the membrane-bound AP molecules, through derangement of the re-cycling of receptors. The results of the present investigation may therefore suggest that the plasma membrane enzyme determination may not be as sensitive an indicator of chloroquine-related tissue damage as the determination of the lysosomal acid hydrolases, particularly with regard to renal damage.

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**Chronic cadmium intake results in dose-related excretion of metallothionein in urine**

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**Summary.** Urinary excretion of metallothionein was measured by radioimmunoassay in rats given drinking water containing 5 or 50 mg cadmium/l for up to 2 years. The metallothionein levels corresponded to the concentration of cadmium in the drinking water and increased linearly over the course of the study. These results demonstrate that urinary metallothionein is a sensitive biological indicator of oral cadmium exposure.

**Key words.** Cadmium; metallothionein; urinalysis; chronic exposure.

Cadmium (Cd) is an important occupational toxicant and environmental pollutant. Chronic exposure to this metal results in its progressive accumulation, mainly in liver and kidney and can lead to renal tubular dysfunction characterized by proteinuria, glucosuria and aminoaciduria in experimental animals and man. For this reason, it is important to develop a sensitive and reliable biological indicator of exposure to this metal. Several studies have examined the usefulness of urinary metallothionein (MT) as a measure of Cd body burden and also in the determination of Cd-induced renal dysfunction. Elevated levels of urinary MT have been demonstrated in human populations exposed to Cd in the environment, in occupationally exposed Cd workers, and in rats injected s.c. with cadmium chloride (CdCl₂). In the present study, the urinary excretion of MT was investigated in rats treated for up to 2 years with Cd-containing drinking water. The purpose of this long-term study was to mimic the human environmental exposure and evaluate whether urinary MT is a biological marker of Cd exposure.

**Methods.** Male Wistar rats, aged 27 days, were obtained from Charles River Breeding Laboratories, Inc. The animals were divided into three groups of 68 each and given distilled drinking water containing 0 (group 1), 5 (group 2) or 50 (group 3) mg Cd as CdCl₂ per liter (Mallinkrodt, Inc., Paris, KY) and commercial laboratory chow (Charles River, RMH 1000) ad libitum. The animals were housed two per cage in large plastic shoebox cages for up to 2 years and cared for in accordance with institutional guidelines. The animals were moved to metabolic cages for urine collection. From 8 rats in each treatment group, 24-h urine specimens were collected over ice, once every 6 to 8 weeks, during the course of the study. Every 3 months, 8 additional rats from each group were sacrificed. Their urines were collected one week before the sacrifice. All urine specimens were stored at −20 °C until analyses.

MT in urine was analyzed by a modification of our radioimmunoassay method described earlier. The modification consisted of substituting Pansorbin (Calbiochem LaJolla, CA) in place of ammonium sulfate, as the precipitating agent for bound antigen.

Linear regression analysis was performed on the data relating urinary MT levels to the concentration of exposure. Where group means were calculated, the reported values are arithmetic means and standard errors.

**Results and discussion.** The water consumption of all animals on various treatment regimens was measured on a weekly basis. The water consumption of rats in group 3 was significantly lower than that of group 2 or control rats. This phenomenon may be ascribed to aversive gustatory or olfactory stimuli associated with Cd-containing drinking solutions. The resulting mean weekly Cd intakes of groups 2 and 3 were calculated to be 1.1 ± 0.04 mg/rat and 8.5 ± 0.2 mg/rat, respectively (fig. 1). Therefore, the actual
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Figure 1. Mean weekly Cd intake of rats maintained on drinking water containing two different concentrations of CdCl₂. The Cd intake (mg/week/rat) was calculated by multiplying the mean weekly water consumption (ml/week/rat) by the concentration of Cd (5 or 50 mg/l) in the drinking water. Mean values of 6–8 rats at each time point are shown for groups 2 (●) and 3 (○).

Figure 2. Urinary MT levels of control rats and rats maintained on drinking water containing CdCl₂. Linear regression analysis was performed on the data relating individual urinary MT levels to the duration of exposure.

A Group 1; 0 mg Cd/l; slope = 0.18; correlation coefficient = 0.152 (p = 0.443).
B Group 2; 5 mg Cd/l; slope = 3.35; correlation coefficient = 0.719 (p < 0.001).
C Group 3; 50 mg Cd/l; slope = 28.26; correlation coefficient = 0.832 (p < 0.001).

difference in dose between the two groups of rats was less than what would be predicted from the concentration of Cd in the drinking water. Urinary MT levels of control rats (group 1) did not change significantly throughout the study (fig. 2). The mean MT level in this group was 90 ± 4 μg/l. However, in Cd-exposed groups the MT levels increased steadily with the duration of exposure. For example, at 78 weeks the mean values for groups 2 and 3 were 403 ± 69 and 2666 ± 379 μg/l, respectively. From the slopes of that data shown in figure 2, the rate of MT excretion in group 3 rats was calculated to be 8.4-fold greater than that of group 2 rats. This indicates that the rate of MT excretion is related to the accumulated dose. The results of this study are consistent with the conclusions of previous clinical studies 3–6 that chronic oral intake of low levels of Cd results in urinary MT excretion. Short-term studies with rats injected s.c. with subacute doses of Cd have also shown that MT excretion is proportional to the renal and hepatic Cd accumulations until a threshold tissue Cd concentration is reached 7–14. In these studies a marked elevation in MT excretion was observed after reaching the threshold tissue Cd levels. In the present study such a critical tissue level apparently was not achieved, since the increase in urinary MT remained linear after 2 years of Cd exposure. Tissue Cd analysis indicated that the maximum concentration of Cd in the renal cortex of group 3 rats was approximately 90 μg/g wet weight which is less than half the reported critical concentration. Furthermore, no morphological or functional changes could be observed in the kidney from any of the experimental animals 15. The presence of MT in the urine may thus reflect an increased burden of Cd in the kidney. Radioimmunoassay is herewith shown to provide a sensitive assay for the detection of such a biochemical marker after chronic exposure.

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