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Renal Function and Metabolism After Relief of Unilateral Ureteral Obstruction (38976)

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Renal handling of electrolytes and water may be altered following the relief of obstructive uropathy with increased excretion of salt and water. Mechanisms proposed to explain this increased salt and water excretion include retention of solutes (1), production of a natriuretic material during obstruction (2), and expansion of the extracellular fluid volume (3). In addition, it has been postulated that structural damage to the kidney may also play a role in the altered handling of electrolytes and water (2).

Recent observations demonstrating increased fractional excretion of salt and water following release of unilateral ureteral obstruction in experimental animals (4) indicated that structural damage or some other intrinsic change within the kidney may be responsible for the altered electrolyte and water handling. The presence of a contralateral normal kidney during the period of obstruction would prevent extracellular fluid volume expansion and solute retention during the period of obstruction, and thus exclude these as major factors in the altered excretory pattern of electrolytes and water. It has been suggested that structural alterations such as flattening of proximal tubular microvilli (5) will decrease the total luminal surface area of the epithelial cells which makes contact with the tubular fluid and thereby decrease tubular reabsorption (2). Of interest is the fact that certain histochemical alterations are observed following obstruction. Alkaline phosphatase has been found to be greatly reduced (6, 7) while glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase activities were increased in the proximal tubules of obstructed kidneys (8). Following release of obstruction the activity of these enzymes remained elevated during the initial 48 hr and returned to normal 6 days postobstruction. Decreases in Na-K ATPase activity also have been described following obstruction of 48–72 hr duration (9). Despite these observations, little attention has been paid to the possible role of renal metabolic alterations in the handling of electrolytes and water following relief of obstruction. The present studies performed in rats 3–6 hr after release of a 24-hr period of unilateral ureteral obstruction were designed to evaluate the metabolic capabilities of both the postobstructed kidney and its contralateral control. The steady state levels of adenosine triphosphate and certain substrates, as well as the gluconeogenic capacity, ammoniagenesis, oxidative metabolism, and the rate of respiration (in the presence of glucose and glutamine) were compared. The results suggest that metabolic alterations may be responsible in part for the altered pattern of electrolyte and water excretion.

Methodology. All experiments were performed in female Sprague–Dawley rats weighing 200–270 g and fed a standard rat chow. Twenty-four hours preceding the study, under light ether anesthesia, the right ureter was completely ligated at the distal third, and food and water were withheld until the time of study. After 24 hr of complete unilateral ureteral obstruction, the animals were anesthetized lightly with ether and the right ureteral obstruction was relieved by placing a polyethylene cannula proximal to the ligature.

In some animals clearance measurements from both the postobstructed and the con-

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tralateral control kidney were performed 3–6 hr following release of the unilateral ureteral obstruction. The animals were infused with [^{14}C] inulin in 0.9% NaCl given at a rate of 39 μl/min. In other rats, 10% dextrose or 2 mg% glutamine were added to the infusion solution. The details of performing clearance studies in these animals have been previously described (4).

For certain *in vitro* studies of metabolism the animals were killed by decapitation 3 hr postrelease of unilateral obstruction. Both the postobstructed and the contralateral control kidney were rapidly removed, decapsulated, and placed in ice-cold saline solution. For the studies pertaining to gluconeogenesis and ammoniagenesis renal cortical slices of 0.3–0.4 mm thickness were used. The slices were preincubated for 1 hr at 37° in substrate free Krebs-Ringer's bicarbonate buffer. Thereafter 25–50 mg of tissue were added to individual flasks containing 10 ml Krebs-Ringer's bicarbonate buffer at pH 7.4 either in the presence of 10 m*M* glutamine or 10 m*M* alpha-ketoglutarate or without substrate. The flasks were then placed in a Dubnoff metabolic shaker and incubated for 90 min at 37° with a gas phase of 95% oxygen, 5% CO_2. Incubations were performed as previously described (10) with aliquots of the buffer solution obtained at the beginning and end of the incubation for the determination of glucose and ammonia. All determinations were run in duplicate. Ammonia and glucose production rates were corrected for endogenous synthesis measured simultaneously in slices incubated in substrate free medium.

Oxygen utilization was measured in a Gilson respirometer by incubation of a 10–15 mg slice of kidney cortex in 2 ml of Tris-Ringer's buffer (10 m*M* Tris, 135 m*M* sodium, 5.0 m*M* potassium, 1.5 m*M* calcium, 2.0 m*M* phosphate, and 0.5 m*M* magnesium, 10 m*M* glucose, 0.5 m*M* L-glutamine). The flasks were flushed with 100% oxygen and shaken at 140 oscillations/min. Oxygen consumption was measured at 30-min intervals. Either uniformly labeled [^{14}C]D-glucose or L-glutamine (New England Nuclear) was used as a radioactive tracer. 10 *N* sulfuric acid was added at the end of incubation, and evolved carbon dioxide was trapped in 10% KOH in the flask center well. ^{14}C counting was in a liquid scintillation medium (3A40, RPI Corp.) in a Packard Tri-Carb (model 3214). Counts were corrected for quenching and background. The micromoles of substrate disappearing from the medium were calculated from specific activities and is henceforth referred to as "uptake."

Glucose, glutamine, glutamate, lactate, and ATP were measured enzymatically after perchloric acid extraction of freeze clamped kidneys. All substrates were determined by enzymatic assays utilizing fluorometric techniques (11).

For the electron microscopy studies the kidneys of rats 4 hr postrelease of unilateral ureteral ligation were fixed *in vivo* under pentobarbital anesthesia. A solution of 1% glutaraldehyde in 0.1 *M* sodium cacodylate containing 2% dextran 40 wt/vol and 1000 U heparin/liter, pH 7.35, was infused into the animal via the left ventricle for 5 min. Cortical portions of the kidney were removed and placed in cold 0.1 *M* sodium cacodylate buffer with 1% glutaraldehyde (Polysciences) and 1% lanthanum (12). Tissue was cut in 1 × 1 mm cubes. The tissues were washed for 1 hr in 0.1 *M* sodium cacodylate and 1% lanthanum and postfixed in 1% osmium tetroxide without lanthanum, dehydrated through a graded series of alcohols, and embedded in Swiss araldite in a routine fashion for electron microscopy. Thin sections were then cut and doubly stained with uranyl acetate and lead citrate.

Results. Parameters of renal function for both the postobstructed and the contralateral control kidney of rats which received 0.9% NaCl during the clearance studies are shown in Table I. Glomerular filtration rate and absolute sodium excretion were lower in the postobstructed kidney than in the control. However, both fractional sodium and water excretion were higher in the postobstructed kidney.

The metabolic parameters for cortical slices obtained from both the control and the postobstructed kidney are reported in Table II. Oxygen consumption, glutamine

TABLE I. RENAL FUNCTION IN RATS 3–6 HR POSTRELEASE OF UNILATERAL URETERAL LIGATION.[a]

	Number of experiments	Control kidney	Postobstructed kidney	Statistical significance[b]
V, μl/min	27	25.22 ± 2.32	17.39 ± 1.67	<.02
C_{in}, ml/min	27	1.46 ± 0.04	0.56 ± 0.04	<.001
$U_{Na}V$, μequiv/min	27	3.71 ± 0.31	2.09 ± 0.17	<.001
FE_{Na}, %	27	1.79 ± 0.14	3.39 ± 0.42	<.005
FE_{H_2O}, %	27	2.06 ± 0.33	4.30 ± 0.67	<.001

[a] Rats were infused with 0.9% NaCl at a rate of 39 μl/min. V = urine flow; C_{in} = inulin clearance; $U_{Na}V$ = urinary excretion of sodium; FE_{Na} = fractional excretion of sodium; FE_{H_2O} = fractional excretion of water.

[b] Paired *t*.

TABLE II. RENAL METABOLISM IN RATS 3–6 HR POSTRELEASE OF UNILATERAL URETERAL LIGATION.

	Number of Experiments	Control kidney	Postobstructed kidney	Statistical significance[b]
QO_2, μl/mg dry wt/hr	11	19.4 ± 0.8	16.3 ± 1.6	<.02
Glucose oxidation[c]	10	40 ± 5	34 ± 6	NS
Glutamine oxidation[c]	11	51 ± 6	34 ± 4	<.001
Glucose "uptake"[c]	4	541 ± 185	474 ± 101	NS
Glutamine "uptake"[c]	9	78 ± 8	49 ± 9	<.005
Glucose production:[c]				
(1) glutamine, 10 m*M*[a]	7	46 ± 5	17 ± 2	<.001
(2) α-ketoglutarate, 10 m*M*[a]	7	64 ± 8	29 ± 6	<.005
Ammoniagenesis[c]	7	910 ± 43	651 ± 60	<.001

[a] Substrate utilized.

[b] Paired *t*.

[c] All values are expressed in μmoles/g dry wt/hr.

uptake, and glutamine oxidation were considerably lower in the postobstructed kidney. No significant differences in glucose oxidation or uptake were observed between the postobstructed and the control kidney. The production of glucose from either glutamine or alpha-ketoglutarate was decreased by more than 50% in the postobstructed kidney. At the same time ammonia production from glutamine was markedly decreased in the postobstructed kidney as compared to the contralateral control kidney. The postobstructed kidney exhibited a marked decrease in glutamine uptake and oxidation, decreased oxygen consumption, and impaired gluconeogenesis and ammoniagenesis.

To further define the metabolic alterations observed the steady state concentrations of ATP, glucose, lactate, glutamine, and glutamate were measured in both the control contralateral kidney and the postobstructed kidney (Table III). A marked and significant decrease in the levels of glutamate and ATP were observed in the postobstructed kidney. No significant differences in the steady state concentrations of glucose, glutamine, or lactate were noted between the two kidneys.

Electron microscopy of the proximal tubules of kidneys studied 4–6 hr postrelease of ureteral ligation revealed swelling, vacuolization and fragmentation of mitochondria (Fig. 1). The structure and size of mitochondria in the contralateral control kidney were not different from those observed in normal rats (Fig. 1).

Clearance studies (Table IV) were performed in rats (after release of unilateral ureteral ligation) during the infusion of either glucose or glutamine in 0.9% NaCl

TABLE III. STEADY-STATE RENAL CORTICAL LEVELS OF CERTAIN INTERMEDIARY METABOLITES, 3–6 HR POSTRELEASE OF UNILATERAL URETERAL OBSTRUCTION

	Number of experiments	Control kidney	Postobstructed kidney	Statistical significance[b]
ATP[a]	6	3.30 ± 0.53	1.62 ± 0.21	$P < .02$
Glucose	6	4.20 ± 0.66	5.34 ± 0.80	NS
Lactate	6	1.80 ± 0.60	2.83 ± 0.80	NS
Glutamine	6	0.32 ± 0.12	0.39 ± 0.08	NS
Glutamate	6	3.63 ± 0.28	2.86 ± 0.36	$P < .02$

[a] All data are expressed as μmoles/g wet wt.
[b] Student's *t*, paired.

TABLE IV. EFFECTS OF GLUCOSE OR GLUTAMINE INFUSION ON SODIUM AND WATER EXCRETION BY THE CONTROL AND POSTOBSTRUCTED KIDNEY OF THE RAT.[a]

	V(μl/min)		C_{in} (ml/min)		$U_{Na}V$(μequiv/min)		FE_{Na} (%)		FE_{H_2O} (%)	
Solution	C	P.O.	C	P.O.	C	P.O.	C	P.O.	C	P.O.
0.9% NaCl + 10% glucose (n = 4)	34 ±0.0	12 ±0.0	1.45 ±0.04	0.58 ±0.08	3.53 ±0.12	1.43 ±0.28	1.95 ±0.27	1.95 ±0.45	2.33 ±0.26	2.88 ±0.55
	$P < .02$		$P < .005$		$P < .01$		NS		NS	
0.9% NaCl + 2 mg% glutamine (n = 5)	30 ±5	19 ±3	1.45 ±0.05	0.72 ±0.06	3.70 ±0.36	1.97 ±0.37	1.78 ±0.18	2.08 ±0.65	2.29 ±0.37	2.88 ±0.91
	$P < .05$		$P < .001$		$P < .05$		NS		NS	

[a] Rats were studied 3–6 h after release of unilateral ureteral obstruction. For definitions of abbreviations, see Table I. C = control kidney; P.O. = postobstructed kidney.

instead of NaCl alone (Table I). While GFR was decreased to the same extent in the animals receiving exogenous substrate as compared to the animals infused with NaCl alone (Table I), there were no differences in mean fractional sodium or water excretion between the control and the postobstructed kidney in the rats infused with glucose or glutamine. Fig. 2 presents a composite of the fractional sodium and water excretions for the control and postobstructed kidney in animals infused with either NaCl alone, or NaCl plus glucose or glutamine. Only in the animals infused with NaCl alone did fractional sodium and water excretion exceed the values seen in the contralateral control kidney.

Discussion. Obstruction of the kidney for a period of 24 hr resulted in discernible functional, metabolic, and ultrastructural alterations. Glomerular filtration rate and absolute sodium excretion were decreased in the postobstructed kidney. These results are in agreement with those previously reported from this laboratory and others (4, 13–16). On the other hand, both fractional sodium and water excretion were higher in the postobstructed than in the contralateral kidney of rats infused with sodium chloride alone. No such differences in fractional excretion of salt and water between the two kidneys were observed in rats infused with NaCl containing 10% glucose or 2 mg% glutamine. While the increased fractional excretion of salt and water in the postobstructed kidney may be related to the level of glomerular filtration rate, it should be noted that the average glomerular filtration rates for the animals infused with saline or saline plus glucose were not significantly different. Therefore at comparable levels of GFR the rats infused with glucose demonstrated a lower fractional excretion of salt and water than the rats receiving sodium chloride alone. These data would suggest that part of the alterations in salt and water reabsorption in the postobstructed kidney could relate to altered renal metabolism or to the fact that these rats had been fasted for 24 hr. A role for increased luminal

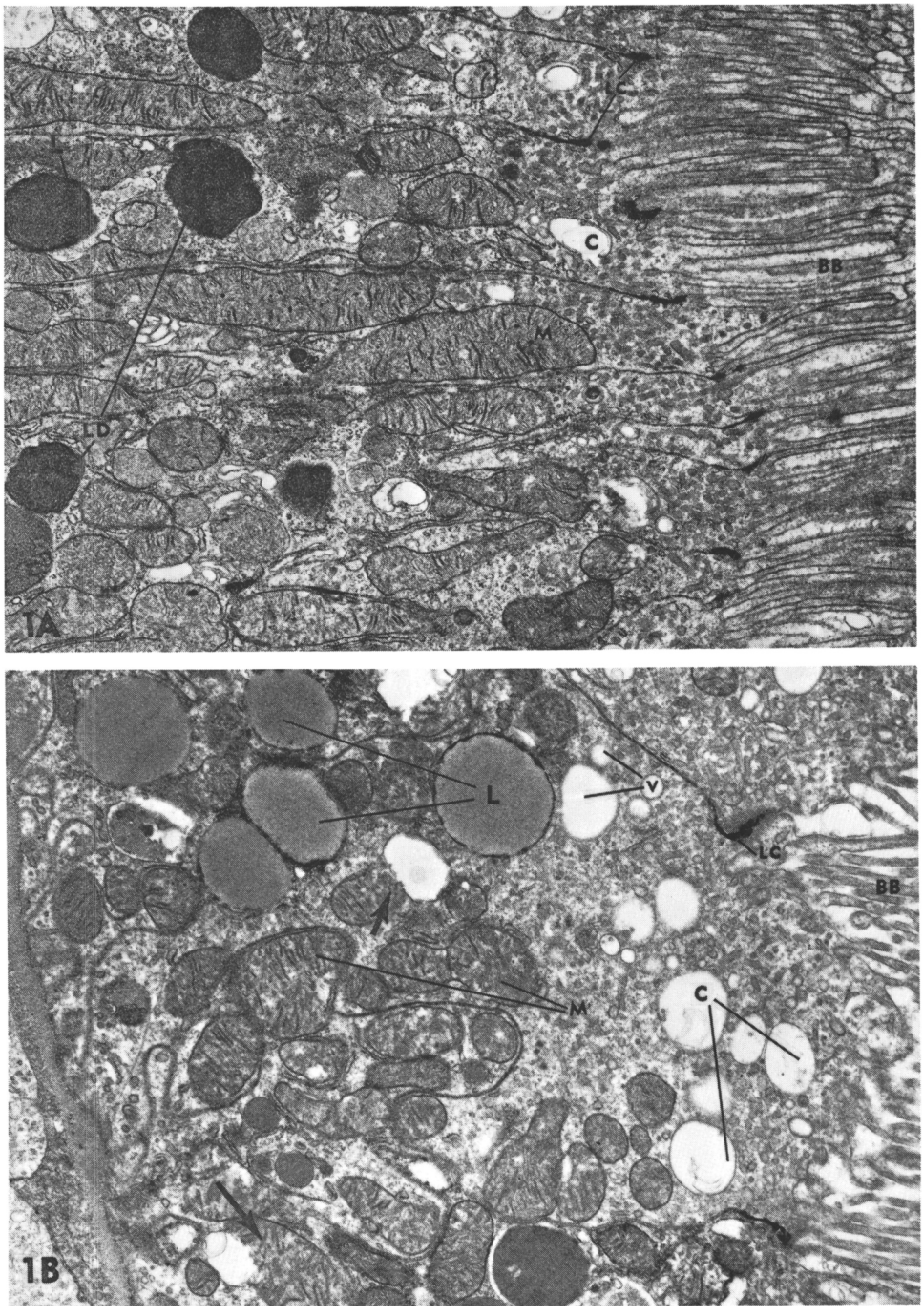

FIG. 1. A. Section through the apical region of proximal convoluted tubule epithelium of a normal kidney. Electron dense lanthanum deposits (LC) are seen in the area of the *zonula occludens.* Mitochondria (M), cytosome (C), lipofusin deposits (LD), lipid droplets (L) in the cytoplasm and brush border (BB) are identified. (× 11,440.) B. Section through the apical region of proximal convoluted tubule epithelium of a postobstructed kidney 4 hr after release of unilateral obstruction. Apical cytoplasm appears edematous, increased number of vacuoles (V) and cytosomes (C) are noted. Mitochondria (M) are swollen, have distorted cristae and at times vacuolation (arrows). Lipid droplets (L) are present. Lanthanum deposits (LC) are noted. (×14,100.)

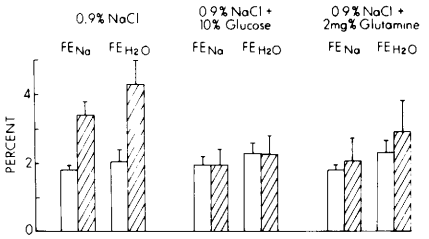

FIG. 2. Fractional sodium and water excretion by the control (clear bars) and postobstructed kidney (stippled bars) after release of unilateral ureteral obstruction. Rats were infused with sodium chloride alone, sodium chloride with 10% glucose and sodium chloride containing 2 mg% glutamine at a rate of 39 μl/min. The significant differences in fractional sodium and water excretion between the control and experimental kidney observed in rats infused with sodium chloride alone were obliterated when substrate was added to the infusate.

glucose augmenting sodium reabsorption should also be considered.

Mitochondrial structure in proximal tubules was markedly altered showing swelling, vacuolization, and fragmentation. Steady state levels of ATP were decreased by 50% in the postobstructed kidney as compared to the contralateral control kidney. On the other hand, the steady state levels of major renal metabolites such as glucose, lactate, and glutamine were unchanged as compared to the contralateral control kidney. However, the levels of glutamate were significantly decreased in the postobstructed kidney. In addition, there was significantly less oxidation of glutamine to carbon dioxide and a markedly decreased oxygen consumption. Both glucose formation and ammoniagenesis were decreased in the postobstructed kidney as compared to the contralateral control. The finding of decreased uptake and oxidative decarboxylation of glutamine, diminished ammoniagenesis, and decreased steady state levels of glutamate suggest that glutamine entry into mitochondria, a process coupled to glutaminase I activity (17), is markedly impaired in the postobstructed kidney. Gluconeogenesis may be decreased in the postobstructed kidney because production of high energy compounds may be rate limiting. Inhibition of the conversion of oxaloacetate to phosphoenolpyruvate, an energy dependent and rate limiting step in gluconeogenesis, may markedly decrease the synthesis of glucose from amino acids in the kidney (18). A preliminary report describing decreased gluconeogenesis and low levels of ATP in the postobstructed kidney of the dog has been published recently (19).

In summary, the present studies indicate the presence of both functional and structural abnormalities in the postobstructed kidney involving mitochondrial integrity and deranged intermediary metabolism. It is suggested that some of these metabolic changes may account in part for the functional abnormalities observed *in vivo*.

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