the membrane-bound AP molecules, through derangement of the re-cycling of receptors[6,9,18,24]. 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[1,2]. 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[3-5], in occupationally exposed Cd workers[5,7-11], and in rats injected s.c. with cadmium chloride ($CdCl_2$)[5,12-14].

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 $CdCl_2$ 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[12]. 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 duration 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 adversive gustatory or olfactory stimuli associated with Cd-containing drinking solutions[15,16]. 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

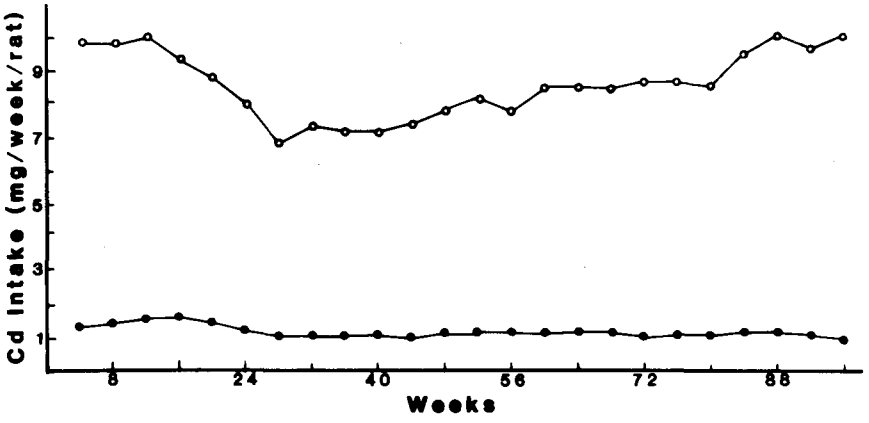

Figure 1. Mean weekly Cd intake of rats maintained on drinking water containing two different concentrations of $CdCl_2$. The Cd intake (mg/week/rat) was calculated by multiplying the mean weekly water consumption (ml/week/rat) by the concentration of Cd (5 or 50 mg/l) in the drinking water. Mean values of 6–8 rats at each time point are shown for groups 2 (●) and 3 (○).

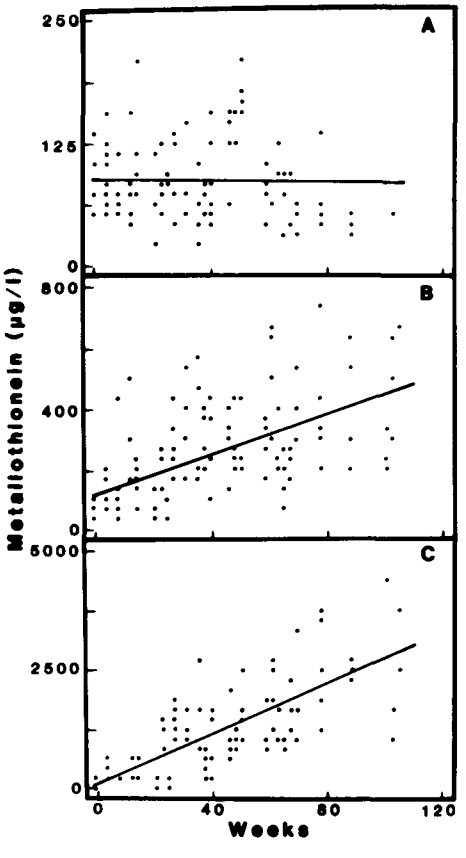

Figure 2. Urinary MT levels of control rats and rats maintained on drinking water containing $CdCl_2$. Linear regression analysis was performed on the data relating individual urinary MT levels to the duration of exposure.
A Group 1; 0 mg Cd/l; slope = 0.18; correlation coefficient = 0.152 ($p = 0.443$).
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[17]. 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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Inhibition of phosphatase by open-chain nucleoside analogues in insects

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Summary. (S)-9-(2,3-dihydroxypropyl) adenine (DHPA), D-eritadenine and some other open-chain nucleoside analogues, which exhibit adverse biological effects in microorganisms, plants and animals, cause pronounced inhibition of intestinal phosphatases in the hemipteran insect *Pyrrhocoris apterus*. The rate of p-nitrophenylphosphate hydrolysis by homogenates from intestinal epithelium and Malpighian tubules was inhibited up to 94% by 2–10 millimolar concentrations of these drugs. This effect is stronger than that of sodium fluoride, which is recognized as a common inhibitor of phosphatase. We conclude that inhibition of phosphatase activity in the digestive and excretory organs may be responsible for the previously reported massive excretion of phosphorylated derivatives of the nucleoside analogues after their oral administration to insects.
Key words. 9-Alkyladenines; D-eritadenine; SAH-hydrolase; acid phosphatase; alkaline phosphatase; *Pyrrhocoris apterus*.

Nucleoside analogues related to (S)-9-(2,3-dihydroxypropyl) adenine (DHPA) exhibit adverse biological effects in most living organisms including virus, plant and animal systems. The effects are manifested, for instance, by pronounced antiviral action [1], inhibition of growth in plant roots [2], sterilization or ovicidal action in insects [3, 13], teratogenic effects in chick embryos [4], or aspermatogenic effects in mice [5]. It is generally believed that these compounds interfere with diverse biological systems by means of inhibition of S-adenosyl-L-homocysteine hydrolase (SAH-hydrolase) [5], which is the key enzyme involved in the essential methylation reactions [6, 7].

In our previous studies on the dietary effects of these drugs in insects, we have found endogenous phosphorylation and rapid excretion of all of the nucleoside analogues administered in the diet in the form of the corresponding phosphates, including optical enantiomers of the natural nucleosides [7, 8]. This metabolic pathway, i.e. phosphorylation and excretion of the phosphate, is not very common, because any more extensive excretion of the phosphorylated metabolites would inevitably deprive the organism of energy and inorganic phosphate. Thinking about a possible selective advantage of this unusual phenomenon, we have assumed that it might perhaps help the organism to eliminate all atypical and therefore hazardous nucleotides from the endogenous pool of essential ones. The problem remains, however, of how the phosphorylated metabolites could pass through the epithelium of the excretory organs into the hind gut and, finally, into the excrements. The observed 'phosphonuria' can in fact be classified as a disease, because the alimentary duct and Malpighian tubules of insects contain extremely active phosphatase enzymes, which are capable of hydrolyzing a wide range of structurally unrelated esters of phosphoric acid over a broad range of pH values [9]. Physiologically, these enzymes are engaged mainly in the turnover and reutilization of inorganic phosphate and in the transport of molecules across the epithelial membranes. In this communication we report briefly on the in vitro and in vivo effects of the selected analogues of nucleosides on phosphate hydrolysis in the epithelium of the intestine and Malpighian tubules.

Materials and methods. The experiments were performed on adult females of *Pyrrhocoris apterus* L., fed with dry linden seeds and kept at 25 °C, as has been previously described [3]. The nucleoside analogues were obtained through the courtesy of Dr A. Holý; their purity and preparation have been described by Holý et al. [8]. The organs selected for the assays, i.e. intestine (actually the midgut portion of the intestine or midgut epithelium alone) and Malpighian tubules were dissected in ice-cold insect Ringer. After careful washing, the tissues were transferred into glass homogenizers and stored frozen at −20 °C until used (no more than 3 weeks). The phosphomonoesterase activity was determined by a common p-nitrophenylphosphate method, using the micromodification of Linhardt and Walter [10]. We used 5.5 mM substrate in 0.05 M citrate buffer, pH 4.8 (acid phosphatase of the intestine), or pH 5.2 (acid phosphatase of Malpighian tubules). For alkaline phosphatase we used 0.05 M glycine buffer, pH 9.3 for both tissues. The results are expressed in µmole equivalents of the hydrolyzed p-nitrophenylphosphate related to one organ, 1 min, at 30 °C. The values are averages from 8–10 separate measurements.

Results and discussion. In the adult females of *Pyrrhocoris*, the first reproduction cycle is terminated by oviposition at day 6 after adult emergence (25 °C). The maximum metabol-