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A Micropuncture Study of a Dietary Induced, Hyperuricemic Model of Acute Renal Failure in the Rat (40745)

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Acute renal failure as the result of an increased plasma urate concentration is a well-documented phenomenon in clinical settings, particularly following chemotherapy of leukemias (1). Stavric *et al.* (2) were the first to develop a suitable model to study this form of nephropathy in the rat. Their method utilized a dietary supplement of uric acid given simultaneously with oxonic acid, to block the rat's endogenous hepatic uricase. This resulted in elevated plasma urate concentrations. Previous micropuncture studies (3–5) have provided experimental evidence for the presence of tubular obstruction in the pathogenesis of this model of acute renal failure. However, neither paper included an assessment of the contribution of changes in glomerular dynamics on impaired renal function. We have measured whole kidney and single nephron function and have attempted to assess the contributions of tubular obstruction and of changes in glomerular dynamics to the pathogenesis of this model.

Materials and methods. Male, albino Sprague–Dawley rats (Charles River Laboratories) were used throughout the study (N = 40, mean body weight = 261 ± 55 g). Animals were anesthetized prior to surgery with an intraperitoneal dose of Nembutal (sodium pentobarbital, Abbott, 60 mg/kg body wt). Maintenance infusions of 0.85% sodium chloride containing inulin for estimation of inulin clearance and *para*-aminohippuric acid (PAH) to estimate renal plasma flow were infused at 2.34 ml/hr via an indwelling jugular cannula. Sustaining doses of anesthetic were administered via a similar route. The left kidney was prepared for micropuncture as previously described (6) and urine was collected separately from each kidney. Elevated plasma urate concentrations were induced in one group of animals (Diet Group) by feeding powdered rat chow (Purina) supplemented with 5% uric acid (99+% pure, Aldrich Chemical Co.) and 3% oxonic acid as the potassium salt (4,6-dihydroxy-*s*-triazine-2-carboxylic acid, Aldrich Chemical Co.) for 2 days.[1] A second group of rats (Control Group) were pair fed with powdered rat chow alone.

(i) Tubular fluid collections. Four to eight tubular fluid collections were obtained from randomly selected proximal tubules in six diet animals and their pair-fed controls. Collection rate was controlled to maintain intratubular pressure, continuously measured upstream, at its precollection value.

(ii) Pressure measurements. Pressure measurements were made in nine diet animals and their pair-fed controls. Intratubular pressures were measured in random proximal and distal tubules using a servonulling device. Glomerular capillary pressures were estimated using the sum of the "stop-flow" pressure measured in the earliest proximal convolution and the calculated colloid osmotic pressure of plasma proteins. In addition, measurements of pressures were made in star vessels and peritubular capillaries.

(iii) Tubular microinjections of inulin. Small volumes, 0.2–2.0 nl, of tritiated inulin (ICN Life Sciences) in 0.85% sodium chloride were injected into random proximal tubules to test the permeability of the tubular epithelium to inulin as previously described (7). Intratubular pressures measured upstream, at its precollection value.

[1] In a series of pilot experiments, the diet of powdered chow supplemented with 5% uric acid and 3% oxonic acid, potassium salt, induced significantly higher plasma urate concentrations than either chow plus 5% uric acid alone or chow plus 3% oxonic acid alone.

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monitored during each injection. Injection rate was controlled to maintain a constant intratubular pressure. Urine was collected from both kidneys and the excreted radioactivity measured using a liquid acintillation counter.

(iv) Histology. Six-micrometer sections of renal tissue removed from diet animals and their pair-fed controls were stained using the Schultz method (8). This technique is specific for monosodium urate and uric acid crystals. The stained sections were observed using low-power light microscopy.

Standard analytical techniques were utilized throughout for estimation of concentrations of inulin, PAH, uric acid, and protein in tubular fluid samples and aliquots of urine and plasma (9–13). Student's *t* test was utilized throughout for analysis of significance.

Results and discussion. Mean plasma urate concentration was 1.68 ± 1.0 mg/dl in the control group and 10.52 ± 2.3 mg/dl in the diet animals ($P < 0.001$). Urate excretion was also significantly higher in the diet group (Diet = 4.45 ± 5.6 μg/min; Control = 0.85 ± 0.3 μg/min, $P < 0.001$). Glomerular filtration rate was significantly lower in the diet group (Table I), similar to previous reports (3–5). However, in contrast to the studies of Spencer *et al.*, urine flow rate was not reduced; in fact, urine flow rate was slightly increased in the diet group. Thus, we have a nonoliguric model of acute renal failure. These changes occurred without significant changes in renal plasma flow, resulting in significant reductions in calculated values for filtration fraction. Mean arterial blood pressure was not different in the diet and control groups (Diet 117.4 ± 17 mm Hg; Control 121.3 ± 10 mm Hg).

Sections of medullary tissue are shown in Fig. 1. Tissue from diet animals (B) exhibited collecting ducts which were swollen and packed with crystals. These crystalline deposits stained uric acid positive with the Schultz strain.

Table II shows the recoveries of microinjected, tritiated inulin in diet and control animals. Recovery of radioactivity from the injected kidney was 98.4 ± 2.3% of the injectate in the control animals. Observation of the kidney surface of the diet animals *in vivo* revealed both dilated and collapsed nephrons. White crystalline deposits were present in many of the tubular lumina. Two nephron populations could be distinguished. In the first group, nephrons had tubular flow and intratubular pressures comparable with those in control animals. Recovery of microinjected inulin from the injected kidney was 98.4 ± 2.3%, not significantly different from their pair-fed controls. In contrast, the second nephron group exhibited zero flow and significantly elevated intratubular pressures. Following microinjection into these nephrons, recovery of radioactivity from the injected kidney was zero. Recovery from the contralateral kidney was also zero, indicating that although the nephron was blocked, no injected inulin had leaked across the tubular epithelium. Thus, we feel confident that our estimations of inulin clearance for whole kidneys and single nephrons are an accurate measure of glomerular filtration rate.

TABLE I. PARAMETERS OF WHOLE KIDNEY FUNCTION IN DIET RATS AND THEIR PAIR-FED CONTROLS

Group	U/P_{IN}[a]	Urine flow (μl/min)	GFR (ml/min)	RPF (ml/min)	Filtration fraction	Sodium excretion (nEq/min)
Diet ($N = 15$)	75.5 ± 44.1[b]	5.51 ± 1.86	0.409 ± 0.25[b]	1.73 ± 0.9	0.23 ± 0.06*	506 ± 336
Control ($N = 15$)	192.2 ± 39.5	4.28 ± 0.9	0.753 ± 0.19	2.40 ± 0.9	0.33 ± 0.06	522 ± 276

[a] Urine to plasma inulin concentration ratio. All values are means of mean/animal ± 1 Standard Deviation (SD). Values for urine flow rate, glomerular filtration rate (GFR), renal plasma flow (RPF), and sodium excretion are for two kidneys/100 g body wt.

* $P < 0.05$, Diet vs Control.

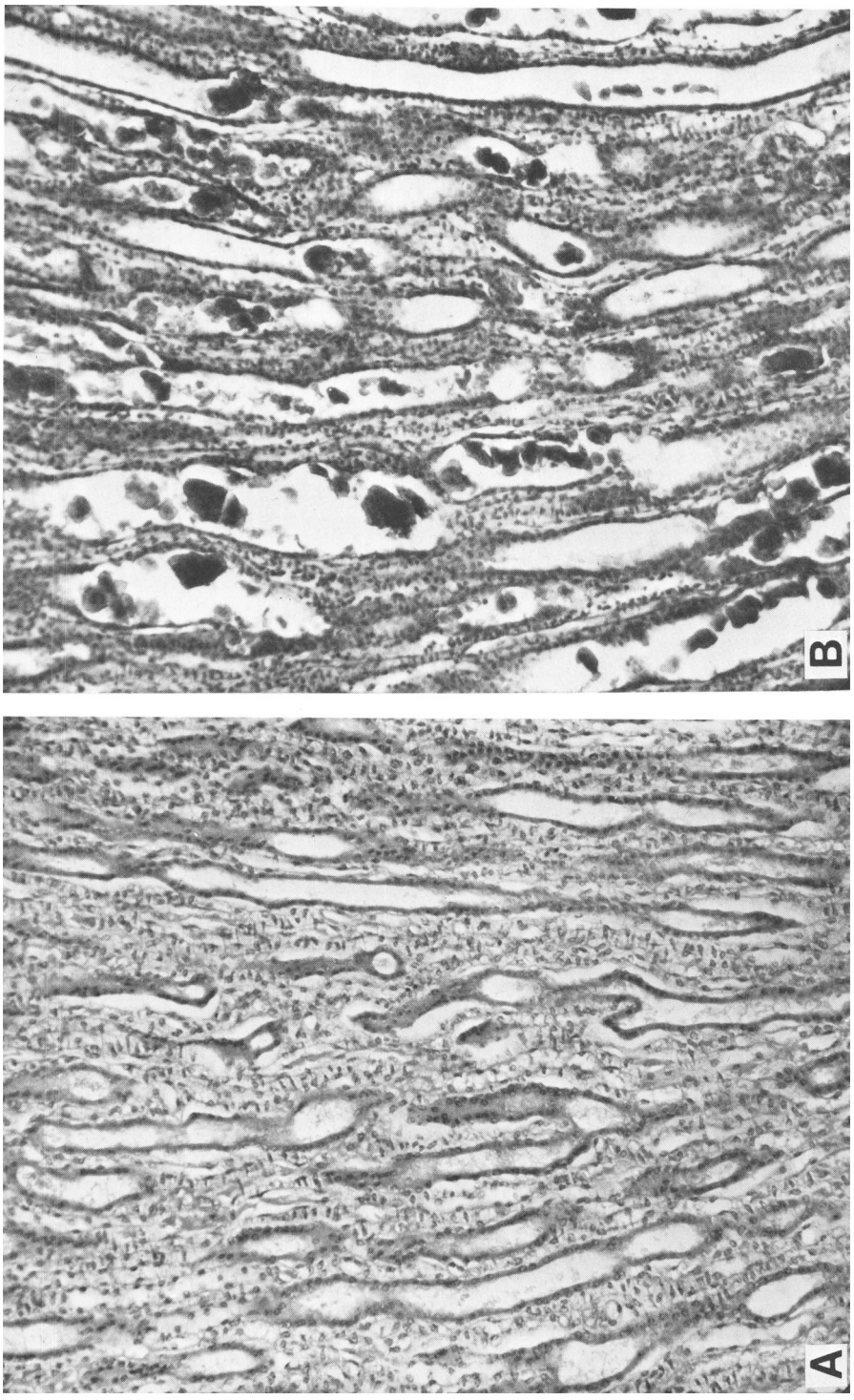

FIG. 1. Sections of medullary tissue removed from Control (A) and Diet animals (B). Magnification ×100.

TABLE II. RECOVERY OF MICROINJECTED, TRITIATED INULIN IN THE URINE FROM THE IPSILATERAL KIDNEY IN DIET AND CONTROL ANIMALS

Group	% recovery of isotope[a]	Proximal intratubular pressure (mm Hg)	% total tubules
Diet (N = 5)			
Zero flow	0	21.5 ± 2.3*	24
Tubules with flow	98.4 ± 2.3	12.0 ± 1.5	76
Control (N = 5)	98.4 ± 2.3	12.4 ± 1.5	100

[a] Values are means of mean/animal ± 1 SD.
* $P < 0.001$, Zero flow vs flow in the diet group and Zero flow vs Control.

Tubular fluid collections were made in six animals and their pair-fed controls (Table III). In the control animals, the nephron population was homogenous, mean single nephron glomerular filtration rate (SNGFR) was 28.7 ± 3.9 nl/min with a mean intratubular pressure at the collection site of 11.1 ± 1.3 mm Hg. In contrast, nephrons in the diet group again fell into two distinct populations. The first had uniformly elevated intratubular pressures. If intratubular pressures were maintained during tubular fluid collection there was no tubular flow. The second group had measurable values for SNGFR and intratubular pressures which were not significantly different from controls. Although the mean SNGFR in these nephrons was not statistically different from that in controls, the range of values was much greater (8.8 to 37.0 nl/min vs 24.8 to 32.6 nl/min in controls). Tubules with no flow constituted 38% of all nephrons sampled. In the previous micropuncture studies (3–5) although SNGFR was uniformly reduced, there appear to have been no nephrons having zero flow. However, Spencer *et al.* (3) did not monitor intratubular pressures during collection, and Conger *et al.* (4, 5) did, but downstream of the oil block injected to obtain collections. This may not have been an accurate estimation of intratubular pressure at the collection site.

TABLE III. PARAMETERS OF SINGLE NEPHRON FUNCTION IN DIET ANIMALS AND THEIR PAIR-FED CONTROLS

Group	SNGFR[a] (nl/min)	Intratubular pressure mm Hg[b]
Diet (N = 6)		
Zero flow	0	15.34 ± 5.7
Tubules with flow	22.9 ± 14.1	10.86 ± 2.1
Control (N = 6)	28.7 ± 3.9	11.13 ± 1.3

[a] Values for single nephron glomerular filtration rate (SNGFR) are means of mean/animal ± 1 SD.
[b] Intratubular pressures were measured simultaneously in the same nephron loop from which the tubular fluid sample was obtained.

We have documented a decreased glomerular filtration rate in whole kidney clearance studies and at the single nephron level. Why is GFR reduced? Several possibilities exist. The first contributing factor might be tubular obstruction, and the second, changes in intrarenal hemodynamics resulting in a reduced driving force for filtrate formation. Also, both factors might be important in effecting a reduced GFR. Accordingly, we proceeded to test for either factor or a combination of both in the pathogenesis of the model. Evidence for tubular obstruction arises from demonstration of the presence of uric acid crystals in medullary collecting tubules (Fig. 1) visualized in 6-μm sections of kidney tissue. Also, the medulla of kidneys removed from diet animals at the termination of each experiment exhibited white crystalline deposits. In many kidneys these deposits extended up into the cortex. The second line of evidence derives from measurements of intratubular pressures. The distribution of intratubular pressure values in diet and control animals is shown in Fig. 2. Variation was much greater in the diet group than in the controls. Table IV shows mean pressures in tubular and intrarenal microvascular segments. Mean distal intratubular pressures were significantly elevated in the diet animals ($P < 0.001$), further evidence of

TABLE IV. MEAN INTRATUBULAR AND INTRARENAL MICROVASCULAR PRESSURES IN DIET ANIMALS AND THEIR PAIR-FED CONTROLS

Group	Intratubular pressure (mm Hg)		Glomerular capillary pressure (mm Hg)	Star vessel pressure (mm Hg)	Peritubular capillary pressure (mm Hg)
	Proximal	Distal			
Diet (N = 9)	12.28 ± 3.2[a]	14.20 ± 5.2*	38.10 ± 7.6***	12.02 ± 3.3	8.03 ± 2.7**
Control (N = 9)	12.58 ± 0.9	5.42 ± 0.7	50.03 ± 4.7	13.60 ± 2.1	11.1 ± 1.8

[a] Values are means of mean/animal ± 1 SD.
* $P < 0.01$, Diet vs Control.
** $P < 0.025$, Diet vs Control.
*** $P < 0.005$ Diet vs Control.

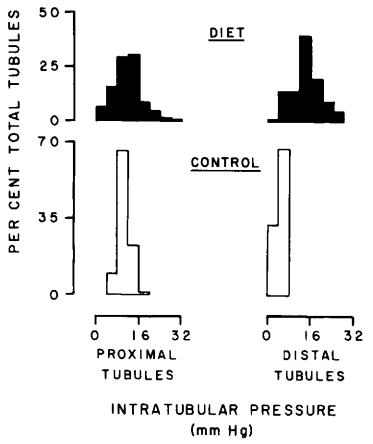

FIG. 2. Distribution of proximal and distal intratubular pressures in Diet (■) and Control (□) animals.

tubular obstruction beyond the distal convolution. Mean estimated glomerular capillary pressure in diet animals was significantly lower ($P < 0.005$) than in the pair-fed control group. Thus, in addition to tubular obstruction, the major force for filtrate formation was also reduced. Next, we attempted to assess the contribution of any changes in intrarenal vascular resistances to the measured decrease in glomerular pressure. Total renal vascular resistance (expressed as mm Hg·mm^{-1}·100g^{-1} body wt) was not significantly different in both groups (Control 94.4 ± 38, Diet 104.4 ± 55). However, preglomerular resistance was elevated in the diet group (69.9 ± 39) compared with pair-fed controls (55.3 ± 23), and postglomerular resistance was reduced (27.6 ± 12 vs 48.7 ± 25). Although these changes were not significantly different statistically they presumably contributed to the measured decrease in estimated glomerular capillary pressure.

Summary. A hyperuricemic model of acute renal failure was induced in the rat by dietary means. Powdered rat chow supplemented with 5% uric acid and 3% oxonic acid as the potassium salt was fed to the "Diet" group for 2 days. The "Control" group was pair fed powdered rat chow alone. The dietary regimen resulted in significantly elevated plasma urate concentrations and reductions in glomerular filtration rate in the diet group. The pathogenesis of the acute renal failure in diet animals was the result of at least two factors; first, tubular obstruction beyond the distal convolution indicated by significantly elevated distal intratubular pressures. These nephrons presumably did not contribute to urine formation. This observation was confirmed by the histological demonstration of uric acid deposits in the medullary collecting ducts. Second, reduced glomerular capillary pressures in the diet group resulting from changes in both pre- and postglomerular resistances.

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