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Oxalate Accumulation in Rat Renal Cortical Slices[1] (41920)

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Abstract. Tubular transport of oxalate is thought to be an energy-mediated process which may contribute to the renal deposition of calcium oxalate in a variety of pathologic states. In order to examine this possibility, the renal handling of oxalate was investigated in rat renal cortical slices *in vitro*. Slices incubated *in vitro* with 1 μM [^{14}C]oxalate in Krebs–Ringer bicarbonate buffer at 25°C for 180 min achieved a mean slice to medium ratio of 2.8 ± 0.08 (SEM) and a mean tissue concentration of 7.7 ± 0.2 μmol/kg dry wt (N = 64). Section freeze-dry autoradiographs demonstrated maximum uptake within proximal tubule cells but no crystals were evident. Substituting N_2 for O_2, adding KCN, or removing Ca^{2+} increased uptake of ^{14}C-oxalate. Dinitrophenol (DNP) and iodoacetamide (IoAc), however, significantly decreased, and O°C eliminated slice uptake. Slices incubated with 100 μM [^{14}C]oxalate showed a further increase in tissue accumulation and the appearance of [^{14}C]oxalate crystals. Crystals formed *in vitro* were deposited throughout the tissue. Oxalic acid did not appear to share the organic acid by renal cortical slices *in vitro* is largely independent of energy-mediated mechanisms.

Renal oxalate deposition appears to be responsible for interstitial nephritis in primary oxalosis, ethylene glycol poisoning, and in certain small bowel diseases characterized by increased oxalate absorption (1). Intrarenal oxalate crystals are also frequently found in end-stage kidney disease regardless of the underlying etiology (2). Although the mechanism of renal oxalate deposition is unknown, it may be related to tubular transport processes. Micropuncture and microperfusion studies have suggested that oxalate undergoes bidirectional transport with net tubular secretion in rat (3–5) and rabbit (6), although net reabsorption in the accessible portion of the tubule has also been reported (7). Inhibition of secretory flux by probenecid (3, 7, 8), *p*-aminohippuric acid (PAH) (3, 7, 9), uric acid (3, 10), and a variety of metabolic inhibitors suggested that oxalate might, at least in part, share the organic acid secretory system. Contradictory results, however, have been reported with probenecid (3, 11). The conclusion that oxalate is actively secreted by the tubule has been further compromised by uncertainty as to the secretory site. Some studies have suggested secretion in the proximal tubule (4, 9), whereas others have found secretion beyond the superficial late distal tubule in rat (7).

In the mammalian kidney, transepithelial transport of organic anions is usually associated with cellular accumulation in the proximal tubule (12–15). It is sometimes assumed that this uptake process reflects the intracellular consequences of the same transport mechanisms that are responsible for transepithelial transfer of organic molecules. In order to determine if oxalate undergoes concentrative transport in rat kidney, we examined renal cortical slice uptake of this nonmetabolizable organic acid (3) *in vitro*. Metabolic inhibitors were employed to assess the energy dependence of the uptake process and the cellular sites of oxalate accumulation were determined by section freeze-dry autoradiography.

Materials and Methods. Female Sprague–Dawley rats weighing approximately 150 g were anesthetized with Inactin (40 mg/kg), the kidneys rapidly excised, and cortical slices (approximately 0.3 mm thick) prepared in a Stadie–Riggs microtome as previously described (13). Unless otherwise stated, four

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slices, weighing about 50 mg, were incubated in a 25-ml Erlenmeyer flask for 180 min at 25°C in 3 ml Krebs–Ringer bicarbonate buffer (KRB) containing 10 m*M* acetate and 5% CO_2 and 95% O_2 in the gas phase. Incubations were conducted with 1, 10, or 100 μM [^{14}C]oxalic acid (84 to 98 mCi/mmole, Amersham/Searle, Arlington Heights, Ill.) in a Dubnoff metabolic shaker. Recoveries measured in the incubation media at room temperature indicated no precipitation of oxalate in KRB at the concentrations used. For inhibition studies, one of the paired flasks from each animal contained the inhibitor and the other served as the control. Five paired experiments were conducted at a time, but in selected studies one or two of the flask pairs were incubated with tritiated paraaminohippuric acid (PAH) to monitor inhibitory effects (15).

Following incubation, the slices were gently blotted, weighed with a torsion balance, and the [^{14}C]oxalate extracted in 1 *N* HCl at 100°C for 15 min. After centrifugation, ^{14}C in the supernatant was measured in a liquid scintillation spectrophotometer. Counting efficiency was determined with internal standards. Tissue water content was determined by measuring the change in weight of two of the four slices in a flask after oven drying at 100°C for 48 hr. When tissue water was measured, [^{14}C]oxalate uptake was determined in the remaining two slices only. In separate slice incubation experiments performed with unlabeled oxalic acid, tissues were fixed in 10% neutral Formalin or paraformaldehyde for examination by light and electron microscopy.

Tissue oxalate concentrations are expressed as micromoles per kilogram dry tissue weight after correcting for tissue water content. This correction is required for comparisons of control with inhibition studies because of the increase in tissue water content induced by the inhibitors. The formula used was, $T = (S - WM)/D$, where T = oxalate concentration in the dry tissue, S = [^{14}C]oxalate concentration in wet tissue, W = tissue water fraction, M = postincubation medium [^{14}C]oxalate concentration, and D = tissue dry weight fraction. Although use of the inulin space instead of the tissue water fraction did not alter the conclusions, the inulin space is not reported because entry of inulin into the intracellular space cannot be excluded in the presence of inhibitors. Statistical significance was determined by paired and unpaired Student's t tests. In all experiments in which significant changes were found these tests were in agreement at the 5% level or better. Probabilities are reported only for unpaired tests unless otherwise noted.

Dinitrophenol (DNP), iodoacetamide (IoAc), and A23187 were obtained from Calbiochem-Behring, La Jolla, California; oxalic acid and Na_2EDTA from Sigma Chemical Company, St. Louis, Missouri, KCN from Fisher Scientific Company, Fairlawn, New Jersey, probenecid from Merck, Sharp and Dohme Research Lab, Rahway, New Jersey, PAH from Mann Research Laboratories, New York, New York, and [^{3}H]PAH from New England Nuclear Corporation, Boston, Massachusetts.

Results. *Slice uptake and distribution.* In 64 control experiments conducted with 1 μM [^{14}C]oxalate, the mean slice to medium concentration ratio (S/M) was 2.8 ± 0.08 (SEM) and the mean tissue oxalate concentration was 7.7 ± 0.2 μmole/kg dry wt (Fig. 1). [^{14}C]Oxalate accumulation was rapid, achieving a tissue concentration of 5.1 ± 1.1

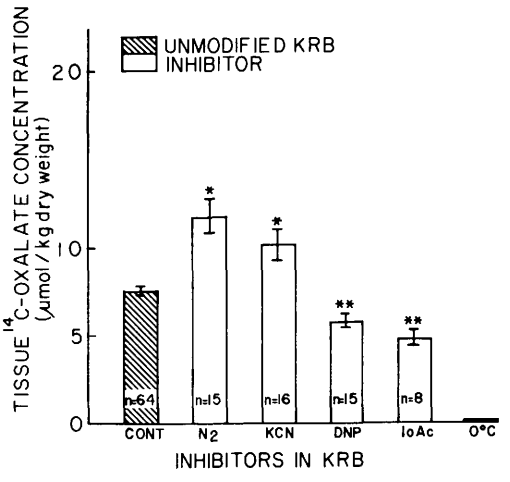

FIG. 1. Effect of metabolic inhibitors of [^{14}C]oxalate accumulation by rat renal cortical slices in KRB (means ± SEM). N_2 and KCN (3 m*M*) increased slice uptake of [^{14}C]oxalic acid while DNP (3 m*M*) and IoAc (10 m*M*) decreased, and 0°C eliminated, uptake. *, $P < 0.01$; **, $P < 0.001$.

μmole/kg (N = 5) after only 15 min. Shorter incubation times were not examined because of incomplete slice penetration by substrate after brief incubations (14). With 10 μM [^{14}C]oxalic acid in KRB, the mean tissue concentration was 26.6 ± 3.0 μmole/kg and this increased to 55.0 ± 18.0 μmole/kg dry wt with 100 μM [^{14}C]oxalate. Electron microscopic examination of kidney slices after 180-min incubations indicated that the higher concentration of oxalic acid increased the extent and frequency of nonspecific mitochondrial changes occurring during the course of incubation. Condensation and swelling of mitochondria in the presence of 100 μM oxalic acid were not, however, distinguishable from changes seen in reversible ischemic injury (16).

Substituting N_2 for O_2 or adding KCN (3 mM) to KRB containing 1 μM [^{14}C]oxalic acid increased slice uptake significantly ($P < 0.01$, Fig. 1). Under these conditions the tissue water content increased from 77 to 84% of wet tissue weight. DNP (3 mM) and IoAc (10 mM) significantly reduced [^{14}C]-oxalate accumulation while reducing the temperature to 0°C eliminated slice accumulation ($P < 0.001$, Fig. 1). DNP and IoAc increased tissue water content to 87%. Removal of Ca^{2+} from KRB (Fig. 2) also increased uptake ($P < 0.005$) without altering tissue water content. The addition of KCN (3 mM) with or without EDTA did not alter oxalate uptake in calcium-free medium. The addition of the calcium ionophore, A23187 (0.1 mM), and the chelating agent, Na_2EDTA (1 mM), to calcium-free medium further increased slice accumulation ($P < 0.001$) also without altering tissue water. Electron microscopy under these conditions showed mitochondrial condensation and swelling comparable to that observed in slices incubated with 100 μM oxalic acid. Oxalic acid did not appear to share the organic acid secretory system in this *in vitro* preparation. Neither 10 mM probenecid nor 1 mM PAH reduced slice uptake of 1 μM [^{14}C]oxalic acid in paired experiments. Probenecid increased tissue water content to 85%. Similarly, 100 μM oxalic acid failed to inhibit slice accumulation of [^{3}H]PAH.

Autoradiography. Section freeze-dry autoradiographs obtained from slices incubated

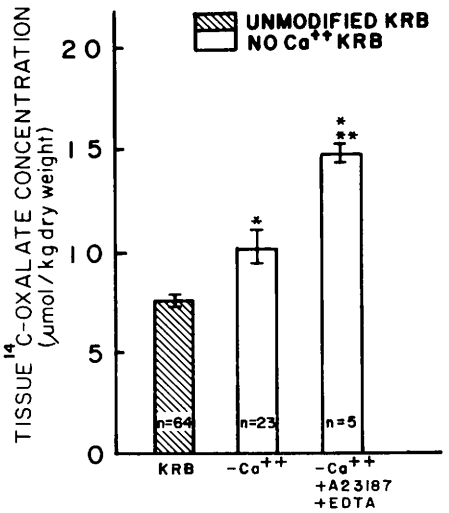

FIG. 2. Effect of removing Ca^{2+} from KRB on [^{14}C]oxalate uptake by renal cortical slices (mean ± SEM). Removing Ca^{2+} significantly increased [^{14}C]oxalate uptake compared to that in standard KRB. The addition of A23187 and Na_2EDTA further increased uptake. *, $P < 0.005$ compared to unmodified KRB; **, $P < 0.001$ compared to calcium-free KRB in unpaired experiments.

with [^{14}C]oxalate revealed accumulation of radioactivity within the cells of proximal tubules (Fig. 3). Subcellular sequestration or uphill secretion into tubular lumina was not evident. With medium oxalic acid concentrations of 1 or 10 μM, autoradiographs showed highest grain density in association with the cells of proximal tubules. Slices incubated with 100 μM [^{14}C]oxalic acid, however, showed crystals throughout the tissue (Fig. 4). Crystals were present within blood vessels and interstitium, as well as within tubular lumens, after *in vitro* incubation with 100 μM [^{14}C]oxalic acid. The presence of calcium oxalate crystals distributed throughout the tissue prevents interpretation of slice uptake when the medium oxalate concentration was 100 μM.

The autoradiographic pattern of [^{14}C]oxalate distribution within the slice was not altered by metabolic inhibitors regardless of the effect on slice uptake. Maximum [^{14}C]oxalate concentrations remained within the cells of proximal tubules. Crystals were not evident by either polarized light or section freeze-dry autoradiography in slices incubated

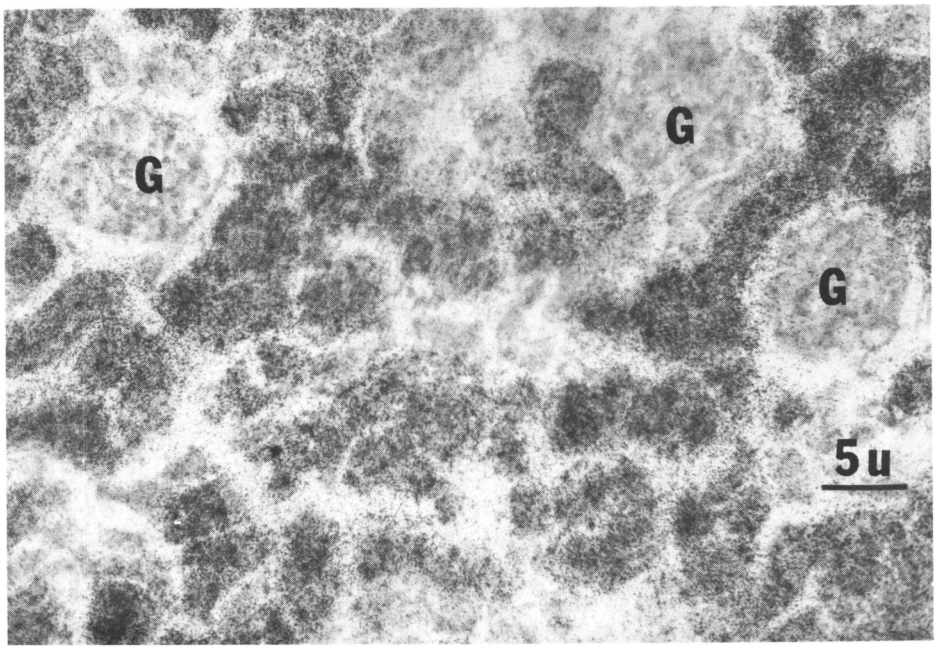

FIG. 3. Section freeze-dry autoradiograph after *in vitro* rat renal cortical slice incubation with 10 μM [^{14}C]oxalate. Autoradiograph is confined to cells of proximal tubules. No luminal or cellular sequestration is evident. Crystals were not seen in polarized light microscopy of freeze-dryed section prior to autoradiography. G, glomerulus. (×310).

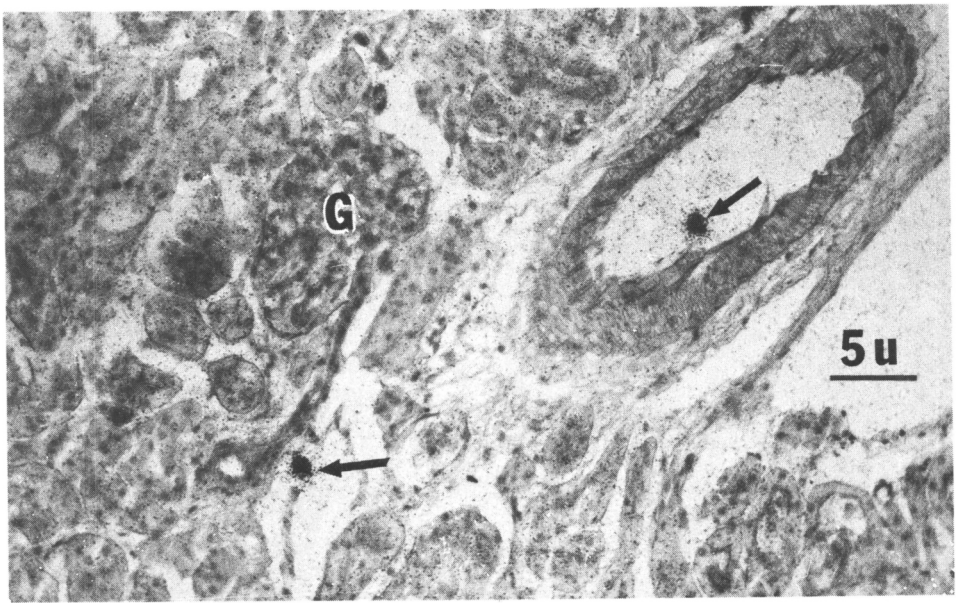

FIG. 4. Section freeze-dry autoradiograph from renal cortical slice incubated in KRB with 100 μM [^{14}C]oxalate. Grain aggregates indicate [^{14}C]calcium oxalate crystals formed during *in vitro* incubation. Crystals are present in the lumen of an arteriole and the interstitial space (arrows). G, glomerulus. ×200.

with 1 or 10 μM [^{14}C]oxalate under any of the incubation conditions studied.

Discussion. These studies show that rat renal cortical slices accumulate oxalate *in vitro*. In contrast to the effect of metabolic inhibitors on concentrative transport of weak organic acids (15), substituting N_2 for O_2 or introducing KCN increases slice accumulation of oxalate in KRB. The uptake process was rapid compared with accumulation rates found with weak organic acids (13, 14). Section freeze-dry autoradiographs prepared from tissues incubated with low oxalate concentrations (1 and 10 μM), show [^{14}C]oxalate in highest concentration within the cells of proximal tubules. At these near physiologic concentrations (4), the [^{14}C]oxalate appears to be homogeneously distributed throughout the cell cytoplasm, neither intra- nor extracellular crystals are evident and there is no evidence of secretion across the luminal membrane. At higher medium concentrations (100 μM), however, [^{14}C]oxalate crystals form throughout the tissue indicating the passive nature of the accumulation process under these conditions. In the slice preparation, cellular accumulation appears to occur only from the antiluminal surface of proximal tubules (13, 17).

The increase in slice uptake of oxalate in the presence of N_2 and KCN indicates that slice accumulation is predominantly a non-energy-dependent process in the slice preparation. In contrast to the evidence cited above suggesting that oxalic acid accumulation in renal cortical slices is passive, some of our results might be interpreted as showing inhibition of an active transport component. Both DNP and IoAc reduced slice uptake of [^{14}C]oxalate in KRB, while lowering the temperature to O°C eliminated uptake. The reduction in uptake by DNP and IoAc were, however, incomplete in contrast to the total inhibition of concentrative transport of organic acids such as PAH under similar incubation conditions (15). Moreover, oxalic acid failed to inhibit slice uptake of PAH and neither probenecid nor PAH reduced slice accumulation of oxalic acid. Oxalic acid does not, therefore, appear to share the organic acid secretory system in the renal cortical slice preparation. The incomplete inhibition of oxalate uptake could, however, be attributed to changes in the microenvironment within the tissue, (e.g., pH, membrane charge, ionization, cations, etc.) rather than to an effect on active transport.[2] Cell membrane permeabilities, intracellular binding, and the conditions necessary for crystallization may be modified by metabolic inhibitors. Temperature, for example, is known to profoundly affect membrane behavior (18) and DNP alters electrical conductance of lipid membranes (19). Thus, metabolic inhibitors may alter local solute composition and membrane structure, thereby impairing transmembrane transfers and/or oxalate binding.

The possibility that cellular accumulation of oxalate is due to complexing with Ca^{2+} was examined. Crystals seen after incubation of slices with 100 μM [^{14}C]oxalate indicate that calcium oxalate may crystallize when sufficiently high local concentrations are attained. Removal of Ca^{2+} from the medium with the addition of calcium ionophore (A23187) and EDTA, however, did not reduce tissue accumulation of 1 μM oxalate. The effect of the calcium ionophore (A23187) and the chelating agent (EDTA) on the intracellular calcium concentration in proximal tubules is unknown. Slices incubated under these conditions did not show unique morphologic changes. Mitochondrial condensation and swelling were comparable to that seen in slices incubated with 100 μM oxalic acid in the presence of calcium. The possibility that intracellular calcium concentrations increased under all the experimental conditions used cannot be excluded. It is possible that calcium oxalate complexes increased within proximal tubule cells even in the presence of EDTA, however, the nature of the intracellular oxalate cannot be determined from these studies. If intracellular crystals are formed, these minute structures could not be

[2] Experiments performed during manuscript review indicate that oxalic acid does not alter oxygen utilization by rat renal cortical slices during *in vitro* incubation. The mean oxygen uptake measured by Clark electrodes (Yellow Springs Oxygen Monitor, Yellow Springs, Ohio) was 13 ± 0.4 (SD) in control slices (7–8 mg) and 13 ± 0.8 μl/hr/mg dry wt with 100 μM oxalic acid added in five paired studies performed at 27°C in KRB for 1/2 hr following equilibration.

resolved by polarized light microscopy or section freeze-dry autoradiography. It is possible that intracellular oxalate is in an amorphous state or bound to constituents in the cell other than calcium.

Enhancement of oxalate uptake by metabolic inhibitors *in vitro* suggests that proximal tubule damage may increase tissue oxalate accumulation. The passive component of oxalate uptake in rat renal cortical slices may, therefore, explain the deposition of oxalate in advanced kidney disease regardless of etiology.

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