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Apparent Competition between Myoglobin and Metallothionein for Renal Reabsorption (40341)

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The function of the metallothioneins (MT), the metal-binding low molecular weight proteins whose synthesis is induced by cadmium and other heavy metals, remains in question. The work described here was initiated in order to evaluate a possible excretory function of CdMT, and to study the mechanism of its tubular reabsorption. Earlier reports provided evidence for the high capacity of rabbit kidneys to transport filtered CdMT out of the tubular lumen (1, 2). Reabsorption of CdMT proved sensitive to Cd intoxication and became saturated at high filtered loads; it could also be reversibly depressed by myoglobin, whereas lysozyme, immunoglobulin L-chain and ovalbumin remained without effect. Saturability, sensitivity to inhibition and relative specificity all support the conclusion that CdMT reabsorption represents a mediated process. The present paper explores in greater detail the interaction between CdMT and myoglobin, and leads to the tentative conclusion that these two proteins compete for tubular transport by the same system.

Materials and methods. Preparation of ^{109}CdMT, and the measurement of its renal transit characteristics and fractional reabsorption in rabbits have been described in detail in a previous publication (2). Similar techniques were applied in the study of myoglobin. Samples of horse myoglobin were iodinated by a standard procedure (3), and had an average final specific activity of 10^9 cpm/mg protein, as determined on a well-type scintillation counter (Packard Auto-γ). Labelled protein, together with ^{3}H-methoxyinulin, was administered as bolus injection via a short catheter advanced into the thoracic aorta. Radioactivity determinations involved simultaneous counting of ^{109}Cd and ^{3}H on a Packard Tricarb liquid scintillation spectrometer with automatic external standardization. Alternatively, for ^{125}I and ^{3}H, total β counts were determined, followed by subtraction of ^{125}I activity as calculated from the results of γ counting and the ratio of β to γ counts for ^{125}I. Recoveries were estimated as before (2) by summation of radioactivities of sequential urine or plasma fractions up to that fraction whose extrapolated tracer concentration fell below 2% of the cumulative total; extrapolation of the descending slope from peak values served to correct for recirculation of tracer (see e.g. Fig. 2). Mean transit time ($\bar{t}$) is defined as $\Sigma\ (C_t \cdot t)/\Sigma(C_t)$, where C_t represents the concentration of tracer in a fraction collected after elapsed time t (4). The male New Zealand white rabbits used in these studies weighed on the average 2.5 kg, and had been maintained on commercial pellets. Experimental procedures were carried out under pentobarbital anesthesia. In animals with two intact kidneys diuresis was induced by continuous infusion of 5% mannitol in saline at a rate of 2 ml/kg/min. Rabbits whose left renal vein had been cannulated for measurement of A-V transit times, and whose contralateral kidney as well as mesenteric artery had been tied off, received 0.4 ml 15% mannitol in saline/kg/min.

Results. The low permeability of muscle capillaries to myoglobin is well documented (5), as is its relatively low glomerular sieving coefficient (6). It is not surprising, therefore, that in its artery-to-vein transit characteristics across the kidney myoglobin resembles plasma protein (Evans Blue) rather than inulin. This is illustrated in Fig. 1 by the results of one of four similar experiments; the mean transit time for Evans Blue was calculated from the ratio $\bar{t}_{EB}/\bar{t}_{In}$ as measured in earlier work (7). In contrast, the mean vascular transit time of CdMT resembles that of inulin (2). If the rapid renal transit of myoglobin reflected primarily its binding to e.g. haptoglobulin, then it should be possible to prolong $\bar{t}_{myo}$ by saturating and exceeding the capacity

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of such ligands. However, in each of the above four studies a second bolus of myoglobin was injected, containing 1000 times the concentration used in Fig. 1 (2 mg versus 2 μg): no significant shift in $\bar{t}_{myo}$ relative to $\bar{t}_{In}$ was observed $(\bar{t}_{myo}/\bar{t}_{In})_{II}/(\bar{t}_{myo}/\bar{t}_{In})_{I} = 1.03$, range 0.89 – 1.10. In the rabbit kidney, as in other tissues studied, free myoglobin is therefore clearly much less diffusible, and presumably less filterable, than is inulin.

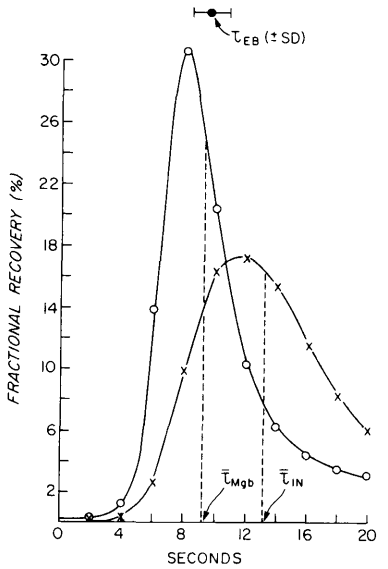

FIG. 1. *A-V transit time of myoglobin. Rabbit Myo 10.* Renal venous blood flow 40 ml/min, hematocrit 25%. The bolus contained 2 μg ^{125}I-myoglobin and 10 μCi ^{3}H inulin in a final volume of 0.3 ml. Venous recoveries are shown for ^{125}I (○) and ^{3}H (×); mean transit times ($\bar{t}$) are indicated, and were calculated for Evans Blue (EB) from the ratio of $\bar{t}_{EB}/\bar{t}_{In}$ obtained in earlier studies (7).

It follows from the restricted diffusibility of myoglobin that the precise filtered load and, therefore, the fractional reabsorption of the protein cannot be accurately defined under present conditions. Accordingly, the following experiments on factors influencing tubular handling of myoglobin compare absolute excretion of ^{125}I-myoglobin under various conditions, rather than its fractional reabsorption. Figure 2 shows the urinary transit characteristics of myoglobin at low and high concentrations. Clearly, using inulin excretion as reference point, the excretion of ^{125}I-myoglobin was increased in presence of excess unlabelled myoglobin. Results of 12 similar studies are collected in Table I and show that, on the average, excretion of label rose by 43% above control values at the high myoglobin concentrations. Attention is further drawn to the fact that the same result was achieved by addition of 1.1 mg CdMT. Such a concentration of CdMT was previously shown to exert no acute toxic effect on the kidney (2); similarly, in the present study, 1.1 mg CdMT caused no inhibition of tubular PAH transport. An additional observation illustrated in Fig. 2 is the tubular transit delay of myoglobin; such a delay was consistently observed in every study and resembles that reported for CdMT (2).

Interaction between CdMT and myoglobin

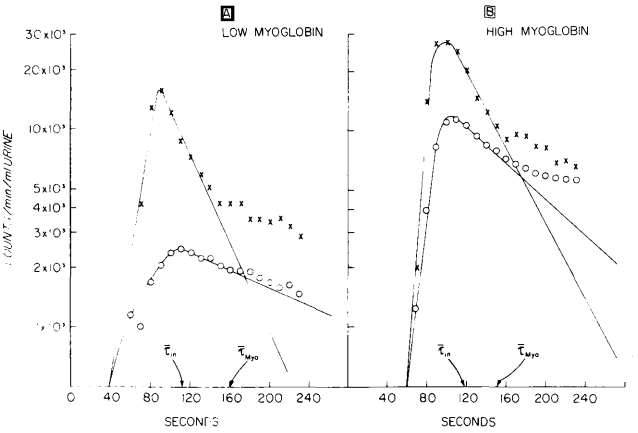

FIG. 2. *Tubular transit of myoglobin. Rabbit Myo 16L.* Urine flow period I: 2.6 ml/min, II: 2.8 ml/min. Urinary tracer recoveries are shown for ^{125}I (○) and ^{3}H (×). Each bolus contained (in 0.5 ml) 2 μg ^{125}I-myoglobin + 4 μCi ^{3}H-inulin; bolus #2 contained in addition 2 mg unlabelled myoglobin, and was injected 15 minutes after bolus I.

TABLE I. MYOGLOBIN EXCRETION.[a]

Periods			Excretion of ^{125}I Period II/I	
I	II	*n*	Mean	Range
Control	+2 mg Myo	12	1.43	0.83–2.00
Control	+1.1 mg CdMT	5	1.46	1.05–1.79

[a] Each bolus contained 2 μg ^{125}I-myoglobin. Unlabelled myoglobin and CdMT were added as shown to second bolus, which was injected 15 min after bolus 1. Excretion was computed as shown in Fig. 2.

is not characteristic only of low molecular weight proteins. Thus, in experiments on four animals (8 kidneys), in which 150–300 mg hemoglobin were injected intravenously 60 sec before the usual arterial bolus containing 100 μg CdMT, the fractional reabsorption of CdMT fell from a mean of 57% (SD, 10%) to 11 ± 10%.

Discussion. Table I shows that an excess of unlabelled myoglobin increases excretion of labelled myoglobin, a result which could reflect either displacement of the labelled compound from plasma ligands with subsequent increase in its filterability, or saturation of a tubular transport mechanism. Attention may here be focused on the action of excess CdMT: this protein does not react with high molecular weight plasma constituents under present conditions (2). Although CdMT does, therefore, not compete with myoglobin for a common plasma protein ligand, it exerted the same effect on ^{125}I-myoglobin reabsorption as did excess myoglobin (Table I). It seems likely, therefore, that after injection of excess myoglobin we are dealing with saturation of its reabsorption, not with increased fractional filterability. In other words, like CdMT, myoglobin appears to be reabsorbed from the renal tubule by a saturable process. Further, this process is inhibited by CdMT, just as previous experiments had shown an affinity of myoglobin for the system mediating CdMT reabsorption (2). Both proteins undergo similar tubular transit delays during excretion. We also recall the ready reversibility of the myoglobin inhibition of CdMT reabsorption (2). A plausible explanation for the great similarities in the renal handling of CdMT and of myoglobin, and for the mutual inhibition of their reabsorption, invokes competition for a common reabsorptive system. Such competition is unlikely to reflect only similarity in size of the two proteins, as hemoglobin also inhibits CdMT reabsorption; on the other hand, lysozyme and immunoglobulin L-chain did not affect CdMT transport (2). Whether the apparent competition between myoglobin and CdMT, and the saturation of their respective reabsorption, are events primarily associated with the first step in protein reabsorption at the brush border cell membrane (8) cannot be decided on the basis of the results described here.

Summary. Artery-to-vein and artery-to-urine transit characteristics of ^{125}I-myoglobin across the rabbit kidney were compared to those of cadmium-metallothionein (CdMT) labelled with ^{109}Cd, and their interaction during tubular reabsorption was determined. Both proteins are reabsorbed by a saturable system, mutually inhibit each other's reabsorption, and suffer similar tubular transit delays. On the basis of these results, and of the previous observation that myoglobin inhibition of CdMT reabsorption is fully reversible, we may tentatively conclude that the two proteins compete for reabsorption by a common transport system. This system also reacts with hemoglobin, indicating that its affinity for substrates is determined by factors other than purely size of the protein molecule.

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