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Accumulation of Lead by Renal Slices in the Presence of Organic Anions (41112)

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Abstract. *In vitro* uptake of lead by rabbit renal cortical slices was measured using ^{203}Pb. In protein-free medium, steady-state slice-to-medium ratios were 30–70. The addition of protein (2 g/dl) or cysteine (11.5 or 23 μM) markedly reduced this ratio. All other experiments were performed with protein-containing medium. Cysteine or citrate, 113 μM, significantly increased the slice-to-medium ratio by 12–19%, but addition of serum ultrafiltrate had no effect. Metabolic inhibitors significantly reduced the ratio by 12%, but because this figure was similar to the percentage swelling by the slices, there was no effect on the mass of lead accumulated. The inhibitors had no significant effect on the increment in S/M ratio produced by cysteine or citrate. We conclude that lead cotransport into renal slices with low-molecular-weight organic ligands of plasma is very small compared to the uptake due to active transport of free ionic lead.

Because the trace elements, in general, and heavy metals, in particular, exist in the body fluids mainly in combination with organic anions, it has been widely assumed that the transport of lead must be intimately associated with that of these anions (1). However, our previous study strongly suggested that lead can enter cells of renal slices as the free ion, most likely via an active transport system specific for group IVA elements (2). The present experiments were designed to evaluate the possibility that other pathways, coupled with organic anions, also play a role in lead transport into renal tissue. The effects of adding different organic anions on the accumulation of ^{203}Pb by renal slices *in vitro* was studied.

Methods. Male Langshaw rabbits weighing 1–2.5 kg were used. They were killed by a sharp blow on the head, followed by thoracotomy. Renal cortical slices were prepared as described by Rosenberg *et al.* (3); the slices were made by hand using a Stadie–Riggs tissue slicer, the blade being changed at least once during the slicing. Two types of buffer solutions were used, the composition and preparation of which have been described previously (2): Krebs–Ringer bicarbonate (KRB) or Tris buffer (50 mM Trizma–HCl at pH 7.4). The atmosphere above the KRB buffer was gassed with 95% O_2–5% CO_2, that above the Tris buffer with 100% O_2. Temperature was maintained at 37°.

All slices (each approximately 0.3 mm thickness) were preincubated for 30 min in a separate flask containing no Pb and were then transferred, six to a flask (total slice weight = 70–125 mg) to 125-ml Ehrlenmeyer flasks containing 20 ml of buffer, 0.1 μM carrier Pb (II) acetate, approximately 0.2 μCi ^{203}Pb obtained as carrier-free (<3 ng Pb) Pb (II) acetate from New England Nuclear, and varying concentrations of cysteine, citrate, cystine, glutathione, bovine serum albumin (fraction V), or ultrafiltrate of rabbit serum. The latter was prepared by ultracentrifugation of serum through Amicon G-50 filter cones (4) and diluting this ultrafiltrate with an equal volume of KRB. The concentrations of the organic anions were chosen to approximate normal plasma concentrations. When metabolic inhibitors were used, they were present during both the 30-min preincubation and the 90-min incubation with Pb. All results are reported as the ratio (S/M) of slice concentration (per gram wet weight) to medium concentration (per milliliter). Each value is the average of two flasks (12 slices). There were no differences observed in any experiments between the uptake from KRB or Tris-buffer, and so the results have been pooled. In all cases when drugs or different media were used, control incubations (no drugs and a standard buffer) were performed in the same experiment for purposes of comparison. All group data are reported as mean ± SE.

Results. Figure 1A summarizes the ef-

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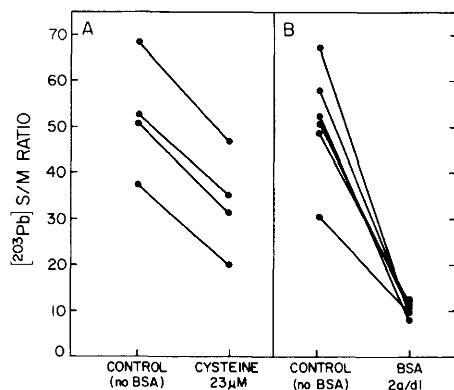

FIG. 1. Effects of cysteine (left panel) or BSA (right panel) on the slice-to-medium ratio for ^{203}Pb in renal cortical slices. Each pair of points denotes a separate experiment.

fects of adding cysteine (23 μM) to the medium. In each of the four experiments the accumulation of ^{203}Pb by the slices was markedly reduced by this addition. As will be described in the Discussion section, we reasoned that this decrease was due to a lowering of the free Pb concentration in the medium, resulting in a proportional decrease in uptake via the ionic-Pb transport pathway previously documented (2). Under such conditions, it would be impossible to evaluate the contribution of a cysteine-coupled uptake pathway for Pb. Therefore, in all subsequent experiments, we used BSA, 2 g/dl, in all media to serve as a source of Pb for binding to the added anion. Figure 1B demonstrates the profound reductions in Pb uptake produced by the presence of BSA.

Table I summarizes the effects of adding organic anions or serum ultrafiltrate to the medium. The S/M ratio was increased slightly (12%) but significantly in the presence of cysteine or citrate. The combination of the two anions produced a slightly larger effect than either alone, but the differences between the groups were not significant. Cystine and serum ultrafiltrate failed to increase the S/M ratio; glutathione and histidine were tried in only one experiment each, and no obvious effect was observed.

Table II summarizes experiments in which a combination of sodium cyanide, 3 mM, and sodium iodoacetate, 3 mM, was added to half the flasks. Any added anions or ultrafiltrate were present in both the control and experimental (metabolic inhibitor) flasks. The metabolic inhibitors slightly, but significantly, reduced the S/M ratios for ^{203}Pb when only the basic protein-containing medium was used. However, as judged by differences in weight from control, the slices swelled 15 ± 3.0% ($P < 0.001$) in the presence of the inhibitors; since this is essentially the same value as the percentage decrease in S/M ratio, the mass of ^{203}Pb accumulated by the slices was not altered by the inhibitors. The inhibitors also tended to reduce the slice-to-medium ratios achieved in the presence of cysteine, citrate, or ultrafiltrate; however, none of these changes was statisti-

TABLE I. EFFECTS OF ORGANIC MOLECULES ON SLICE-TO-MEDIUM RATIO OF ^{203}Pb AT 90 min

Test substance added to experimental flasks	$(S/M)_{Exp}/(S/M)_{Control}$	P
Cysteine, 11.5 μM	1.12 ± 0.037 (4)	<0.05
Cysteine, 23.0 μM	1.16 ± 0.030 (8)	<0.01
Citrate, 113.0 μM	1.12 ± 0.022 (5)	<0.001
Cysteine, 11.5 μM } Citrate, 113.0 μM	1.19 ± 0.06 (5)	<0.05
Cystine, 40 μM	0.89 ± 0.06 (3)	N.S.
Glutathione, 30 μM	0.92 (1)	—
Histidine, 85 μM	1.05 (1)	—
Serum ultrafiltrate	0.93 ± 0.05 (3)	N.S.

Note. Both the control and experimental media contained BSA, 2 g/dl. The test of significance used was paired samples analysis. The values in parentheses denote the numbers of separate experiments, each performed on a different day.

TABLE II. EFFECTS OF METABOLIC INHIBITORS ON SLICE-TO-MEDIUM RATIO OF ^{203}Pb AT 90 min

Test substance present in control and experimental flasks	$(S/M)_{Exp}/(S/M)_{Control}$	P
—	0.88 ± 0.04 (9)	<0.02
Cysteine, 11.5 μM	0.93 ± 0.05 (6)	N.S.
Citrate, 113 μM	0.91 ± 0.05 (5)	N.S.
Cysteine, 11.5 μM + Citrate, 113 μM	0.89 ± 0.07 (3)	N.S.
Ultrafiltrate	0.92 ± 0.03 (3)	N.S.

Note. Both the control and experimental flasks contained BSA, 2 g/dl. The experimental flasks contained Na cyanide and Na iodoacetate, both 3 mM. The test of significance used was paired samples analysis. The values in parentheses denote the numbers of separate experiments, each performed on a different day.

cally significant (probably because of the small sample size), and none exceeded the decrease to be expected from slice swelling alone. Most important, none of the decreases exceeded the effect seen when only the basal protein-containing medium was used. In summary, then, metabolic inhibitors seemed to exert no significant effect on lead accumulation by the slices under any of the conditions of these experiments.

Discussion. The logic of these experiments was as follows: if a substantial amount of lead uptake by renal cortical slices can occur through cotransport with cysteine, citrate, or some other putative low-molecular-weight ligand for lead in plasma, then addition of these substances to the incubation medium should increase lead accumulation. This is the same approach used to study mercury (5) and copper (6), the only other trace metals previously evaluated (to our knowledge) using renal slices. In the case of copper (6), S/M ratio in unenriched medium was less than unity, but could be tripled when histidine (but no other amino acid) was added. For mercury (5), the basal slice-to-medium ratio was higher—3 to 4—but could also be increased as much as fourfold by addition of amino acid, particularly cysteine; moreover, this increment could be abolished by metabolic inhibitors.

Clearly, the situation for lead in our experiments is very different. First, lead is avidly accumulated by renal slices in the complete absence of any organic anion. Second, the addition of cysteine or other low-molecular-weight organic ligands markedly reduced accumulation. A likely interpretation of these two phenomena is that the free-ion transport pathway is very dominant under the conditions of our experiment, so that addition of substances which bind lead, thereby lowering its free concentration, results in a decrease in uptake. This made it impossible to evaluate any contribution of cotransport with small organic anions from the standard medium.

To try to circumvent this problem, protein was added to the medium, the logic being as follows. The protein should bind much of the lead and reduce free-ion transport to some low level; this protein-bound lead could then act as a source of lead for any added low-molecular-weight anion, so the free-ion concentration change should be minimized, and we might therefore be able to see any increase in uptake due to cotransport. Addition of protein did, in fact, greatly reduce accumulation of lead, and the addition of cysteine or citrate significantly increased accumulation from this control level. This increment was relatively small (12–19%), but is consistent with the existence of some cotransport.

On the other hand, metabolic inhibitors, which profoundly reduce uptake by the free-ion pathway (2), had no effect on the small extra accumulation ascribable to cysteine or citrate. This suggests that that increment was not due to an active cotransport system but to an increase in the diffusibility of the lead bound to the low-molecular-weight anion.

Accumulation of cysteine or citrate was not measured in these experiments, but many others have documented high slice-to-medium ratios for these substances using identical conditions and concentrations (7, 8). The reason for focusing our attention on cysteine and citrate is that these two anions are thought to be the most important low-molecular-weight ligands for lead in plasma (9). A few experiments were done with cystine (mainly because, under our experimental conditions, much of the cysteine in the medium was transformed to cystine),

glutathione, and histidine, but no striking effect was seen. Finally, serum ultrafiltrate was used to test whether normal plasma might contain an as yet unidentified low-molecular-ligand crucial for lead cotransport. No effect at all was observed, not even the increment to be expected from the presence of citrate or cysteine.

In conclusion, these experiments along with our previous study (2) suggest that lead accumulation by renal slices (an indication, presumably, of uptake across basolateral cell membranes) is mainly by free-ion transport, cotransport with low-molecular-weight ligands playing a minor role, if any at all. One question raised by the present data is why the slice-to-medium ratio—5–10—for lead in the presence of protein is still so much above unity. This value was not altered to any great extent by metabolic inhibitors and coincides with the value reported previously for nonprotein media in the presence of metabolic inhibitors (2). This adds further support to the hypothesis (2) that the ratio of 5–10 is due mainly to lead binding by plasma membranes and not to actual transport into the cells.

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1. Task group on metal accumulation, Environ. Physiol. Biochem. **3**, 65 (1973).
2. Vander, A. J., Mouw, D. R., Cox, J., and Johnson, B., Amer. J. Physiol. **236**, F373–378 (1979).
3. Rosenberg, L. E., Blair, A., and Segal, S., Biochim. Biophys. Acta **54**, 479 (1961).
4. Vander, A. J., Taylor, D. L., Kalitis, K., Mouw, D. R., and Victery, W., Amer. J. Physiol. **233**, F532 (1977).
5. Richardson, R. J., Wilder, A. C., and Murphy, S. D., Proc. Soc. Exp. Biol. Med. **150**, 303 (1975).
6. Neumann, D. Z., and Silverberg, M., Nature (London) **5034**, 414–416 (1966).
7. Segal, S., and Crawhall, J. C., Proc. Nat. Acad. Sci. USA **59**, 231 (1968).
8. Segal, S., and Thier, S. O., *in* "Handbook of Physiology; Renal Physiology" (J. Orloff and R. W. Berliner eds.), pp. 653–676. Amer. Physiol. Soc., Washington, D.C. (1973).
9. May, P. M., and Linder, P. W., J. Chem. Soc., Dalton Transactions, p. 588 (1977).

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