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17. Requests for reprints should be addressed to: Dr. J. G. Aarnoudse, Department of Obstetrics and Gynaecology, State University, 59 Oostersingel, Groningen 9700 RB, The Netherlands
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The Influence of Age on Acute Renal Toxicity of Uranyl Nitrate in the Dog

JUAN C. PELAYO,[27] PETER M. ANDREWS, ALINE K. COFFEY, PHILIP L. CALCAGNO, GILBERT M. EISNER, AND PEDRO A. JOSE

Departments of Pediatrics and Anatomy, Georgetown University Medical Center, Washington, D.C. and Department of Internal Medicine, Veterans Administration Hospital, San Diego, California, USA

Summary

The influence of age upon uranyl nitrate (UN) induced acute renal failure (ARF) was evaluated in 30 canine puppies 1–2 wk and 3–5 wk old. Renal function and morphologic studies were performed 2 h (initiation phase) and 24 h (maintenance phase) after UN administration. Age-matched controls received vehicle alone. Administration of UN to 1–2-wk-old puppies produced no changes in whole kidney glomerular filtration rate (GFR), despite a significant reduction in renal plasma flow (RPF) ($P < 0.01$). In contrast, during the maintenance phase, GFR was 60% lower than in the control group ($P < 0.02$) whereas values for RPF were nearly identical to control values. In 3–5-wk-old puppies the magnitude of response to the heavy metal was much greater and GFR was nearly completely suppressed during the maintenance phase. This major alteration of GFR was independent of changes in RPF, because RPF remained similar to control values.

Morphologic alterations consistent with the nephrotoxic effects of UN were observed in the proximal tubules of the most differentiated nephrons. These age-related morphologic alterations correlated well with the functional response (GFR) observed after UN administration, *i.e.*, a proportionately greater degree of both morphologic and functional alterations followed the administration of the heavy metal in the oldest group of puppies.

Abbreviations

ABG, arterial blood gas
ARF, acute renal failure
5% D in 1/3 NS, 5% dextrose in 1/3 normal saline solution
FENa, fractional excretion of sodium
FF, filtration fraction
GFR, whole kidney glomerular filtration rate
MAP, mean systemic arterial pressure
RBF, renal blood flow
RPF, renal plasma flow
UKV, urinary potassium excretion
UN, uranyl nitrate
UNaV, absolute urinary sodium excretion
V, urinary flow rate

Abundant experimental evidence on the pathogenesis of nephrotoxic forms of acute parenchymal renal failure has been obtained mainly from studies using mature mammals. These studies have examined the mechanisms by which antibiotics and heavy metals induce ARF (4, 7, 10, 11, 24); however, little attention has been given to the pattern and pathogenesis of nephrotoxic ARF in the developing mammal. There is some clinical and experimental evidence suggesting that the immature kidney is more tolerant to the nephrotoxic effects of aminoglycosides than its adult counterpart (9, 19).

It is well established that during the period of maturational growth, developmental changes occur in the perfusion of superficial glomeruli, distribution of renal blood flow, and in tubular transport (1, 2, 13–16, 20, 21, 25). Age-related differences in sensitivity to nephrotoxic agents could arise from the normally occurring differences in renal function in the developing mammal. UN-induced ARF has been well characterized in the mature mammal (7, 10, 11, 24). The present investigation, therefore, was undertaken to further explore and delineate the influence of age upon UN-induced ARF in the immature dog.

MATERIALS AND METHODS

Experiments were performed on 30 healthy mongrel puppies of either sex, born in our animal resource facility, ranging in age from 3–39 days and weighing 330–1225 g. The pregnant mothers were shipped to our animal resource facility 1–2 wk before whelping. The animals were allowed free access to their mother's milk and were isolated from the mother immediately before the experiment.

General procedures. Anesthesia was induced with an intravenous injection of 15–20 mg/kg body wt sodium pentobarbital (Nembutal, Abbott Laboratories) and maintained with bolus intravenous infusions as needed. The puppies were placed on a temperature controlled animal board and body temperature was kept constant within 36.5–37.5°C by means of a thermosensitive feedback device (YSI model 72A, Yellow Spring Inst. Co., Inc., OH). An endotracheal tube was inserted and ventilation was artificially assisted, with room air, by means of a small animal respirator. Polyethylene catheters (PE 50-90) were inserted into the left femoral artery for blood sampling and continuous monitoring of the MAP by means of an electronic transducer (Model p23ID, Gould Statham Inst. Co., CA) connected to a direct writing recorder (Model 220, Brush Gould recorder). Then, initially a 0.3 ml arterial blood was sampled for measurement of ABG and hematocrit. The respirator settings were then adjusted to maintain adequate ventilation.

Both the left femoral and right external jugular veins were cannulated with PE (50-90) for fluid and [^{3}H]methoxy-inulin administration. The urinary bladder was exposed through a midline suprapubic incision and cannulated with PE 90 for timed urine collections. In addition, the dorsal aspect of the left kidney was exposed by a retroperitoneal approach. The renal artery was dissected gently and fitted with a noncannulating electromagnetic flow probe (Biotronex Lab., Inc.) with minimal disturbance of other structures. The RBF was continuously recorded on a second channel of the recorder. Flow probes ranged in size from 0.85–2.0 mm, inner diameter, and were calibrated by the *in vitro* method using isolated neonatal dog vessels perfused with normal saline at various constant flows. The baseline zero flow was obtained by a temporary complete occlusion of the renal artery utilizing blunt forceps (16).

In all experimental groups, surgical fluid losses were replaced by the infusion of 0.5% body wt of 2.5% albumin in normal saline administered over 45 min. Upon completion of the surgical procedure, the equilibration period was begun: a priming dose of 10 μCi of [^{3}H]methoxy-inulin (New England Nuclear, Boston, MA) in 0.5 ml of 5% D in 1/3 NS was given as a bolus and 5% D in 1/3 NS containing [^{3}H]methoxy-inulin in an amount sufficient to deliver 20 μCi/h was begun at a rate of 1 ml/h through the right external jugular vein. Additional fluid (5% D in 1/3 NS) was infused through the left femoral vein at a rate of 0.06 ml·kg^{-1}·min^{-1} to maintain an adequate degree of hydration. After an 1-h equilibration period and stable MAP and RBF, the study period was started: two 60-min clearance collections of urine were obtained, with midpoint blood sampling (blood volume samples less than 0.15 ml each) for determination of electrolytes, [^{3}H]methoxy-inulin concentration and hematocrit. At the end of the last clearance period, 0.3 ml of arterial blood was sampled for measurement of ABG. Then, fixation of the kidneys *in situ* was achieved by the infusion of a phosphate buffered glutaraldehyde solution into the abdominal aorta.

Fixation and preparation for light and electron microscopic observations. The kidneys were initially fixed *in situ* by retrograde perfusion of phosphate buffered 1% glutaraldehyde for approximately 3–5 min. The fixed kidneys were then excised from the body and immersed in phosphate buffered glutaraldehyde for an additional 6 h. The kidneys were cut into small blocks, rinsed in phosphate buffer and post-fixed in phosphate buffered 1% osmium tetroxide for 1 h. The samples were stained in block in 2% aqueous uranyl acetate for 3 h, dehydrated through graded acetones, and embedded in a mixture of Epon and Araldite resins. Thick sections were stained with toluidine blue and viewed and photographed using a Zeiss RA light microscope equipped with a 35 mm camera attachement. Thin sections (50–80 nm) post-stained with uranyl acetate and lead citrate (21) were examined using JEOL 100S electron microscopic operating at 60 or 80 KV.

Experimental protocols. The animals were divided into six groups and were studied during the initiation phase (2 h) and maintenance period (24 h) of UN (Sigma) induced ARF. Animals in groups I-III were approximately 1–2-wk-old; those in groups IV-VI were 3–5-wk-old. Group I (age, 9.20 ± 1.98 days) consisted of five control puppies that were given only vehicle I.V. as a placebo. Group II (age, 10.40 ± 0.74 days) ($n = 5$) received UN 10 mg mg/kg body wt IV. In groups I and II clearance and hemodynamic studies were begun at 2 h after either placebo or UN administration. Group III (age, 9.80 ± 0.49 days) consisted of five puppies that received UN 10 mg/kg body wt. I.V., and were studied 24 h later. They were maintained in an incubator (Isolette) during that period and 5% D in 1/3 NS was administered at a rate of 120–150 ml·kg body wt^{-1}·24 h^{-1} through a 25 gauge butterfly needle placed in the cephalic vein. Group IV (age, 29.20 ± 2.43 days) consisted of five animals that solely received placebo. Group V (age 27.20 ± 0.33 days) had five animals that were given UN 10 mg/kg body wt IV. In both groups functional studies were initiated exactly 2 h after the administration of either vehicle or UN. Additionally, five puppies from Group VI (age, 26.00 ± 3.47 days) were given UN 10 mg/kg body wt. These animals were studied 24 h post-UN administration. They were maintained in an incubator (Isolette) during that period and 5% D in 1/3 NS was administered at a rate of 125–150 ml·kg body wt^{-1}·24 h^{-1} through a 25 gauge butterfly needle placed in the cephalic vein.

Analytical methods. Urine was collected in preweighed plastic containers under mineral oil. Arterial blood samples were collected in heparinized capillary tubes (100 μl). [^{3}H]methoxy-inulin activity in urine and plasma was measured, usually in duplicate, in Liquiscint (National Diagnostics, Somerville, NJ) using a Beckman Liquid Scintillation Counter (Model LS 7500). Urine and plasma sodium and potassium concentrations were determined by flame photometry (Instrumentation Laboratory).

Calculations. GFR, RPF, UNaV, UKV excretion, and FENa were calculated by standard formulas.

Statistical analysis. Statistical analysis was performed by the unpaired Student's t test or Mann Whitney test as indicated. Statistical significance is defined as $P < 0.05$. All values are expressed as mean ± S.E.M.

RESULTS

General results. Tables 1 and 2 summarize the values of body weight, MAP, hematocrit, arterial pH, P_{CO_2} and P_{CO_2} as well as parameters of whole kidney function for each group under investigation.

A. Puppies (1–2-wk-old), Groups I, II, and III. Mean values for body wt., MAP, and hematocrit obtained in all experimental groups were nearly identical (Table 1). Although GFR was numerically lower in Group II (1.11 ± 0.15 ml/min) than in Group I (1.61 ± 0.26 ml/min), this difference did not reach statistical significance ($P > 0.05$) (Table 2). Mean values for RBF and RPF in Group II were significantly lower than in Group I ($P < 0.01$ and $P < 0.02$, respectively) although FF remained unchanged. GFR in Group III averaged 0.61 ± 0.22 ml/min, a value 60% lower than in Group I ($P < 0.02$) whereas RBF and RPF were similar to control values (Group I), but were higher than values in Group II; thus, filtration fraction was lower in Group III (0.07 ± 0.02) as compared with Group I ($P < 0.05$) and Group II ($P < 0.02$) (Table 2). V, UNaV, UKV, and FENa did not differ among these three groups.

B. Puppies (3–5-wk-old), Groups IV, V and VI. Statistical analysis revealed no significant differences for mean values of body wt and hematocrit in all three experimental conditions.

Table 1. *Values for body weight, mean arterial pressure (MAP), hematocrit (Hct), pH, $Paco_2$ and Pao_2, in all experimental groups*[1]

Group	Body wt (kg)	MAP (mmHg)	Hct (%)	pH (units)	$Paco_2$ (mmHg)	Pao_2 (mmHg)
Group A						
1–2 wk[2]						
I. (2 h N.S.)[3]	0.556 ± 0.044	53.25 ± 1.97	34.30 ± 1.86	7.33 ± 0.03	34.9 ± 6.9	86.6 ± 9.9
II. (2 h U.N.)[4]	0.527 ± 0.070	52.30 ± 2.74	30.00 ± 1.76	7.32 ± 0.02	34.5 ± 5.2	100.2 ± 8.3
III. (24 h U.N.)[5]	0.482 ± 0.068	59.50 ± 2.03	33.60 ± 0.96	7.38 ± 0.03	31.8 ± 2.2	107.2 ± 3.9
Group B						
3–5 wk[2]						
IV. (2 h N.S.)	0.710 ± 0.068	74.00 ± 4.33	27.40 ± 1.31	7.34 ± 0.03	34.5 ± 1.8	90.0 ± 7.6
V. (2 h U.N.)	0.645 ± 0.059	67.20 ± 3.60	26.70 ± 2.50	7.39 ± 0.04	31.6 ± 2.1	88.2 ± 6.2
VI. (24 h U.N.)	0.853 ± 0.146	59.50 ± 3.97	25.60 ± 2.80	7.35 ± 0.04	26.2 ± 2.9	105.8 ± 5.5
I *vs* II[6]	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
I *vs* III	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
II *vs* III	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
IV *vs.* V	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
IV *vs* VI	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.
V *vs* VI	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
I *vs* IV	N.S.	<0.005	<0.02	N.S.	N.S.	N.S.
II *vs* V	N.S.	<0.01	N.S.	N.S.	N.S.	N.S.
III *vs* VI	<0.05	N.S.	<0.05	N.S.	N.S.	N.S.

[1] Values are expressed as mean ± S.E.M.
[2] Five animals were studied in each group.
[3] 2 h N.S. = studied 2 h after normal saline.
[4] 2 h U.N. = studied 2 h after uranyl nitrate.
[5] 24 h U.N. = studied 24 h after uranyl nitrate.
[6] *P* values calculated from unpaired data by Student's *t* test.

Table 2. *Summary of clearance and hemodynamics data in all experimental groups*[1]

Group	GFR (ml/min)	RBF (ml/min)	RPF (ml/min)	FF	V (μl/min)	UNaV (μEq/min)	UKV (μEq/min)	FENa (%)
Group A								
1–2 wk[2]								
I. (2 h N.S.)	1.61	14.88	9.51	0.16	16.60	0.80	0.71	1.05
	±0.26	±0.67	±0.37	±0.03	±3.50	±0.25	±0.14	±0.40
II. (2 h U.N.)	1.11	9.95	6.86	0.18	26.66	2.57	0.96	1.56
	±0.15	±1.24	±0.78	±0.03	±5.56	±0.74	±0.15	±0.32
III. (24 h U.N.)	0.61	14.05	9.43	0.07	11.80	1.02	0.73	4.60
	±0.22	±0.95	±0.65	±0.02	±4.56	±0.28	±0.28	±2.49
Group B								
3–5 wk[2]								
IV. (2 h N.S.)	2.19	16.71	12.16	0.18	36.15	0.76	1.55	0.49
	±0.38	±1.39	±1.07	±0.03	±11.85	±0.26	±0.26	±0.26
V. (2 h U.N.)	1.50	16.88	12.43	0.13	29.49	1.35	1.14	0.58
	±0.40	±1.69	±1.28	±0.03	±8.19	±0.46	±0.29	±0.12
VI. (24 h U.N.)	0.01	20.00	15.17	0.00	1.23	0.05	0.06	36.17
	±0.007	±1.19	±1.38	±0.00	±0.52	±0.01	±0.03	±18.63
I *vs* II[3]	N.S.	<0.01	<0.02	N.S.	N.S.	N.S.	N.S.	N.S.
I *vs* III	<0.02	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.
II *vs* III	N.S.	<0.05	<0.05	<0.02	N.S.	N.S.	N.S.	N.S.
IV *vs* V	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
IV *vs* VI	<0.001	N.S.	N.S.	<0.001	<0.025	<0.05	<0.001	N.S.
V *vs* VI	<0.01	N.S.	N.S.	<0.005	<0.01	<0.025	<0.01	N.S.[4]
I *vs* IV	N.S.[4]	N.S.[4]	<0.05	N.S.	N.S.	N.S.	<0.02	N.S.
II *vs* V	N.S.	<0.01	<0.01	N.S.	N.S.	N.S.	N.S.	<0.02
III *vs* VI	<0.005	<0.01	<0.02	<0.01	<0.01	N.S.[4]	<0.05	N.S.[4]

[1] The legends are the same as in Table 1, values are expressed as mean ± S.E.M. Abbreviations, see "Abbreviations."
[2] Five animals were studied in each group.
[3] *P* values calculated from unpaired data by Student's *t* test.
[4] $P < 0.05$, Mann-Whitney test.

MAP was lower in Group VI than in Group IV ($P < 0.05$); however, there was no significant difference between Group V and Group VI (Table 1). As shown in Table 2, GFR averaged 2.18 ± 0.38 ml/min in control puppies (Group IV), a value that was not significantly different from 1.50 ± 0.40 ml/min in Group V. GFR was reduced nearly to zero in Group VI ($P < 0.001$). Also, the mean GFR value for Group VI was significantly lower than in Group V ($P < 0.01$). In contrast, mean values for RBF

and RPF were not different in the three experimental groups. A predictable decline in values for FF was observed in Group VI as compared with either Group IV ($P < 0.001$) or Group V ($P < 0.005$) (Table 2). As depicted in Table 2, urinary flow rate in Group VI was significantly lower than in Group IV ($P < 0.025$) and in Group V ($P < 0.01$). Similarly, UNaV significantly decreased in Group VI as compared with either Group IV ($P < 0.05$) or Group V ($P < 0.025$). Changes in absolute UKV closely paralleled the observed alterations in Group VI for urine flow rate and UNaV (Table 2). FENa was increased in Group VI ($P < 0.05$ Mann-Whitney test).

Comparison of Groups I, II, and III *versus* IV, V, and VI, respectively: Group I had lower MAP, but higher hematocrit than Group IV ($P < 0.004$ and $P < 0.02$, respectively). Body wt was not significantly different between groups (Table 1). Mean values for GFR, RBF, FF, V, UNaV, and FENa were not statistically lower in Group I than in Group IV ($p > 0.05$) by t test, but significant differences in RBF and GFR were noted using Mann Whitney test ($P < 0.05$).

As shown in Table 1, MAP was lower in Group II than in Group V ($P < 0.01$). Similar values for GFR were obtained in Group II and V, however RBF and RPF were significantly reduced in Group II as compared with Group V ($P < 0.01$ and $P < 0.01$, respectively). Mean values for FF, V, UNaV, and UKV were not different in Group II *versus* Group V. FENa reached a lower value in Group V than in Group II (Table 2).

Group III had lower body weight and higher hematocrit values than Group VI ($p < 0.05$ and $P < 0.05$, respectively). MAP was not different between both groups (Table 1). It should be noted that mean GFR values were markedly reduced in Group VI in comparison to Group III ($P < 0.005$). Conversely RBF and RPF were higher in Group VI than in Group III ($P < 0.01$ and $P < 0.02$, respectively). The reduction of GFR, accompanied by the increase in RPF, led to a significantly lower value of FF in Group VI than in Group III ($P < 0.01$). Urinary flow rate and UKV in Group VI were lower than in Group III ($P < 0.01$ and $P < 0.05$, respectively). UNaV was lesser in Group III than in Group VI ($P < 0.05$, Mann Whitney test); because of marked reduction in GFR in Group VI, the FENa was higher compared with Group III.

Morphologic results. There was a general, although not invariable, correlation between the chronologic age of the animals and the extent to which nephrons were differentiated (Fig. 1 and 2). As has been noted previously (12) there was an obvious gradient in the degree of differentiation of nephrons in the dog cortex with nephrogenesis being the most advanced adjacent to the medulla and least advanced at the cortical periphery. Even in the oldest animals (Groups IV, V, and VI), a significant amount of nephrogenesis was still in progress.

UN-induced alteration of nephron morphology was only seen in the most differentiated nephrons and therefore was most clearly evident in the older animals studied (*i.e.*, Group V and VI) compared with controls (Fig. 1 and 2). Kidneys from older puppies (Group V) and the younger puppies (Group II, Fig. 3) examined 2 h after UN injection, exhibited an abnormal accumulation of variable sized vacuoles in cells lining the proximal convoluted tubules (Fig. 4). These cytoplasmic vesicles were often quite large and in the most severe instances entirely filled the lining cells. Despite this alteration in the proximal tubules, the renal corpuscles and distal tubules and the collecting ducts, did not exhibit any consistent pattern of alterations that could be solely attributed to UN treatment. In addition to proximal tubule vacuolation, the animals examined 24 h after UN injection also exhibited a population of necrotic proximal tubules (Fig. 5 and 6). In addition, hyaline casts were seen filling the lumina in some segments of Henle's loop. Although the number of the necrotic proximal tubules and hyaline casts were variable, their frequency correlated with functional studies and were therefore more prev-

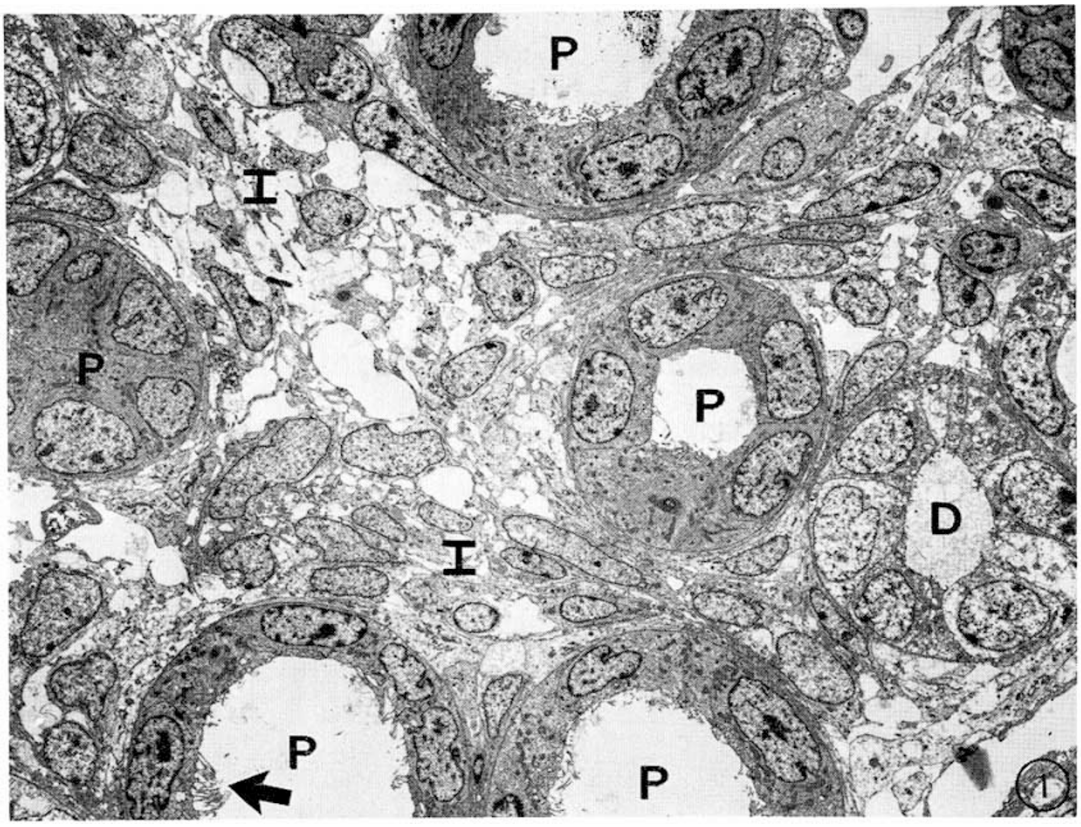

Fig. 1. Midcortical region of a kidney from an untreated young puppy (13-day-old). Proximal tubules (*P*) in various stages of differentiation, distal tubules (*D*), and relatively large areas of interstitial tissue (*I*) are characteristic of the young puppy kidney. Note that even the more differentiated proximal tubules exhibit a partially elaborated microvillous brush border (*arrow*). Section stained with uranyl acetate and lead citrate, ×1600.

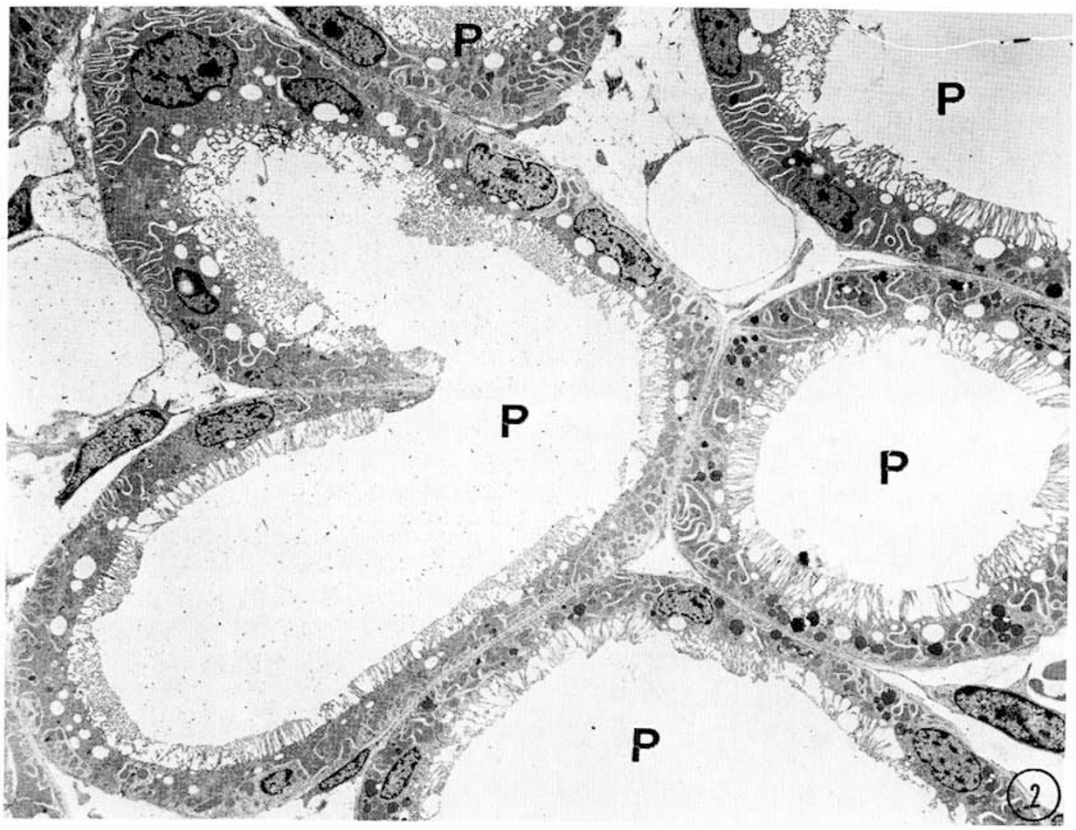

Fig. 2. Midcortical region of a kidney from an untreated older puppy (33-day-old). More mature proximal tubules (*P*) with a microvillous brush border are typical of the older puppy kidney. Note that adjacent proximal tubules are more closely juxtaposed, leaving smaller areas of interstitial tissue. Also note that small apical vacuoles are normally present in the more mature proximal tubules. Sections stained with uranyl acetate and lead citrate, ×1600.

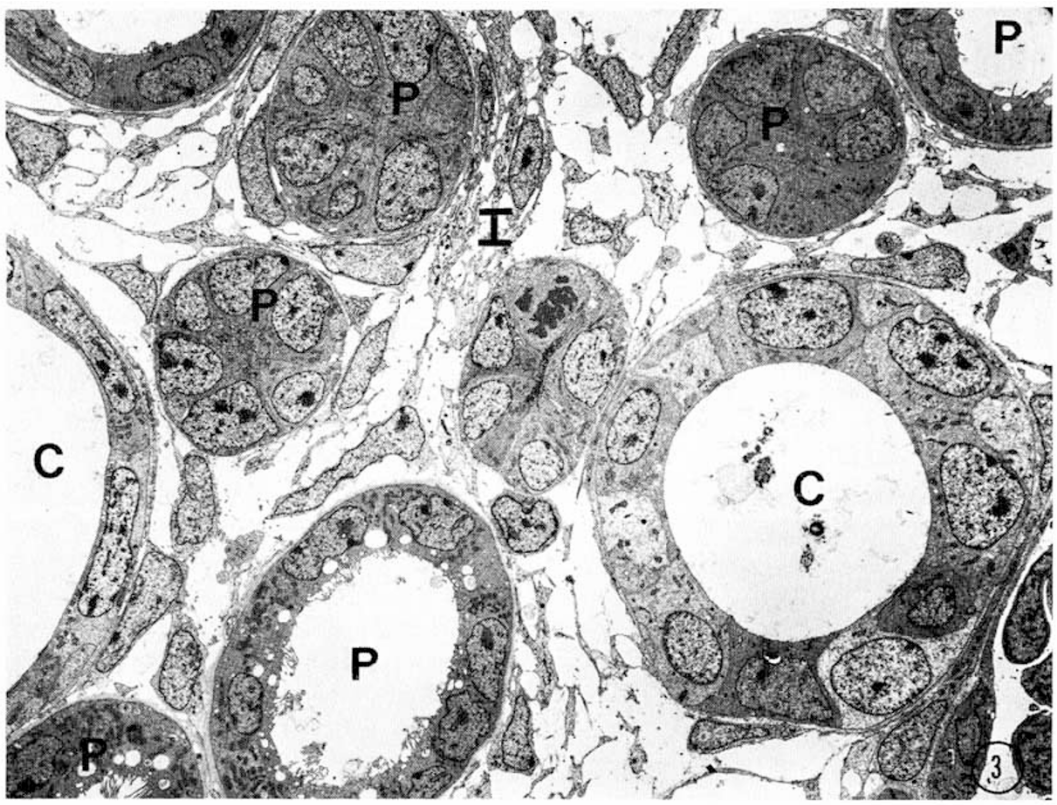

Fig. 3. Midcortical region of a kidney from a young puppy (9-day-old) 2 h after uranyl nitrate administration. Note that after 2 h, uranyl acetate treatment appears to have had little morphologic effect on the young puppy kidney (*P*) proximal tubule, (*C*) collecting duct, and (*I*) interstitial tissue. Sections stained with uranyl acetate and lead citrate, ×1600.

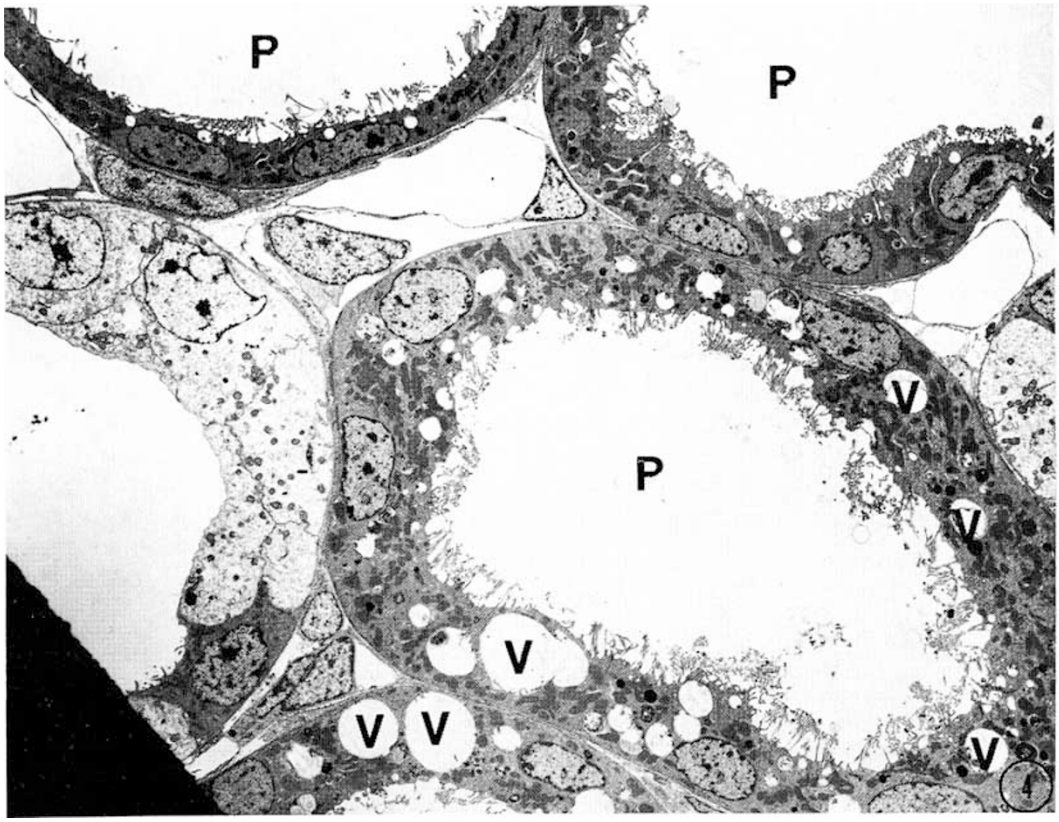

Fig. 4. Midcortical region of a kidney from an older puppy (34-day-old) 2 h after uranyl nitrate administration. Some of the more differentiated proximal tubules (*P*) exhibit large cytoplasmic vacuoles (*V*) and (*C*) collecting duct. Stained with uranyl acetate and lead citrate, ×1600.

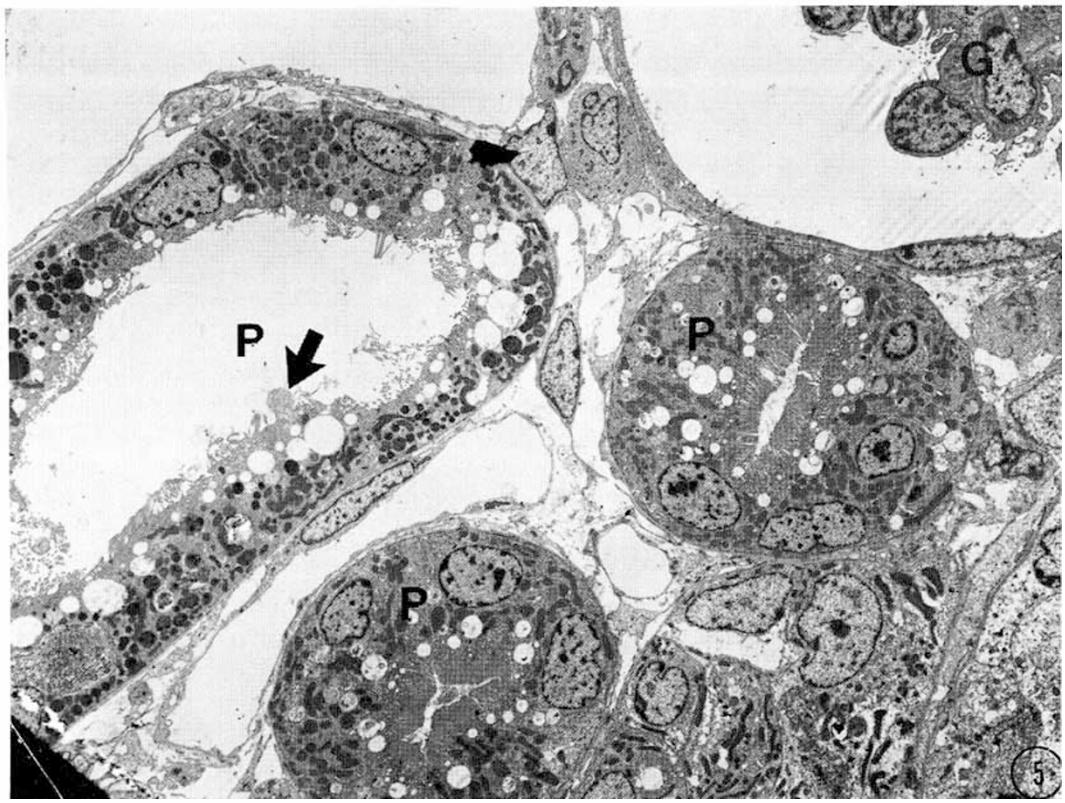

Fig. 5. Midcortical region of a kidney from a young puppy (11-day-old) 24 h after uranyl nitrate administration. Some differentiated proximal tubules (*P*) exhibit large vacuoles and some exhibit focal signs of cell disruption (*arrow*). (*G*) glomerulus. Stained with uranyl acetate and lead citrate, ×1600.

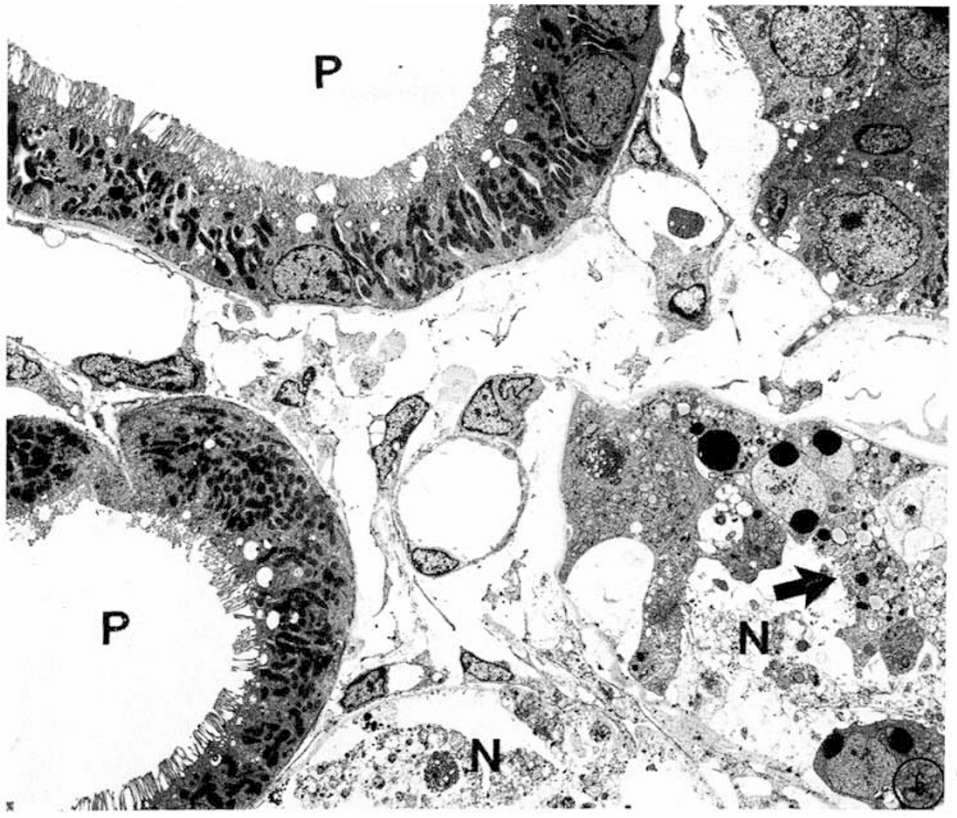

Fig. 6. Midcortical region of a kidney from an older puppy (25-day-old) 24 h after uranyl nitrate administration. Some differentiated proximal tubules (*P*) are normal in appearance whereas others have undergone extensive necrosis (*N*). Note the brush border debris in the lumen of a necrotic tubule (*arrow*). Stained with uranyl acetate and lead citrate, ×1600.

alent in the older (*i.e.*, Group VI) than the younger puppies (*i.e.*, Group III).

DISCUSSION

Experimental ARF induced by administration of UN is a reproducible and predictable nephrotoxic model in the mature animal (7, 10, 11, 24). Although the exact role of changes in RBF in the initiation and maintenance of UN-induced ARF continues to be controversial, alterations in RBF do not seem to be the major determinant of the initial reduction of GFR. Restoration of RBF to normal, either spontaneously or by pharmacologic agents, does not restore GFR in the course of ARF (11, 18). Although no unifying hypothesis to explain the pathogenesis of this nephrotoxic model has been conclusively substantiated, available data have suggested both a tubular and glomerular effect of UN (7, 10).

Transepithelial backleak of inulin has been documented and tubular obstruction is not a mechanism in the early pathogenesis of this nephrotoxic model (7, 10). Recent studies by Blantz (7) in the rat demonstrated that UN administration resulted in reduction of nephron filtration rate as well as an epithelial "backleak" effect. The reduction in nephron filtration rate was actually the consequence of decreases in the glomerular permeability coefficient. Avasthi *et al.* (3) using an identical protocol, observed reduction in the number and density of glomerular endothelial fenestrae, which correlated with the decrease in GFR. More recent studies have suggested that these changes in fenestrae may be functional in nature in that pretreatment of rats with converting enzyme inhibitor and/or plasma volume expansion after UN administration will return the glomerular permeability coefficient to normal values, but the transepithelial "backleak" of solutes persists (8).

Relatively few studies have been reported in the immature animal with experimental ARF. In contrast to the adult experience, gentamicin and other aminoglycosides are commonly used to treat several disease entitities in premature and term newborns, but only rarely do these agents appear to cause renal function impairment (19).

Recently, experimental studies in neonatal dogs have substantiated a relative tolerance of immature kidney to the nephrotoxic effects of gentamicin (9). This protective effect appeared to be afforded by the pattern of renal blood flow distribution seen during normal development. In contrast, Bidani *et al.* (5, 6) evaluating UN and $HgCl_2$-induced ARF in developing rats obtained conflicting results: the degree of ARF was similar in all age groups and a pattern of delayed renal function recovery and higher mortality rate was exhibited by the youngest animals.

The present studies attempted to ascertain the relative influence of age upon a nephrotoxic form (UN) of acute parenchymal renal failure during the initiation and maintenance phase in the developing puppy. Administration of UN to 1–2-wk-old puppies produced no significant changes in GFR during the initiation phase, despite a significant reduction in RPF (Group II). In contrast, within 24 h of UN administration (Group III), GFR was found to decrease by 60% as compared with control GFR in Group I. Although RPF remained nearly identical to control values, the observed reduction in GFR could not be attributed to a plasma flow effect. This reduction in GFR in the presence of near constant RPF led to a lower filtration fraction in Group III than in Groups I and II.

Assessment of changes in GFR in 3–5-wk-old puppies revealed a marked difference, relative to 1–2-wk-old puppies, in the magnitude of response to the heavy metal. In the older puppies the average value for GFR 2 h post-UN administration (Group V), although lower than in the control group (Group IV), was not significantly different. GFR was practically abolished during the maintenance phase (Group VI) as compared with control GFR. It is important to appreciate that this major alteration in GFR was entirely independent of changes in RPF because RPF

remained similar to control values. In this older group of puppies during the maintenance phase, filtration fraction fell to a negligible value. Similar to the changes in GFR and RPF, the changes in sodium and potassium excretion were greater in older than younger puppies.

The GFR and RBF were slightly greater in older than younger puppies (Group I *versus* Group IV). The body weights of the control animals in Group I did not differ from those in Group VI. This fortuitous similarity in body weight despite differences in age reflects the heterogeneity of these animals but may have partially obscured the well known age-related differences in basal GFR and RBF (13); however, morphologic studies demonstrated a correlation between the chronologic age of the puppies and the degree to which nephron units were differentiated. A typical pattern of centrifugal growth was observed in both age groups studied, in that nephrogenesis was more advanced in juxtamedullary than in superficial nephrons. Morphologic alterations consistent with the nephrotoxic effects of the heavy metal were observed in the proximal convoluted tubules of the most differentiated nephrons, being most evident in the oldest puppies under investigation. Proximal tubular cells exhibited an accumulation of variable size and often quite large vacuoles. By 24 h, the presence of hyaline casts and necrotic tubules correlated with the decline in renal function.

These age-related structural modifications correlated well with the functional responses (GFR) observed after UN administration, *i.e.*, a proportionately greater degree of both morphologic and functional alterations followed the administration of the heavy metal in the oldest group of puppies. Although the youngest group of puppies sustained some degree of renal structural damage and functional loss as a consequence of UN administration, these alterations were not as severe as in the oldest puppies and (by indirect comparison from other studies) in the adult dog (11, 24). In accordance with our observations is the recent finding by Cowan *et al.* (9) that chronologic age, and therefore the degree of development, plays a significant role in determining the final response of the renal toxic effects of gentamicin in developing puppies.

Collectively, our findings substantiate the view that the early phase of developmental growth could provide a protective "functional ambient" to the nephrotoxic effects of UN. Also, these experiments indicate that RBF *per se* is not a major determinant in the reduction of GFR in this model. The question remains as to the mechanisms leading to the decrease in renal function. Although the present studies do not permit one to fully characterize the mechanisms involved, one could certainly speculate that immaturity of both biologic systems regulating glomerular hemodynamics and uptake-transport processes by the renal tubule might have played a significant role in the amelioration of ARF in the more immature animals. It is of interest that diabetes in the rat confers complete and partial protection against the nephrotoxicity of gentamicin and UN, respectively (22). It has been suggested that this may in part be due to decreased binding and transport of the nephrotoxin into proximal tubules (17).

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27. Requests for reprints should be addressed to: Dr. Juan C. Pelayo, Veterans Administration Hospital, Nephrology Division, San Diego, California 92161.

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