Excretion and retention of cadmium, zinc, and mercury by rabbit kidney

E. C. FOULKES

Departments of Environmental Health and Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45219

Foulkes, E. C. Excretion and retention of cadmium, zinc, and mercury by rabbit kidney. Am. J. Physiol. 227(6): 1356-1360, 1974.—Mean renal artery-to-vein transit times (f) of $^{109}$Cd, $^{65}$Zn, or $^{203}$Hg were compared to those of Evans blue (EB) and inulin in rabbits. All three elements were fully recovered in venous plasma, where they slightly preceded inulin, although f exceeded fEB. Competition for the injected metals is suggested between circulating plasma protein and fixed endothelial ligands. Upon addition of mercaptoethanol (ME) to the bolus injection, $^{109}$Cd appeared in urine; total recovery in blood and urine dropped to two-thirds of the dose reaching the kidney. Renal retention of the remaining one-third was reduced by 30 % after ureteral occlusion. Both luminal and peritubular cell membranes thus are involved in Cd uptake, a conclusion in agreement with the previous finding of lesions at these two sites. In the presence of ME, essentially all $^{203}$Hg was retained in the kidney but renal handling of Zn was not affected. The stronger chelator EDTA led to recoveries of $^{109}$Cd in blood and urine approaching those of inulin. These results bear on the role of metal ligands in determining renal excretion and accumulation of heavy metals.

thiol compounds; metal binding; secretion; renal accumulation; mean transit times

THE SENSITIVITY OF THE KIDNEYS to toxic effects of heavy metals is well documented. In addition the extent of the renal excretion of such metals plays, of course, a major part in determining their accumulation in the body. In spite of these facts the nature of the reaction between kidney and toxic metals remains unclear, and relatively little is known of the extent to which filtration, reabsorption, and secretion possibly determine metal excretion.

Because of their high reactivity with proteins and other plasma constituents, heavy metal ions usually circulate in plasma as complexes with a variety of small- or large-molecular-weight compounds. As a result patterns of renal excretion of heavy metals injected directly into the renal circulation (e.g. 17) may differ greatly from those observed after metal administration by other routes. Study of the normal excretion of heavy metals in urine, or of their reaction with the kidney, therefore largely resolves itself into an analysis of the renal handling of metal complexes. Classical illustrations of the role of ligands in metal excretion are furnished by the tubular secretion of organic mercury compounds (1) and the therapeutic use of strong chelating compounds to remove toxic metals from the body (e.g. 3). Another instance where presence of an organic ligand determines the affinity of the kidney for a heavy metal is the effect of thiol compounds in increasing the renal toxicity of cadmium (12, 16).

The need to elucidate the interaction of heavy metals with the kidney and to clarify the role of ligands in this process led to formulation of the following specific questions about the renal handling of cadmium, zinc, and mercury. 1) What is the fate of free metal ions injected into the renal artery? 2) To what extent do metal ligands alter the renal disposition of the metals? 3) Under what conditions can heavy metals react with and become concentrated by the kidney? Answers to these questions are considered in relation to mechanisms of renal excretion and accumulation of these metals.

METHODS

Male white New Zealand rabbits weighing 2-3 kg were used for these experiments. They were surgically prepared under Nembutal anesthesia as previously described (6). In short, this involved preparation of an aortic pocket at the level of the left renal artery and ligation of the right kidney and mesenteric artery. Urine was collected from a ureteral catheter. Venous effluent drained into a reservoir from which it was returned to the jugular vein. Urine flow was stimulated by addition of 1 ml/min 12.5% mannitol in saline to the venous reservoir. At the beginning of surgery sufficient blood was exchanged for 6% dextran in saline to permit subsequent volume replacement with blood in order to avoid significant changes in plasma protein concentration and hematocrit. Hematocrit values ranged from 20 to 25%. Isotopes, purchased from ICN Corp., were injected through a PE-50 catheter into the aortic pocket. After injection and depending on blood flow, successive 2- or 3-s venous blood samples were collected. Radioactivity determinations were performed on a Packard Tri-Carb and an automatic gamma well-type scintillation spectrometer. Mean transit times were computed as previously (5) from the formula $T = t \cdot \Delta t / \Sigma C_i \cdot \Delta t$, where $C_i$ represents recovery in a fraction collected at time $t$ and over a time interval of $\Delta t$. All times were corrected for passage through catheter dead space. Collections were continued until recovery per fraction dropped below 2% of the cumulative total. Recoveries of Cd, Zn, and Hg are related to that of inulin on the assumption that total inulin collected in plasma and urine represents 100% of the injected dose reaching the kidney. The filtration fraction is expressed as $^3$H excreted in urine/total $^3$H recovered in blood and urine.
Earlier attempts to use total injected inulin as base line for computation of recoveries led to variable results. To some extent this may be attributed to the difficulty of accurately measuring the small volume of the bolus. A more serious difficulty arises from the fact that the dead-space volume in the aortic catheter and aortic pocket is not insignificant in relation to the bolus injected. Of course, loss of metal in this dead space will be proportional to loss of inulin. The total fraction of bolus actually reaching the kidney therefore is appropriately represented by the sum of inulin recoveries in blood and urine. Before each subsequent injection blood was permitted to drain through the arterial catheter in order to flush out the dead space.

RESULTS

Renal transit times. In Fig. 1 are shown some typical curves relating the fractional recovery of inulin, Cd, and Evans blue to the time of collection of renal venous samples. From these curves mean transit times like those illustrated in Fig. 1 were computed for each solute. The mean transit time of plasma protein as measured with Evans blue is seen to be shorter than that of cadmium, which in turn slightly but significantly precedes inulin. Values for mean transit times relative to the simultaneously measured were obtained in repeated experiments; mean values and ranges are collected in Table 1. Similar experiments were also carried out with Hg and Zn, whose mean transit times bear the same general relationship to \( t_{\text{in}} \) as does \( t_{\text{cd}} \). The mean of \( t_{\text{in}} \) values obtained successively in 11 kidneys corresponds to an average value for the inulin space in these kidneys of 20 ml/100 g fresh wt (range 16–32) as calculated from the product of plasma flow and \( t_{\text{in}} \).

The small but significant difference in \( t_{\text{cd}} \) and \( t_{\text{in}} \) shown in Table 1 disappears when the injection bolus contains an excess of mercaptoethanol (see also Table 3). In 12 such studies the ratio of \( t_{\text{cd}}/t_{\text{in}} \) averaged 1.01 (range 0.97–1.08). In two further experiments the addition of 20 \( \mu \)mol ethylenediaminetetraacetic acid (EDTA) instead of mercaptoethanol (see Table 3) yielded ratios of 0.95 and 1.00. In the presence of these low-molecular-weight ligands the transit kinetics of Cd thus resemble those of inulin.

A preliminary report of the present results (8) stated that absence of delayed appearance of Cd such as seen in Fig. 1 could be equated with absence of appreciable filtration and reabsorption of Cd. Attention was drawn in contrast to the prolonged mean transit times of Na, a solute freely filtered and reabsorbed back into the bloodstream. As shown, however, in Fig. 2 by the results of one of two such studies tailing of the Na transit curve does not reflect filtration and reabsorption. Thus \( t_{\text{in}} \) remained unchanged in relation to \( t_{\text{in}} \) even after inhibition of filtration by ureteral occlusion (note the increased relative recovery of inulin as a measure of completeness of inhibition of filtration).

In a further series of experiments mean transit times were determined for Cd and inulin in isolated kidneys in the presence or absence of plasma protein. The kidneys were perfused through the renal artery at 3–5 ml/min at room temperature. In a control period the perfusate consisted of heparinized plasma; a 6 % dextran solution in bicarbonate-Ringer solution was used during period 2. Transit times for Cd and inulin from artery to vein were determined in absence of ME as usual. In period 1 (plasma perfusion) the ratio of \( t_{\text{cd}}/t_{\text{in}} \) averaged 0.98. Reasons for the lack of close agreement between this result and the value shown in Table 1 are not clear but are presumably related to the extensive differences in experimental conditions. In any case, the value of 0.98 strongly contrasts with that of 2.9 in period 2, after removal of circulating protein. At the same time venous recovery of Cd dropped from 103 to 57 % of injected dose (all values represent means from 2 separate studies).

Renal retention of heavy metals. Table 2 illustrates the finding

### Table 1. Relative renal artery-to-vein transit times

<table>
<thead>
<tr>
<th>Solute</th>
<th>( t_{\text{cd}}/t_{\text{in}} )</th>
<th>( n )</th>
</tr>
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<tbody>
<tr>
<td>(^{109}\text{Cd} )</td>
<td>0.84 (0.60–0.98)</td>
<td>28</td>
</tr>
<tr>
<td>(^{68}\text{Hg} )</td>
<td>0.82 (0.74–0.91)</td>
<td>10</td>
</tr>
<tr>
<td>(^{65}\text{Zn} )</td>
<td>0.71 (0.59–0.83)</td>
<td>5</td>
</tr>
<tr>
<td>Evans blue</td>
<td>0.61 (0.50–0.83)</td>
<td>14</td>
</tr>
</tbody>
</table>

All transit times were corrected for dead-space delays; respective mean ratios of \( n \) determinations are shown in each case with range in parentheses. A small number of ratios were based on creatinine rather than inulin transit times but were included here since \( t_{\text{in}} \) and \( t_{\text{creatinine}} \) did not differ significantly.

### FIG. 1. Renal artery-to-vein transit curves. Animal Cd no. 21, 3.3 kg. Injection contained in 0.3 ml saline 1 \( \mu \)mol CdCl\(_{2}\), 1 \( \mu \)Ci \(^{109}\text{Cd} \), 25 \( \mu \)Ci \(^{131}\text{Hg} \) methoxyinulin, and 1 mg Evans blue. Blood flow 25 ml/min, hematocrit 22 %, urine flow 1.3 ml/min, kidney wt 14.5 g.

### FIG. 2. Effect of ureteral occlusion on \( t_{\text{in}} \). Rabbit Cd-Na no. 4, 2.9 kg. Injection contained 25 \( \mu \)Ci methoxyinulin plus 0.7 \( \mu \)Ci \(^{22}\text{Na} \) in 0.3 ml NaCl. Blood flow 33 ml/min, hematocrit 22 %, urine flow (period 1) 1.1 ml/min, kidney wt 16.0 g.
that after intra-arterial administration of 1 μmol of Cd, Hg, or Zn (as chlorides) essentially all the injected material reaching the kidney is recovered in renal venous plasma. In contrast with this complete recovery in control studies, a significant portion of Cd is retained in the kidney when injected together with an excess of mercaptoethanol (see also Table 3). In the case of Hg the addition of the thiol compound leads to essentially complete removal of all isotope from blood and urine. Large amounts of Cd and especially of Hg are found in the kidney after such injections, of an order of magnitude sufficient to account for uptake from blood and urine. Clearance of Hg from blood was not affected by addition of 40 μmol probenecid to the injection, a concentration that greatly decreased transport of PAH (2 experiments). Unlike uptake of Cd and Hg, that of Zn is not facilitated by mercaptoethanol. Nor was any Cd uptake seen in two experiments in which the mercaptoethanol was injected together with an excess of mercaptoethanol (see Table 3). In this particular experiment most of the injected Cd was recovered in renal venous plasma in the control period; no appreciable counts were present in red cells. Only in the case of Cd + ME was metal excreted in urine (see Table 3). * Mercaptoethanol; for details of injections see Table 3.

A more detailed analysis of effects of metal ligands (mercaptoethanol and EDTA) on Cd excretion and retention is presented in Table 3. In this particular experiment most of the injected Cd was recovered in renal venous plasma in the control period; no appreciable Cd appeared in urine. The addition of mercaptoethanol led not only to significant urinary excretion of Cd but also to greatly reduced total recovery (cf. Table 2). Use of the much stronger metal ligand EDTA causes recoveries of Cd to approach those of inulin; similar results were obtained in a small number of studies with Zn.

Site of Cd uptake by the kidney. In order to evaluate the relative contributions to total renal Cd retention of a) reabsorption from tubular urine and b) uptake by cellular elements more directly from blood, the effect of ureteral occlusion on Cd uptake was determined. The assumption is made that abolishing glomerular filtration in this manner will not alter processes occurring on the blood side of renal cells. Table 4 illustrates results and computations from means of four similar experiments. Under free-flow conditions, and in agreement with Table 3, a total of 64% of Cd reaching the kidney was recovered in blood and urine so that 36% may be assumed to have been retained in the tissue. This retention was reduced to 24% after ureteral occlusion; the difference of 12% presumably represents that portion of Cd that during the control period was filtered and reabsorbed and retained in the kidney. In other words, this computation leads to the conclusion that under present conditions about one-third of total Cd uptake by tissue represents uptake from tubular urine, whereas the remaining two-thirds reflect uptake across the peritubular side of tubule cells and/or accumulation by other cellular structures in the kidney. A further calculation as shown indicates that even in presence of excess mercaptoethanol only 14/24 or roughly two-thirds of plasma Cd were filterable.

**Discussion**

The critical influence of various low-molecular weight metal ligands on the renal handling of Cd and other metals is fully confirmed by the present results. In the absence of such compounds the metals are neither excreted nor re-

<table>
<thead>
<tr>
<th>TABLE 2. Recovery of metals in venous plasma</th>
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<tbody>
<tr>
<td>Solute</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>114Cd</td>
</tr>
<tr>
<td>(n = 5)</td>
</tr>
<tr>
<td>203Hg</td>
</tr>
<tr>
<td>(n = 7)</td>
</tr>
<tr>
<td>65Zn</td>
</tr>
<tr>
<td>(n = 4)</td>
</tr>
</tbody>
</table>

Values shown represent mean and range of n separate results (±SD), calculated from [(metal/W) plasma/(metal/W) infused] X 100 (1 - filtration fraction). No appreciable counts were present in red cells. Only in the case of Cd + ME was metal excreted in urine (see Table 3). * Mercaptoethanol; for details of injections see Table 3.

<table>
<thead>
<tr>
<th>TABLE 3. Retention and excretion of 114Cd</th>
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<tbody>
<tr>
<td>Period</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1) Control</td>
</tr>
<tr>
<td>Cd</td>
</tr>
<tr>
<td>2) ME</td>
</tr>
<tr>
<td>Cd</td>
</tr>
<tr>
<td>3) EDTA</td>
</tr>
<tr>
<td>Cd</td>
</tr>
</tbody>
</table>

This is one of 5 such experiments in which a bolus of 0.3 ml containing 23 μCi[3H]methoxyinulin, 1 μCi 114Cd, and 1 μmol CdCl2 was injected intrarenally. Mercaptoethanol (ME), 350 μmol, and ethylenediaminetetraacetic acid (EDTA) disodium salt, 20 μmol, were added as shown. No counts were found in red cells. Inulin recovery was set at 100%. Urinary recovery of Cd is given by ([114Cd/3H] urine/(114Cd/3H) infused) X 100 (filtration fraction); for plasma recoveries see Table 2.

<table>
<thead>
<tr>
<th>TABLE 4. Site of 114Cd uptake by kidney in presence of mercaptoethanol</th>
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<tr>
<td>Percent of Renal Dose</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Free flow</td>
</tr>
<tr>
<td>114Cd</td>
</tr>
<tr>
<td>Recovery</td>
</tr>
<tr>
<td>In blood</td>
</tr>
<tr>
<td>In urine</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Retention</td>
</tr>
<tr>
<td>After uptake from blood</td>
</tr>
<tr>
<td>After reabsorption from urine</td>
</tr>
<tr>
<td>Filtered load (excreted + reabsorbed)</td>
</tr>
</tbody>
</table>

These results represent means of 4 similar studies in which 1 μmol Cd and 350 μmol mercaptoethanol were injected as in Table 3 first during a control, free-flow period, then a second time 6-8 min after ureteral occlusion. Means and ranges are given for experimentally determined values; remaining values represent computations from these means.
tained by the kidney. Renal transit characteristics and rec-
coversies of the metals are strongly affected by the presence
of ligands.

Considerable significance has been attached to the shape
of renal transit curves such as those illustrated in Fig. 1. As
pointed out above, the lack of tailing in the transit curve of
Cd had been mistakenly taken in a preliminary communi-
cation (8) as proof that Cd is not filtered or reabsorbed. That
such a correlation between delayed appearance and filtra-
tion-reabsorption is invalid at least for Na had been pre-
viously reported by Eisner et al. (5), who studied the
influence of ureteral occlusion on transit kinetics of Na in
dogs. In the rabbit also, as shown in Fig. 2, abolition of
filtration fails to abolish the delayed recovery of Na in
venous blood. Further evidence that filtration and reabsorp-
tion may not be reflected in artery-to-vein transit curves is
furnished by an earlier analysis of the renal transit charac-
teristics of alpha-aminoisobutyrate in the rabbit (7).

Interpretation of mean transit times also depends on the
significance attributed to the volume of distribution cal-
culated as the product of plasma flow and $t$ (2). If this
volume represents a space within which the ions are freely
diffusible it follows that a major portion of the insulin space
of the tissue must be freely accessible to the metals. Given
the recent report of the relative permeabilities of glomerular
and peritubular capillaries (4) we would then also have to
assume free filtration of circulating metals at the glomerulus.
The observations of Perry et al. (13) that Cd remains diffus-
ible for many seconds after intravascular injection into rats
would be compatible with this interpretation. Nor, as shown
above, can filtration and reabsorption be excluded a priori
by the absence of tailing of the transit curve. Of course,
the postulated process of reabsorption of filtered Cd, Zn, and
Hg would have to be complete and extremely rapid without
any retention of reabsorbed ions in the kidney. Only in this
manner could the total and early recovery of the metals in
venous plasma be satisfactorily explained. Finally, on this
basis, it would have to be concluded that the function of
mercaptoethanol in permitting retention of, for example,
Cd in the tissue lies in a direct effect on the reaction of the
metal with the kidney.

The necessary attributes of the postulated process of metal
reabsorption do not appear very likely. A more plausible
interpretation of the results starts from the probability that
$V_{Gd}$ represents only a virtual volume of distribution in which
Cd is not freely diffusible in absence of mercaptoethanol.
This could arise from transient binding of Cd to endothelial
cells during passage of the ion across the kidney. Reaction of
Cd, Zn, and Hg with rabbit vascular tissue has been pre-
viously observed (13). In other words, circulating plasma
protein and fixed metal ligands are assumed to compete for
the injected ion. This competition should lead to prolonga-
tion of $t_{cd}$ as plasma protein levels are reduced. Such an
inverse correlation was indeed observed in kidneys perfused at
room temperature as described in RESULTS. The suggested
competition for Cd between circulating plasma protein and
fixed endothelial sites in the kidney would explain the delay
of Cd behind Evans blue and would avoid the difficulty of
reconciling apparent diffusion of Cd out of the vascular
space with probable absence of Cd filtration. It is important
to specify endothelial sites: if one assumed that tran-
sient retention of Cd involved parenchymal cells, Cd would
obviously have to leave vascular spaces and the reason for its
probable nonfiltration would remain unsolved.

In the absence of low-molecular-weight ligands Cd thus
appears to be restricted to vascular spaces. In the presence of
mercaptoethanol, by contrast, Cd is excreted in urine.
Presumably Cd filtration now occurs, and the similarity of
mean transit times of insulin and Cd also suggests that the
metal complex is freely diffusible. However, the identity of
$\lambda_{cd}$ and $\lambda_{in}$ in the presence of mercaptoethanol may be
incidental, as the calculation in Table 4 shows that even
under these conditions approximately one-third of circulat-
ing Cd may be protein bound. It seems likely that the major
role of the thiol compound in permitting filtration and
retention of Cd lies in its ability reversibly to complex the
metal in competition with high-molecular-weight or fixed
ligands and thus to render it diffusible. This view is sup-
pported by the finding that addition of 0.15 M mercapto-
ethanol to heparinized rabbit plasma approximately tripled
the ultrafilterability of Cd in vitro. Finally, mention may be
made in this connection also of the lack of effect of prior
administration of mercaptoethanol. Apparently the ligand
must actually be present in plasma together with Cd before
an effect on Cd retention can be observed; a primary direct
action of the thiol compound on the kidney does not seem to
be involved.

The observations reported here on the role of a thiol com-
pound in facilitating uptake of Cd by the kidney help ex-
plain the influence of such compounds on distribution of
injected Cd in the body and on its renal toxicity (12, 16).
The main effect of mercaptoethanol cannot consist of al-
terations in the systemic distribution of Cd, as even after
intra-arterial injection the metal did not enter the tissue over
short time periods in the absence of mercaptoethanol. The
action of the thiol compound therefore must be intrarenal,
permitting Cd to reach renal Cd receptors. This effect may
be contrasted with that of EDTA, a much stronger com-
xplexing agent. In this case the metal is very tightly bound,
and although the complex is freely diffusible and filterable
it does not readily react with the tissue. Free filtration of
EDTA complexes of different metals has often been ob-
erved (e.g. 15).

According to the effects of ureteral occlusion, Cd in the
presence of mercaptoethanol is taken up by the kidney both
at cell membranes facing tubular lumina (luminal mem-
branes) as well as across cell borders in contact with blood or
interstitium (peritubular membranes). It is interesting to
recall that injection of Cd with mercaptoethanol also leads
to functional lesions at both sites: at the luminal membrane
depressed reabsorption of all filtered amino acids was ob-
erved (11); in the case of peritubular membranes specifi-
cally the reaction with dicarboxylic amino acids was
inhibited (10).

The present results also have some bearing on possible
contributions of tubular secretion to renal excretion of heavy
metals. In the case of Hg secretion is well established; thus,
certain organic mercurials are known to be transported by a
probeneicid-sensitive system in the proximal tubule (1). It is
not certain that this fact relates to the finding reported here
that in the presence of mercaptoethanol Hg is almost com-
pletely cleared from renal blood. In light of the failure of
probenecid to prevent this uptake of Hg, and because of the absence of Hg excretion into urine, it is possible that the kidney may here simply act as a passive sink.

For other metals the evidence suggesting secretion is less clear. Thus it may not be correct to infer secretion in mammals from urinary precession of metals ahead of simultaneously injected glomerular markers (14) or from the appearance in urine of metals injected into the portal circulation of birds (17). We have previously attributed appearance in urine of metals injected into the portal circulation of birds (17). We have previously attributed urinary precession to the existence of an ion-permeable nephron segment in the renal medulla across which blood and tubular urine can equilibrate (9). This equilibration does not lead to net secretion. Similarly, permeation of metal ions across the chicken nephron cannot necessarily be equated with net secretion of the ions. The question must also be considered whether demonstration of possible secretion of free ions is relevant to the problem of excretion of these ions under physiological conditions. As pointed out in the introduction, metal excretion can normally be equated with excretion of metal complexes because this is the form in which the metals circulate in plasma. No conclusion on the possible occurrence of Cd secretion can be drawn at this time.

It was a pleasure to work with Mrs. Sheila Blanck and Mr. Clive Pullinger in this investigation.

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