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Reabsorption of Filtered Cadmium-Metallothionein in the Rabbit Kidney[1] (39883)

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Introduction. Metallothioneins (MT) constitute a distinct group of low-molecular-weight metal-binding proteins synthesized in liver and other tissues of animals exposed to heavy metals such as Cd and Zn (1). Although a variety of roles has been suggested for MT, their function in Cd metabolism, if any, remains uncertain. One attractive proposal considers a contribution of Cd-MT to transport of Cd from liver to kidneys and subsequently to its urinary excretion as reabsorption diminishes following onset of renal damage (2). Vostal and Cherian (3) followed the excretion of intraarterially injected Cd-MT, but little is known of mechanisms involved in the renal handling of the compound. Critical testing of the hypothesis that inhibition of Cd-MT reabsorption accounts for urinary Cd excretion requires determination of rates of filtration and reabsorption of Cd-MT in Cd-poisoned animals. We report here the results of such determinations.

Materials and methods. The experiments were performed on male Japanese white rabbits weighing approximately 3 kg and maintained on regular laboratory chow. Half the animals were injected subcutaneously for approximately 12 weeks with 0.5 mg of Cd as $CdCl_2$/kg initial body weight/day; at that time the Cd content of the renal cortex averaged 262 ± 21 (SD) μg/g of fresh weight. Labeled Cd-MT was isolated from livers of animals which had received Cd for 19 days. At that time they were given 150 μCi of ^{115m}Cd (carrier free) by subcutaneous injection; 2 days later they were killed by a blow on the head and the livers were quickly removed and placed on ice. Isolation of the labeled material followed the method of Cherian (4). The final product contained no appreciable Zn, optical absorption at 252 nm equalled 1.51, and the specific activity of Cd was 3500 cpm/μg, as counted in Bray's solution on a Packard Model 3320 liquid-scintillation spectrometer. Concentrations of Cd-MT are calculated on the basis of a mean molecular weight of 10,000, equivalent to seven molecules of Cd. All metal assays were performed by flameless atomic-absorption spectrometry (Varian-Technitron Models 1100 and 63) after wet-ashing, extraction into dithizone-chloroform, and reextraction into 2% HCl. Detection limits for Cd were 0.5 ng/ml of plasma, and 8 ng/ml of urine. Glomerular filtration was equated to clearance of [^{3}H]methoxyinulin (New England Nuclear). For clearance studies rabbits were anesthetized with nembutal; urine flow was stimulated by continuous intravenous infusion of 5% mannitol at 2.4 ml/min. Urine was collected from each kidney through ureteral catheters, but results from the two kidneys were combined for calculation of tubular maxima and other characteristics of reabsorption in each animal. In order to reduce the need for administration of large amounts of Cd-MT, the compound was infused through a mixing chamber (6 ml) at 0.3 ml/min (5) and a femoral catheter was advanced into the thoracic aorta. This permitted maintenance of steady arterial plasma levels of Cd-MT and inulin, with an average variation of 6% from the mean over a period of 1 min. A catheter in the contralateral femoral artery permitted rapid collection of arterial blood. Blood was obtained 1 min after the beginning of

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infusion, and again 1 min later at the beginning of a 1-min urine collection period. No significant uptake of Cd-MT by erythrocytes could be observed. The expected free filterability of plasma Cd-MT and absence of binding to high-molecular-weight proteins could be inferred from the observation that, even 20 min after injection, all plasma ^{115m}Cd remained in one low-molecular-weight fraction upon gel chromatography. Filtered load of Cd-MT could therefore be calculated as the product of its plasma concentration and inulin clearance. Following stepwise addition of Cd-MT to the mixing chamber, repeated clearance determination at different filtered loads were performed on each animal. Excretion of unlabeled Cd by exposed animals was determined on 24-hr urine samples collected in metabolism cages.

Results. Preliminary experiments had suggested that fractional excretion of filtered Cd-MT increases with the dose administered, i.e., that reabsorption might be mediated by a saturable process. In order to study this saturation phenomenon more quantitatively, clearance and reabsorption of Cd-MT were determined as discussed in the Methods section. Results of a control experiment are shown in Fig. 1.

Reabsorption, equated as usual with the difference between filtration and excretion, was complete below a filtered load of 1.2 μg/min. At that level some process must have reached saturation, so that further increases in filtered load now led to gradually increasing excretion. If this saturable process, however, could account for all Cd-MT reabsorption, no further increases in reabsorption should result from raising filtered load above the saturation point. Reabsorption, however, continued to increase with filtered load even though a saturation point had been reached and exceeded. It is necessary to assume, therefore, that a second process is involved in Cd-MT reabsorption which did not become saturated in our experiments. The same interpretation was previously offered for similar results obtained in the study of tubular glycine reabsorption (6).

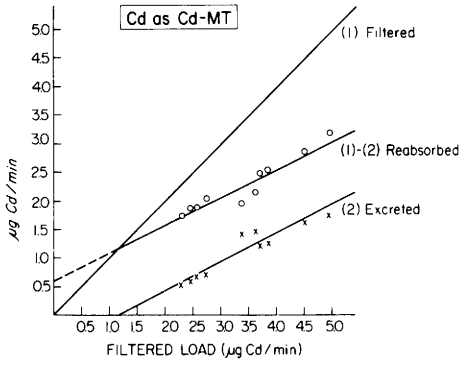

FIG. 1. Excretion of Cd-MT at different filtered loads. Control animal; five successive clearance periods were obtained, and each point represents an observed (×) or calculated (○) value from one kidney. Lines were calculated by least-squares analysis. Extrapolation of the reabsorption line to zero leads to a calculated T_m of 0.6 μg of Cd/min, equivalent to 15.2 μg of Cd-MT/two kidneys/min.

According to convention, extrapolation of the reabsorption line to zero leads to a value of T_m. The slope of the reabsorption line provides a measure of continuing constant fractional reabsorption even at the highest filtered loads attained in these studies. Results of a total of four control studies and of similar determinations on seven Cd-poisoned animals are compared in Table I. Exposure to Cd significantly reduced the maximum capacity of that portion of the reabsorptive process which is saturated under present conditions, without apparently exerting an effect on the nonsaturated component. Mean results are described by the following equations linking reabsorption (R) to filtered

TABLE I. REABSORPTION OF CD-METALLOTHIONEIN.[a]

	Control	Poisoned
n	4	7
Inulin clearance (ml/min)	20 ± 2	17 ± 5
Cd-MT reabsorption: T_m of saturated process (μg/min)	18.6 ± 2.4	7.2 ± 5.0*
Nonsaturated process (μg/μg filtered)	0.47 ± 0.13	0.55 ± 0.10
Plasma Cd (ng/ml)	ND[b]	9.7 ± 1.7
Urine Cd (ng/ml)	ND	350 ± 190
(ng/min)	–	45 ± 15

[a] Mean values, calculated on basis of two kidneys, are shown for n animals ± SD.

[b] ND, not detectable.

* Significantly different from control, $P < 0.01$.

load (F) and excretion (E): In control animals, $R = 0.47\,F + 18.6$ μg of Cd-MT/min; similarly for poisoned rabbits, $R = 0.55\,F + 7.2$ μg of Cd-MT/min. The equation for poisoned animals may be rewritten as $E = F - R = 0.45\,F - 7.2$ μg/min.

Discussion. In work to be reported elsewhere, no significant movement of Cd-MT from peritubular blood in the direction of secretion could be found. Analysis of renal excretion data of Cd-MT may therefore focus on the processes of filtration and reabsorption. The data reported here are conveniently explained in terms of two simultaneous reabsorptive processes, only one of which becomes saturated at relatively low levels of Cd-MT; the second process does not approach saturation even at the highest plasma levels of Cd-MT reached in the clearance experiments. A similar fractional reabsorption of other low-molecular-weight proteins (insulin, ribonuclease) independent of wide absolute changes in filtered load was previously described by Cortney *et al.* (7).

Given the renal capacity for tubular reabsorption of filtered Cd-MT, it is now possible to consider the question of whether excretion of Cd in urine can be ascribed to Cd-MT. Analysis of 24-hr specimens of bladder urine from Cd-treated animals yielded a mean value of Cd excretion of 0.045 μg/min, equivalent to 0.57 μg of Cd-MT if present as such. According to the equation relating excretion to filtered load, this corresponds to a value of 17.26 μg of Cd-MT or 1.3 μg of Cd which must have been filtered per minute if excretion of the metal is to be entirely attributed to Cd-MT. Given a mean total GFR of 17 ml/min (see Table I), this presupposes a plasma level of 0.076 μg of Cd in the form of Cd-MT/ml. The actual plasma level of Cd amounted only to 0.01 μg/ml. Because of the low concentration, it proved difficult to determine with any accuracy the proportion of this Cd present as Cd-MT; at best, however, only a portion of circulating Cd was thus bound. But even if we assumed that Cd-MT could account for all plasma Cd, there would still remain an eightfold discrepancy between calculated and observed plasma levels. It seems unlikely, therefore, that Cd-MT can account for Cd excretion in Cd-poisoned rabbits.

Summary. Metallothioneins isolated from the liver of rabbits injected with ^{115m}Cd were infused into normal and Cd-treated rabbits, and renal filtration, excretion, and reabsorption of the isotope were determined. Reabsorption involved at least two processes, only one of which became saturated at low plasma concentrations of Cd-MT. This process was greatly depressed in poisoned animals. Nevertheless, the reabsorptive capacity even of poisoned kidneys remained so high that renal excretion of Cd in the poisoned rabbit cannot primarily involve filtration of Cd-MT and its tubular rejection, as had been suggested by other workers.

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