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HEPATIC UPTAKE OF CADMIUM AND ITS BILIARY RELEASE AS AFFECTED BY DITHIOERYTHRITOL AND GLUTATHIONE

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Abstract—Net cadmium uptake in the isolated perfused rat liver was half-maximal at 5 μM, and the maximal rate of uptake was 22 nmoles/min per gram liver wet weight. Uptake was augmented when a permeable thiol, dithioerythritol, was infused, whereas it was restricted when glutathione as a nonpermeable thiol or also when bovine serum albumin were infused. The ratio of extra cadmium taken up versus dithioerythritol added was 1:2. Uptake of cadmium was insensitive to anoxia or to the infusion of cyanide. Biliary cadmium release in the perfused liver was not augmented by dithioerythritol but was rather suppressed, whereas bile flow or the release of added ^{3}H-taurocholate were unaffected.

Cadmium was first recognized as a toxic element as the causative agent of Itai-itai disease in Japan, a disorder mainly characterized by damage to the proximal renal tubule (see [1] for brief review). The element accumulates in liver and kidney and is found associated predominantly with metallothionein. There are difficulties in accelerating cadmium loss from the body without kidney damage. Hepatic elimination via biliary excretion was increased by the dithiol, 2,3-dimercapto-1-propanol, without increasing renal cadmium contents [2], and the role of thiols in the biliary excretion of metals has been appreciated [3, 4].

In the present work, we examine the uptake of cadmium into the liver and the effects of thiols on it, together with biliary release. For this purpose, the isolated perfused liver is particularly useful, as short-term effects and steady states can be analyzed readily, an advantage over whole-animal studies.

EXPERIMENTAL METHOD

Hemoglobin-free perfusion of rat liver. Livers of male Wistar rats of 170–250 g body weight, fed on stock diet (Altromin, Lage, F.R.G.), were perfused without recirculation of the perfusate, using the bicarbonate-buffered Krebs–Henseleit solution, equilibrated at 37° with O_2/CO_2 (19/1, v/v) [5]. Perfusate flow was 4–5 ml/min g of liver, wet weight and was kept constant throughout the individual experiment.

Additions were made by micropumps directly before the portal vein.

The bile duct was cannulated with a polyethylene tube (Portex, Hythe, England) as described previously [6].

Assays. O_2 concentration and pH in the effluent perfusate were monitored continuously using a Clark-type platinum electrode and a pH-glass electrode, respectively.

Thiol in caval perfusate was detected continuously by adding 5,5′-dithiobis (nitrobenzoic acid) (DTNB) to the effluent perfusate (30 μM) and following the extinction at 405 nm in a flow-through cuvette in a spectrophotometer (Eppendorf model 6114, Hamburg, F.R.G.). Thiol concentration was calculated using an extinction coefficient for DTNB of 12.7/mM/cm.

^{109}Cadmium radioactivity was determined using a Multi-Prias gamma counter (Packard Instruments), window width 15–40 keV. Influent perfusate contained 0.5–5 nCi/ml ^{109}cadmium. Cd^{2+} uptake was calculated by the influent–effluent concentration differences, and release into bile was calculated on the basis of specific radioactivity of labeled cadmium in bile.

Materials. Chemicals were obtained from Merck (Darmstadt, F.R.G.), biochemicals and enzymes were from Boehringer (Mannheim, F.R.G.) or Sigma (München, F.R.G.). ^{109}Cadmium (carrier-free) was purchased from NEN (Dreieich, F.R.G.). *t*-Butyl hydroperoxide was a gift from Peroxidchemie (Höllriegelskreuth, F.R.G.). Bovine serum albumin (fraction V) was from Miles (Frankfurt, F.R.G.).

RESULTS

Cadmium uptake by the isolated perfused rat liver. Upon infusion of cadmium chloride into the portal vein, perfused rat liver takes up the metal at a steady rate. This is indicated by a stable concentration of cadmium in the perfusate leaving the liver via the caval vein. In the experiment shown in Fig. 1(A), the rate of Cd^{2+} infusion was 31 nmoles/min/g wet weight and the rate of its appearance in the caval perfusate was 21 nmoles/min/g wet weight, indicating an uptake at a rate of 10 nmoles/min/wet weight over the 30 min period in which the plateau level was maintained. In the experiment of Fig. 1(B), influx

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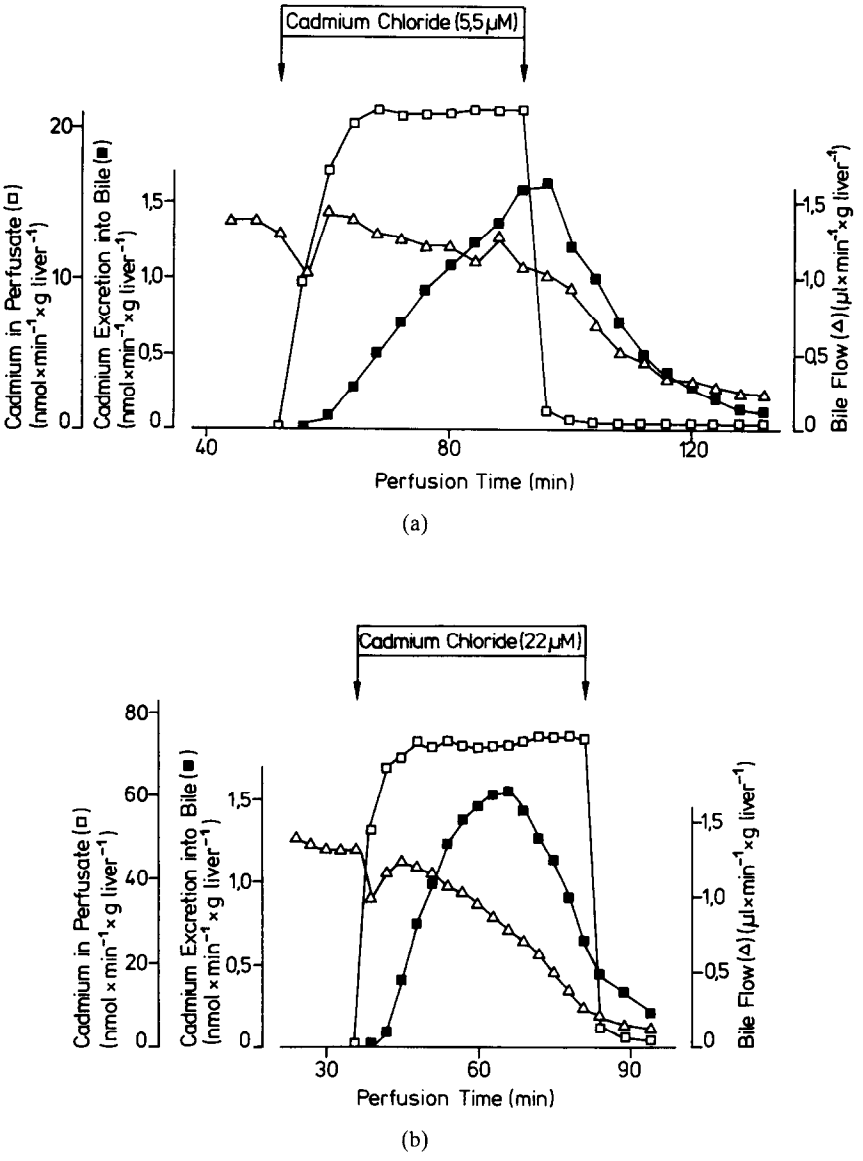

Fig. 1. Effluent perfusate (□) and bile (■) concentrations of cadmium in isolated perfused rat liver. The time interval in which cadmium chloride was infused from an infusion pump to give constant cadmium concentration in the portal vein is indicated on top. Cadmium concentrations were 5.5 and 22 μM in experiments a and b respectively, corresponding to 31 and 85 nmoles/min/g liver wet weight, respectively. Biliary flow is given as (△).

and efflux rates were 85 and 72 nmoles/min/g wet weight, respectively, also maintained over an extended period of time. Thus, the perfused liver responds to infusion of cadmium by an uptake into an intracellular reservoir of apparently considerable size. The uptake is concentration-dependent (Fig. 2), being half-maximal at a rate of infusion of 22 nmoles/min/g wet weight, corresponding to a concentration of approx. 5 μM Cd^{2+} in the influent perfusate. The maximal rate of uptake is 21.7 nmoles/Cd^{2+}/min/g wet weight as obtained from a double-reciprocal plot of the data shown in Fig. 2.

The rate of Cd^{2+} uptake can be significantly altered by compounds capable of binding to cadmium. As shown in Fig. 3, the infusion of glutathione leads to a rise in effluent perfusate Cd^{2+} concentration, whereas dithioerythritol has an opposite effect. These modifications in Cd^{2+} uptake require the free thiol group, as the infusion of the disulfide, GSSG, was without effect. The differences with GSH and dithioerythritol are readily explained by their penetrability. Whereas GSH does not penetrate across the plasma membrane from the sinusoidal space [7], dithioerythritol penetrates readily, and the Cd^{2+} complexes with these thiols obviously do likewise.

The stoichiometry of the extra cadmium uptake versus the dithiol is 1:2, as shown in Fig. 4. In these experiments, low concentrations of dithioerythritol

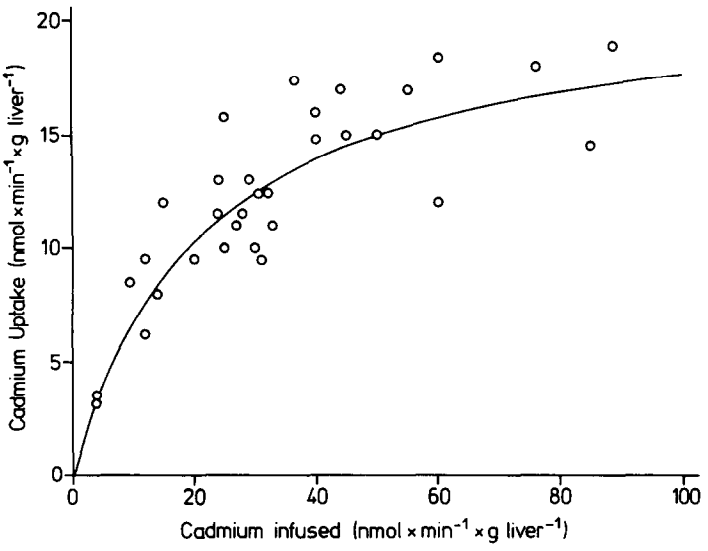

Fig. 2. Hepatic cadmium uptake as a function of the rate of cadmium infusion. Each point is taken from a steady state of effluent perfusate cadmium concentration, and the uptake is calculated from the difference to the portal influent concentration (compare experiments in Fig. 1). With average perfusate flow rate of 4.5 ml/min per gram wet wt, 1 μM cadmium corresponds to approx. 4.5 nmoles/min/g wet weight. Data from 18 different rat liver perfusion experiments.

were employed. Measuring the dithiol in the effluent caval perfusate, it was found that up to a concentration of about 5 μM dithioerythritol the liver takes up the dithiol completely, whereas at higher concentrations a small proportion of the infused dithiol passes through the organ. In other experiments in which the dithiol was initially infused, i.e. in converse order to the experiments in Figs. 3 and 4, it was observed that the addition of cadmium also stimulates the loss of the thiol, as demonstrated by a decrease in effluent thiol concentration (not shown).

Cadmium uptake by the liver was decreased when bovine serum albumin was infused (Fig. 5). With 5 μM cadmium in the influent portal perfusate, the uptake was such that the effluent perfusate contained 2.5 μM Cd^{2+}, and 300 μM albumin restricted the uptake of cadmium by 80%. This is presumably due to the binding of the metal to albumin.

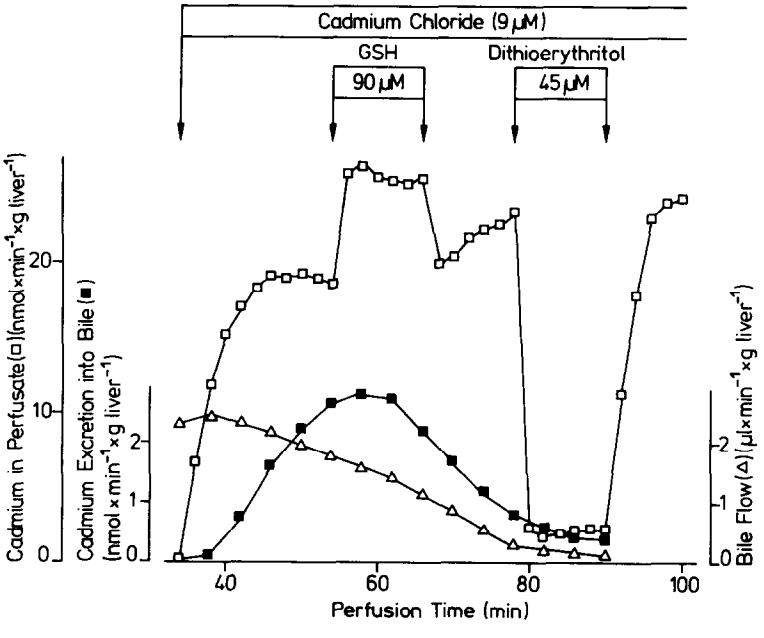

Fig. 3. Effect of glutathione (GSH) and dithioerythritol on hepatic cadmium uptake. Concentrations of the thiol compounds were similar regarding thiol groups. Conditions as in Fig. 1(a).

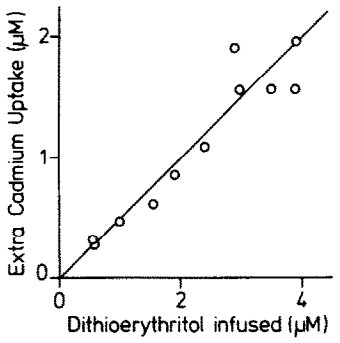

Fig. 4. Extra cadmium uptake elicited by dithioerythritol at low concentration. Cadmium in influent portal perfusate was approx 5 μM, and the dithiol was infused after a steady state effluent perfusate cadmium level had been established. Data from 3 different perfusion experiments. Stoichiometry is 1 Cd^{2+} taken up per 2 dithioerythritol infused.

The uptake of cadmium was not found to be sensitive to anoxic conditions (nitrogen instead of oxygen in the gassing mixture for 10 min), and the infusion of cyanide at 1 mM concentration also did not alter the rate of uptake (data not shown). Thus, the uptake process was not sensitive to transitions known to decrease the level of cellular ATP.

Biliary cadmium release. The biliary release of cadmium occurred at a rate much lower than the rate of uptake (Fig. 1), and over the period of time shown in Fig. 1(A) no steady state of efflux into bile established. When the livers were assayed for cadmium radioactivity at the end of the experiment, the accumulated cadmium accounted for the balance between uptake and release. Biliary cadmium concentration increased towards a maximum of about 2 mM, as can be calculated from the peak value of biliary cadmium release and the bile flow in Fig. 1(B). The decrease in bile flow with increasing time of perfusion was not significantly different in livers that received cadmium as compared to controls. Also, the addition of glutathione or of the permeant thiol, dithioerythritol, did not change the bile flow. However, the biliary excretion of cadmium was almost totally suppressed when dithioerythritol (45 μM) was added to the perfusion medium before cadmium (5 μM) was infused (not shown). This unexpected suppression of cadmium excretion into bile by dithioerythritol was selective for the metal, since both the bile flow (as mentioned above) and also the biliary elimination ^3H-labeled (5 μM) taurocholate were unaffected by the dithiol.

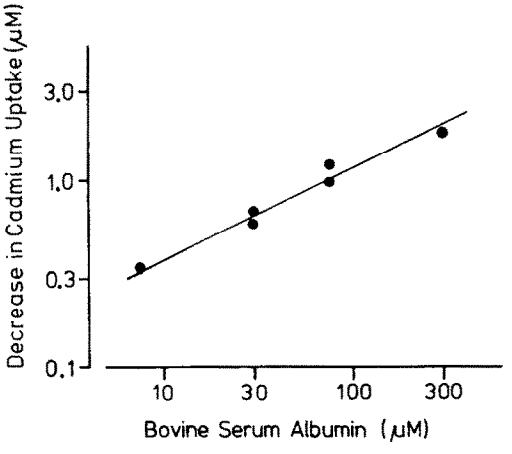

Fig. 5. Decrease in cadmium uptake by albumin. Bovine serum albumin (fraction V) was infused at the concentrations indicated in the abscissa when a steady infusion of cadmium (5 μM) had established a plateau level in effluent cadmium concentration. Cadmium uptake was 2.5 μM. Data from 3 different liver perfusions. Three hundred micromolar albumin corresponds to 20 mg/ml perfusate.

DISCUSSION

The present work demonstrates that rat liver takes up cadmium being half-maximal with 5 μM and with a maximal rate of uptake of 22 nmoles/min per gram liver wet weight (Fig. 2). This rate of uptake can be maintained over extended periods of time during which there is intracellular accumulation. The uptake was substantially facilitated when thiol groups were added in a permeant form, e.g. compounds like dithioerythritol. Nonpermeant thiols like glutathione, on the other hand, led to a restriction of cadmium uptake. Albumin as a binder of cadmium did likewise.

These observations may explain the previous report on the facilitation of biliary excretion of cadmium upon administration of certain thiol compounds [8]; the effects of thiol compounds may be localized predominantly at the uptake step rather than the release step. At low dithioerythritol concentrations (Fig. 4), the stoichiometry between cadmium and dithioerythritol was 1:2. It is not clear, however, whether membrane thiols are involved in the uptake of cadmium without the extra addition of SH compounds.

The present work does not argue against the involvement of thiols, notably glutathione, in the biliary excretion process of heavy metals, of course. Previous work is consistent with the participation of glutathione complexes of heavy metals such as zinc [9], chromium [10] or methylmercury [11, 12]. However, in the intact animal the rate limitation might also be at the uptake step, and thiols could have an important function in this regard, too. This is of particular interest in view of the substantial intracellular storage capacity in the form of metallothionein. Therefore, the present work may demonstrate the usefulness of the isolated organ in the study of the disposition of heavy metals as opposed to the intact animal or the suspensions of single cells.

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REFERENCES

1. M. R. S. Fox, in *Clinical, Biochemical and Nutritional Aspects of Trace Elements*, pp. 537–547. Alan Liss, New York (1982).

2. M. G. Cherian, *J. Toxicol environ. Hlth* **6**, 393–401 (1980).
3. C. D. Klaassen, *Drug Metab. Rev.* **5**, 165–196 (1976).
4. M. Cikrt, in *Industrial and Environmental Xenobiotics, Metabolism and Pharmacokinetics of Organic Chemicals and Metals. Biliary Excretion of Metals* (Eds. I. Gut, M. Cikrt and G. L. Plaa), pp. 17–36, Springer, Heidelberg (1981).
5. H. Sies, *Meth. Enzym.* **52**, 48–59 (1978).
6. T. P. M. Akerboom, M. Bilzer and H. Sies, *J. biol. Chem.* **257**, 4248–4252 (1982).
7. R. Hahn, A. Wendel and L. Flohé, *Biochim. biophys. Acta* **539**, 324–337 (1978).
8. M. G. Cherian, S. Onosaka, G. K. Carson and W. A. W. Dean, *J. Toxicol. environ. Hlth* **9**, 389–399 (1982).
9. J. Alexander, J. Aaseth and T. Refsvik, *Acta Pharmac. Toxicol.* **49**, 190–194 (1981).
10. T. Norseth, J. Alexander, J. Aaseth and S. Langärd, *Acta Pharmac. Toxicol.* **51**, 450–455 (1982).
11. T. Refsvik, *Acta Pharmac. Toxicol.* **52**, 22–29 (1983).
12. N. Ballatori and T. W. Clarkson, *Am. J. Physiol.* **244**, 435–441 (1983).