+}\) bound to MT in the mucosa. These findings suggest that the sequestration of Cd\(^{2+}\) by the mucosal MT prevents the transfer of the cation to the systemic circulation. As the mucosal cells turn-over rapidly, Cd\(^{2+}\) sequestered by the mucosal MT is considered to be excreted during the desquamation process. The reduction of Cd\(^{2+}\) absorption after the Cd\(^{2+}\) challenge by pretreatment shown in the previous study (5) can be explained by these results. According to Kotsonis and Klaassen (24), this inhibitory mechanism of the intestinal MT against Cd\(^{2+}\) absorption is quickly overloaded, since the concentration of Cd\(^{2+}\) in the small intestine reaches a maximum at 3 weeks in rats continuously exposed to a very low concentration of Cd\(^{2+}\) (10 ppm) in drinking water.

In conclusion, the results of this study indicate that MT induced in the small intestinal mucosa by pretreatment prevents the absorption of Cd\(^{2+}\) after the Cd\(^{2+}\) challenge by sequestering the cation, and Cd\(^{2+}\) taken up by the liver and kidney is bound to MT in an inert form, thus the decrease in the amount of Cd\(^{2+}\) not bound to MT, protecting mice against the acute oral toxicity of the cation. A small oral dose of Cd\(^{2+}\) 24 hr prior to the challenge with a large oral dose of the cation was found to be the most effective pretreatment.

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