Experimental Acute Renal Failure Induced by Uranyl Nitrate in the Dog

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ABSTRACT

The diminished glomerular filtration rate observed in previous studies of acute renal failure induced by uranyl nitrate has been ascribed to backflow of glomerular filtrate through necrotic tubular epithelium, since renal blood flow was essentially normal. Renal blood flow (133Xe washout) and renal function were studied serially for 96 hours after the administration of uranyl nitrate (10 mg/kg, iv) in unanesthetized dogs with chronic renal artery catheters. Inulin clearance and total renal blood flow decreased to 25% and 52% of control, respectively, by 6 hours and remained depressed. By 3 hours, cortical flow decreased to 330 ± 20 ml/min 100 g⁻¹ (control 507 ± 12 ml/min 100 g⁻¹) and outer medullary flow increased to 147 ± 8 ml/min 100 g⁻¹ (control 97 ± 18 ml/min 100 g⁻¹), indicating intrarenal blood flow redistribution. From 6 hours on, these flow components were no longer separable. The ratio of flow in the outer two-thirds of the renal cortex to that in the whole cortex, determined using ⁸⁰Sr-labeled microspheres (15μ), decreased to 0.34 ± 0.06 and 0.40 ± 0.04 at 6 and 96 hours, respectively (control 2.21 ± 0.12). Plasma renin activity was 1.8 ± 0.6 ng/ml hour⁻¹ at 3 hours and remained elevated (control 0.6 ± 0.2 ng/ml hour⁻¹). Histological examination revealed minimal tubular change at 8 hours and widespread disruption at 96 hours. The decrease in renal blood flow prior to any significant tubular pathology suggests that alterations in renal hemodynamics, which may be mediated by the renin-angiotensin system, are responsible for the diminished renal function observed in this model of acute renal failure.

KEY WORDS: renal blood flow, intrarenal blood flow distribution, ¹³³Xe washout, radioactive microspheres, plasma renin activity.

Experimental acute renal failure induced by uranyl nitrate in the dog supports the theory that the increased absorption of a normally formed glomerular filtrate through injured tubules is the pathogenic mechanism involved in the development of acute renal failure (1). This conclusion is based on the demonstration of an essentially normal renal blood flow in association with a diminished renal clearance of a variety of substances (2-4). There is, however, little other evidence suggesting that the passive backflow of filtrate plays a role in either the initiation or the maintenance of renal insufficiency in other models of experimental acute renal failure (5-8). Considering the demonstrated importance of renal hemodynamics in human (9, 10) and experimental (11) acute renal failure and the difficulties inherent in estimating renal blood flow in acute renal failure with standard clearance techniques (12-14), a reinvestigation of renal hemodynamics in acute renal failure induced by uranyl nitrate in the dog was undertaken using ¹³³Xe-washout and radiomicrosphere-distribution methods. The results of this study indicate that experimental acute renal failure induced by uranyl nitrate is characterized by significant alterations in both total renal blood flow and intrarenal distribution of blood flow.
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Methods

SERIAL 183XENON WASHOUT, INULIN CLEARANCE, AND URINE VOLUME AFTER URANYL NITRATE ADMINISTRATION

All experiments were performed on mongrel female dogs weighing 18-23 kg. Five dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv), and selective renal arteriography was performed to ascertain the presence of a single renal artery. Immediately afterwards, the renal artery was exposed through a flank incision, and care was taken not to disturb normal innervation. A polyvinyl catheter (0.9 mm, o.d.) was introduced into the renal artery by a modification of the technique described by Herd and Barger (15). In brief, a vascular clamp was placed on the renal artery, the catheter was introduced through an arteriotomy, and the catheter cuff was sutured to the adventitia of the artery. The catheter was tunneled subcutaneously and exteriorized between the scapulae where it was attached to an implanted two-way valve (16). Catheter patency was maintained by daily infusions of 0.5 ml of heparin in saline.

All renal blood flow and clearance studies were performed with the dogs in a sling; the dogs had been trained to stand quietly. After a minimum of 10 days of recovery from surgery, a control 24-hour urine specimen for the determination of sodium, potassium, and osmolality was obtained, and renal blood flow and its intrarenal distribution were determined using 133 xenon washout. An 0.5-1.0-mc bolus of 133 xenon dissolved in 0.25-0.50 ml of 0.85% saline was injected through the valve and flushed with 0.5 ml of saline. A 2-inch cylindrically collimated sodium iodide crystal was placed over the kidney, and the disappearance of radioactivity was monitored for a minimum of 40 minutes. The 133 xenon-washout curve was plotted on semilogarithmic paper and analyzed by curve stripping (17). Mean renal blood flow was calculated from the initial slope of the curve (9, 10, 18, 19). Although this technique assumes uniform distribution of 133 xenon at time zero, the results correlate with measured renal venous effluent at flow rates ranging from 50 to 240 ml/min. Regional blood flow was determined from the slopes of each component curve obtained after curve stripping by using a partition coefficient corrected for hematocrit at the time of study (20). Percentages of total radioactivity of each component, as estimated from the zero-time intercepts (17), are presented for comparison purposes only. Simultaneously, inulin clearance was determined to estimate glomerular filtration rate. The bladder was aseptically catheterized, and inulin was administered through a hind-limb vein in a sustaining dose of 40 mg/kg hour−1 in saline at 1.0 ml/min after a priming dose of 80 mg/kg in 10 ml of saline. All clearance values are the mean of at least two 15-20-minute clearance periods. Urine and serum inulin concentrations were determined on a Technicon Autoanalyzer (21). Prior to the 183 xenon-washout and inulin-clearance studies, 10 ml of blood was collected by venipuncture for use as an inulin blank and for determination of blood urea nitrogen concentration (22).

Only dogs having a mean renal blood flow greater than 3.5 ml/g min−1 and an inulin clearance greater than 30 ml/min were accepted for further study. Acute renal failure was induced by the intravenous administration of anhydrous uranyl trinitrate, 10 mg/kg body weight, in a saline solution (10 mg uranyl trinitrate/ml saline). 186 Xenon washout was determined 3 hours after uranyl nitrate administration. Inulin clearance, blood urea nitrogen concentration, and 186 xenon washout were determined 6, 24, 48, 72, and 96 hours after uranyl nitrate administration. The rate of delivery of the inulin-sustaining solution was halved from 48 hours of study on. Serial 24-hour urine volumes were obtained for determination of osmolality (Fiske Osmometer model 86a) and sodium and potassium concentration (IL flame photometer model 143). Statistical analyses were performed according to Snedecor and Cochran (23), and all results are presented as means ± se.

AUTORADIOGRAPHY, RENAL BLOOD FLOW DETERMINATION, AND HISTOLOGICAL EXAMINATION 96 HOURS AFTER URANYL NITRATE ADMINISTRATION

Ninety-six hours after uranyl nitrate administration, renal blood flow determinations by the microsphere technique and renal tissue for histological examination were obtained in all five dogs, and 85 krypton autoradiographs were prepared in three dogs. The dogs were anesthetized with sodium pentobarbital, and a left ventricle catheter (2.0 mm, o.d.) was placed under direct fluoroscopy through the femoral artery. A bolus of 15-20 μc of 85 strontium microspheres (1.0 mc in 20 ml of 10% dextran) in 3 ml of saline was injected, followed by a 5-ml saline flush. Adequate dispersal of the microspheres in the solution was obtained by 2-3 minutes of sonification (Biosonik II, Bronwill). Immediately after the microsphere infusion, the abdomen was opened in preparation for obtaining the kidneys. A 1.0-mc bolus of 85 krypton was injected into the renal artery, and the kidney was removed and quick-frozen in a Dry Ice-acetone mixture within 5 seconds of injection. The renal autoradiographs were obtained on 2-3-mm coronal sections after overnight exposure to high-speed photographic film (TriX Ortho, Kodak).
The contralateral kidney was prepared for light and electron microscopy by in situ perfusion. The fixative solution was either 3.5% glutaraldehyde in 0.1M cacodylate buffer or 0.735% glutaraldehyde in 75% Tyrode's solution (24). A cannula was advanced to the level of the renal artery and secured distally with a silk ligature around the lower abdominal aorta, and a second ligature was placed above the level of the renal artery. Simultaneously with the occlusion of the upper abdominal aorta, fixative perfusion was begun and maintained at a pressure of 100–120 mm Hg for 15 minutes. Subsequently, the kidney was sectioned coronally, and tissue blocks were immersed in fixative for 2 hours. Tissue blocks for resin embedding were then rinsed in buffer and osmicated (25). Resin-embedded tissues were cut on an LKB ultramicrotome at 0.5–1.0μm and stained with alkaline toluidine blue for light microscopy or at 500 μm and stained with lead citrate and uranyl nitrate for electron microscopy. Electron micrographs were taken on either an RCA 3H or an AEI EMU 6B electron microscope. Tissue blocks for paraffin embedding were dehydrated and embedded in a routine fashion and processed for hematoxylin and eosin staining.

Tissue sections were also obtained from the fixed kidney for calculation of cortical blood flow in two separate, noncontiguous, coronal sections by the method described by Slotkoff et al. (26). The mean of the individual flow rates was used by the method described by Slotkoff et al. (26). Blood samples were obtained 3, 6, 24, 48, 72, and 96 hours after intravenous administration of uranyl nitrate (10 mg/kg body weight). The dogs were allowed to lie quietly for ½ hour, after which 5 ml of blood was obtained by venipuncture, asatraumatically as possible.

Plasma renin activity was determined by the immunoassay procedure of Haber et al. (28), which measures the concentration of angiotensin I generated during a 3-hour incubation at 37°C. For each sample, two different aliquots of plasma were analyzed, each in duplicate. The recorded result is an average of all four assays. To determine the reproducibility of the assay, plasma renin activity was measured 15 times in plasma from a single pool; the mean ± s.e and the coefficient of variation were 2.1 ± 0.1 ng/ml hour−1 and 19%, respectively. Synthetic angiotensin II (Hypertensin, Ciba) added to both plasma and saline to give a final concentration of 40 ng/ml was undetectable, indicating that the antibody (Schwarz-Mann) was specific for angiotensin I. Using a bioassay procedure, the mean recoveries of the exogenous angiotensin II in plasma and in saline were 92% and 120%, respectively. To further validate the immunoassay procedure, both bioassayable plasma renin activity, measured by the method of Skinner (29), and immunoassayable plasma renin activity were determined in 115 samples of plasma. Comparing the results of both methods in the same plasma, the correlation coefficient of 0.798 was highly significant (P < 0.005), and the mean plasma renin activity determined by bioassay was not significantly different from that determined by immunoassay (P > 0.05). In addition, the mean immunoassayable plasma renin activity of nine human subjects in the upright position (3.8 ± 0.8 ng/ml hour−1) was significantly greater (P < 0.01) than the mean plasma renin activity of the same.
TABLE 1
Blood Urea Nitrogen, Inulin Clearance, and Urine Volume, Sodium, Potassium, and Osmolality after Uranyl Nitrate Administration

<table>
<thead>
<tr>
<th>Time after uranium (hr)</th>
<th>Blood urea nitrogen (mg/100 ml)</th>
<th>Urine volume (ml/24 hr)</th>
<th>Urine sodium (mEq/liter)</th>
<th>Inulin clearance (ml/min)</th>
<th>Urine osmolality (mosmole/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16 ± 2</td>
<td>1031 ± 167</td>
<td>23.6 ± 5.3</td>
<td>50 ± 2</td>
<td>1096 ± 173</td>
</tr>
<tr>
<td>6</td>
<td>28 ± 3*</td>
<td>898 ± 41</td>
<td>114.4 ± 18.6*</td>
<td>7 ± 3*</td>
<td>593 ± 44*</td>
</tr>
<tr>
<td>24</td>
<td>68 ± 9*</td>
<td>776 ± 138</td>
<td>105.5 ± 17.0*</td>
<td>4 ± 2*</td>
<td>426 ± 16*</td>
</tr>
<tr>
<td>48</td>
<td>101 ± 9*</td>
<td>622 ± 160</td>
<td>39.4 ± 10.0*</td>
<td>2 ± 1*</td>
<td>453 ± 79*</td>
</tr>
<tr>
<td>72</td>
<td>137 ± 9*</td>
<td>482 ± 159*</td>
<td>55.6 ± 9.8*</td>
<td>2 ± 1*</td>
<td>362 ± 83*</td>
</tr>
<tr>
<td>96</td>
<td>157 ± 6*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each result is the mean of determinations in five dogs.

Results

INULIN CLEARANCE, 133XENON WASHOUT, AND URINE VOLUME, SODIUM, POTASSIUM, AND OSMOLALITY AFTER URANYL NITRATE ADMINISTRATION

The various parameters followed at 6 hours and daily after uranyl nitrate administration are shown in Table 1. The blood urea nitrogen concentration was significantly elevated compared to the control value of 16 ± 2 mg/100 ml 6 hours after uranyl nitrate administration and progressively increased to a mean value of 157 ± 6 mg/100 ml 96 hours after uranyl nitrate administration. Although a tendency toward decreasing 24-hour urine volume was apparent, a significant depression (F < 0.025) was not noted until 96 hours after treatment. Urine sodium concentration promptly increased to 114.4 ± 18.6 mEq/liter and...
105.8 ± 17.0 mEq/liter 24 and 48 hours, respectively, after uranyl nitrate administration and then decreased toward control values. Urine sodium excretion 96 hours after uranyl nitrate administration (26.9 ± 4.7 mEq/24 hours) was not significantly different from the control value of 24.4 ± 4.0 mEq/24 hours (P > 0.30). Within 6 hours of intravenous administration of uranyl nitrate, there was a 75% decrease in inulin clearance to 12 ± 2 ml/min. There was a continued marked depression in inulin clearance from 6 to 96 hours after uranyl nitrate administration. Urine osmolality decreased to 593 ± 44 mosmols/liter at 24 hours and remained depressed for the remainder of the study. The course of alterations in these parameters relative to mean renal blood flow is depicted in Figure 1.

Data obtained from serial 183 xenon-washout curves are contained in Table 2. Three hours after uranyl nitrate administration, the mean total renal blood flow determined from the initial slope of the 183 xenon-washout curve was 224 ± 11 ml/min 100 g⁻¹, significantly less than the mean control value of 357 ± 20 ml/min 100 g⁻¹ (P < 0.005). Total renal blood flow was similarly depressed for the remainder of the study. The mean values during the control period for compartmental renal blood flow and for distribution of radioactivity are comparable to those previously reported by others in unanesthetized dogs (17). Figure 2 represents a typical control 133 xenon-washout curve. Three hours after uranyl nitrate administration, compartment-I flow decreased from 507 ± 12 ml/min 100 g⁻¹ to 330 ± 20 ml/min 100 g⁻¹ (P < 0.001), and compartment-II flow increased from 97 ± 18 ml/min 100 g⁻¹ to 147 ± 8 ml/min 100 g⁻¹ (P < 0.025). From 6-96 hours of study, only three compartments were obtained from analysis of the 133 xenon-washout curves; compartments I and II were replaced by a single monoexponential function (Fig. 3) intermediate in value between the control values for compartments I and II. Compartment-III flow increased to 77 ± 18 ml/min 100 g⁻¹ at 6 hours from a control value

<table>
<thead>
<tr>
<th>Time after uranyl nitrate administration (h)</th>
<th>Compartment-I flow (ml/min 100 g⁻¹)</th>
<th>Compartment-II flow (ml/min 100 g⁻¹)</th>
<th>Compartment-III flow (ml/min 100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>368 ± 20*</td>
<td>108 ± 26*</td>
<td>185 ± 10*</td>
</tr>
<tr>
<td>6</td>
<td>294 ± 22*</td>
<td>108 ± 26*</td>
<td>146 ± 26*</td>
</tr>
<tr>
<td>24</td>
<td>108 ± 20*</td>
<td>108 ± 20*</td>
<td>166 ± 20*</td>
</tr>
<tr>
<td>96</td>
<td>72 ± 7*</td>
<td>72 ± 7*</td>
<td>116 ± 7*</td>
</tr>
</tbody>
</table>

*Significantly different from control; †P < 0.05.

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Semilogarithmic plot of 133 xenon disappearance in a representative study during the control period. The exponentials are also plotted and labeled CI-CIV. The corresponding percents of total initial radioactivity are labeled $A_1-A_4$. RBF corresponds to the mean renal blood flow determined from the initial slope of the disappearance curve.

of $24 \pm 6$ ml/min 100 g$^{-1}$ ($P < 0.0125$) and remained elevated ($P < 0.05$ or less). The redistribution of compartmental blood flows was reflected by similar alterations in the

Semilogarithmic plot of 133 xenon disappearance in same dog as in Figure 2 representing studies obtained 6 hours after intravenous uranyl nitrate administration. Compartments I and II are merged into a monoeponential function (CI-II). Abbreviations are as in Figure 2.
Distribution of renal blood flow by radiomicrospheres and by \(^{188}\) Xenon Washout

<table>
<thead>
<tr>
<th>Time after uranium (hr)</th>
<th>CC (ml/g min(^{-1}))</th>
<th>OC (ml/g min(^{-1}))</th>
<th>IC (ml/g min(^{-1}))</th>
<th>RM (ml/g min(^{-1}))</th>
<th>OC/CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.01 ± 0.11</td>
<td>4.40 ± 0.05</td>
<td>2.01 ± 0.11</td>
<td>0.14 ± 0.01</td>
<td>1.10 ± 0.1</td>
</tr>
<tr>
<td>6</td>
<td>1.43 ± 0.10*</td>
<td>0.57 ± 0.05*</td>
<td>1.75 ± 0.16</td>
<td>0.40 ± 0.06*</td>
<td>0.40 ± 0.1</td>
</tr>
<tr>
<td>96</td>
<td>1.68 ± 0.15*</td>
<td>0.74 ± 0.06*</td>
<td>1.89 ± 0.12</td>
<td>0.48 ± 0.08*</td>
<td>0.44 ± 0.1</td>
</tr>
</tbody>
</table>

**CC** = whole cortex, **OC** = outer cortex, **IC** = inner cortex, **RM** = red medulla; other abbreviations as in Table 2
*Significantly different from control at the 5% confidence limits or less.

In autoradiographs of kidneys obtained from control dogs within 5 seconds of \(^{85}\) krypton injection, radioactivity was limited to the cortex and homogeneously distributed (Fig. 4A). In contrast, autoradiographs of kidneys 6 hours after uranyl nitrate administration demonstrated a heterogeneous distribution of radioactivity throughout the cortical area and in the area of the outer medulla (Fig. 4B). Autoradiographs obtained 96 hours after uranyl nitrate administration were not appreciably different from those obtained 6 hours after uranyl nitrate was given (Fig. 4C).

**FIGURE 4**

Autoradiographs from kidney removed 5 seconds after \(^{85}\) krypton injection. A: Normal dog with radioactivity limited to the cortex (C). IM = inner medulla. B: Six hours after uranyl nitrate administration, radioactivity is heterogeneously distributed in the cortex as well as in the outer medulla (OM). Radioactivity is apparent in blood vessels (BV). C: Ninety-six hours after uranyl nitrate administration.

The values for cortical and intracortical blood flow obtained in control dogs (Table 3) are comparable to those reported by others (26). Micorspheres were evident in glomerular capillaries in control dogs and in dogs that received uranyl nitrate (Fig. 5A and B). The mean whole cortical blood flow 6 hours after uranyl nitrate administration of 1.43 ± 0.10 ml/g min\(^{-1}\) was significantly depressed as compared with the control value of 4.01 ± 0.11 ml/g min\(^{-1}\) \(P < 0.001\). The major determinant of the decrease in whole cortical flow was a marked diminution in mean outer cortical flow to 0.57 ± 0.05 ml/g min\(^{-1}\) \(P < 0.001\). This reduction was re-
Experimentally induced acute renal failure was characterized by a significant decrease in mean renal blood flow (from 3.86 ± 0.06 ml/g min⁻¹ to 1.56 ± 0.09 ml/g min⁻¹, *P < 0.001*) and a decrease in cortical blood flow (from 3.54 ± 0.12 ml/g min⁻¹ to 0.34 ± 0.06 ml/g min⁻¹, *P < 0.001*). Similarly, inner cortical blood flow decreased from 1.66 ± 0.09 ml/g min⁻¹ to 1.85 ± 0.10 ml/g min⁻¹, while outer cortical flow decreased from 2.21 ± 0.12 ml/g min⁻¹ to 0.40 ± 0.06 ml/g min⁻¹, resulting in a significant increase in the ratio of mean inner cortical to whole cortical flow from 0.50 ± 0.05 to 1.22 ± 0.04 (*P < 0.001*), and a significant decrease in the ratio of mean outer cortical to inner cortical flow from 2.21 ± 0.12 to 0.40 ± 0.06 (*P < 0.001*). Similar alterations were apparent in the cortical and intracortical blood flows.

**Table:**

<table>
<thead>
<tr>
<th>IC/CC</th>
<th>OC/IC</th>
<th>Mean renal blood flow (ml/g min⁻¹)</th>
<th>C1 (ml/g min⁻¹)</th>
<th>CII (ml/g min⁻¹)</th>
<th>CIII (ml/g min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ± 0.05</td>
<td>2.21 ± 0.12</td>
<td>3.86 ± 0.06</td>
<td>4.24 ± 0.11</td>
<td>1.18 ± 0.19</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>22 ± 0.04*</td>
<td>0.34 ± 0.06*</td>
<td>1.66 ± 0.09*</td>
<td>3.54 ± 0.88</td>
<td>0.73 ± 0.15*</td>
<td></td>
</tr>
<tr>
<td>6 ± 0.11*</td>
<td>0.40 ± 0.04*</td>
<td>1.85 ± 0.10*</td>
<td>3.52 ± 0.38</td>
<td>0.50 ± 0.09*</td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 5**


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and ratios 96 hours after uranyl nitrate administration.

There was a significant increase in the regional flows calculated for red medulla from a mean control value of $0.14 \pm 0.01 \text{ ml/g min}^{-1}$ to $0.40 \pm 0.06 \text{ ml/g min}^{-1}$ 6 hours ($P < 0.005$) and to $0.48 \pm 0.08 \text{ ml/g min}^{-1}$ 96 hours ($P < 0.001$) after uranyl nitrate administration. The 6- and 96-hour values are not different ($P > 0.15$) from each other. The values obtained for outer medullary blood flow are relative flow rates since microspheres of the size used in this study are trapped in the renal glomerular capillaries (26, 30, and Fig. 5). Red medullary blood flow rates, therefore, may represent agglomerular medullary perfusion from the inner cortex (26, 30–32) rather than shunting, and the increments in flow rates after uranyl nitrate administration may reflect maintenance of inner cortical flow at a time when overall cortical perfusion is markedly decreased.

Table 4 presents the mean regional flow values obtained in the noncontiguous areas (A and B) in control dogs and in dogs receiving uranyl nitrate. Using the paired t-test (23), good agreement was obtained between individual regional flow rates for each group of dogs, indicating that the mean regional flow rates in Table 3 are representative of the cortical blood flow distribution in the entire kidney despite the heterogeneity apparent in the $^{86}$krypton autoradiographs (Fig. 4B and C).

In the five control dogs, the mean whole cortical blood flow of $4.01 \pm 0.11 \text{ ml/g min}^{-1}$ determined by the microsphere technique was not different from the mean total kidney blood flow of $3.88 \pm 0.06 \text{ ml/g min}^{-1}$ (Table 3) determined from $^{133}$xenon washout ($P > 0.05$) by the paired t-test (23) but was significantly different ($P < 0.025$) from compartment-I flow (Table 3). The mean outer cortical flow of $4.40 \pm 0.05 \text{ ml/g min}^{-1}$ was significantly different from the mean total renal blood flow ($P < 0.005$) but not different from compartment-I flow ($4.24 \pm 0.11 \text{ ml/g min}^{-1}$) of the $^{133}$xenon-washout curve ($P > 0.05$). The fusion of compartments I and II after uranyl nitrate administration did not allow comparison of microsphere- and $^{133}$xenon-determined renal blood flows. Mean whole cortical blood flow determined using microspheres, however, was not significantly different from total renal blood flow using the $^{133}$xenon-washout technique at either 6 hours ($P > 0.05$) or 96 hours ($P > 0.05$) after uranyl nitrate administration.

### TABLE 4

Comparison of Renal Blood Flow Distribution Determined by Microsphere Injection in Two Noncontiguous Areas of the Kidney

<table>
<thead>
<tr>
<th>Time after uranium (hr)</th>
<th>Area</th>
<th>CC (ml/g min$^{-1}$)</th>
<th>OC (ml/g min$^{-1}$)</th>
<th>IC (ml/g min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>4.05 ± 0.10</td>
<td>4.36 ± 0.15</td>
<td>2.07 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3.98 ± 0.05</td>
<td>4.15 ± 0.12</td>
<td>1.94 ± 0.06</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>1.32 ± 0.12</td>
<td>0.59 ± 0.05</td>
<td>1.62 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.55 ± 0.08</td>
<td>0.54 ± 0.07</td>
<td>1.84 ± 0.11</td>
</tr>
<tr>
<td>96</td>
<td>A</td>
<td>1.79 ± 0.18</td>
<td>0.79 ± 0.09</td>
<td>1.99 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.54 ± 0.16</td>
<td>0.86 ± 0.04</td>
<td>1.73 ± 0.08</td>
</tr>
</tbody>
</table>

Abbreviations are as in Table 3; $P$ values are given in parentheses. All values are means ± se for five dogs.
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which renal hemodynamic studies were conducted (Tables 1 and 5). Mean renin activities both 3 and 6 hours after uranyl nitrate administration, 1.8 ± 0.6 ng/ml hour⁻¹ and 2.2 ± 0.5 ng/ml hour⁻¹, respectively, were significantly greater than the control value of 0.8 ± 0.2 ng/ml hour⁻¹ (P < 0.025). Between 6 and 24 hours after administration of uranyl nitrate, there was a striking elevation in plasma renin activity (P < 0.01) that persisted without significant variation for the duration of the experiment (Table 5).

HISTOLGICAL EXAMINATION

Six hours after uranyl nitrate administration, light microscopic evidence of tubular disruption and epithelial cell loss was negligible, and interstitial edema was not observed (Fig. 6A). Epithelial cell height and nuclear outlines were uniform. Most of the convoluted tubules in the outer cortex were open and unobstructed. Approximately 15-30% of the observed tubules showed some epithelial swelling and increased eosinophilia. Half of these tubules had reduced or obliterated lumens. In the inner cortex, collapsed pars recta segments were somewhat more frequent. These segments were characterized by slight to moderate cellular swelling and nuclear pleomorphism. All macula densa segments appeared patent. Resin-embedded sections stained with toluidine blue confirmed the impression gained from paraffin-embedded sections. Occasional tubules exhibited reduced or obliterated lumens. The epithelial cells were moderately swollen with pleomorphism and swelling of other nuclei. Mitochondria varied from delicate rods to spheres. An infrequent tubular section demonstrated severe epithelial degeneration and denuding of the basement membrane (Fig. 6B).

Light microscopic observations 24 hours after uranyl nitrate administration revealed widespread tubular disruption and epithelial cell loss. Although present, the areas of interstitial hemorrhage and inflammatory foci were small and infrequent. In the outer cortex, most of the convoluted tubules were uninvolved. However, a few showed early reparative changes, and amorphous eosinophilic material was evident within the lumens of some altered tubules. The inner cortex contained many necrotic pars recta segments. Other pars recta segments with low cuboidal to squamous and highly attenuated epithelial cell linings were also noted. These segments frequently contained amorphous eosinophilic material and some cellular debris. Some tubules remained open and unaffected. Many macula densa segments were either totally collapsed or contained amorphous eosinophilic material. Interstitial edema was not a prominent feature 24 hours after uranyl nitrate administration.

Ninety-six hours after uranyl nitrate was given, convoluted tubules of the outer cortex appeared to be involved to a variable extent on light microscopic examination (Fig. 6C). Widespread attenuation of epithelial linings with marked tubular necrosis and disruption was evident in some dogs. Others exhibited only minor tubular disruption and mild epithelial swelling. The inner cortex, however, was massively involved. The epithelial linings of most tubules were completely disrupted and in the process of being shed into the intratubular lumen. Some tubules had highly attenuated epithelial linings and were filled with amorphous eosinophilic material. Macula densa segments were collapsed and shrunken or contained amorphous eosinophilic debris.

| TABLE 5 |
| Plasma Renin Activity (PRA) and Blood Urea Nitrogen (BUN) Concentration after Uranyl Nitrate Administration |

<table>
<thead>
<tr>
<th>Control</th>
<th>3</th>
<th>6</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA (ng/ml hour⁻¹)</td>
<td>0.6 ± 0.2</td>
<td>1.8 ± 0.6</td>
<td>2.2 ± 0.5</td>
<td>7.3 ± 1.9</td>
<td>6.2 ± 1.6</td>
<td>9.1 ± 2.6</td>
</tr>
<tr>
<td>BUN (mg/100 ml)</td>
<td>15 ± 1</td>
<td>10 ± 1</td>
<td>21 ± 1</td>
<td>58 ± 6</td>
<td>95 ± 10</td>
<td>112 ± 18</td>
</tr>
</tbody>
</table>

Circulation Research, Vol. XXXI, November 1972
Interstitial hemorrhage and edema were commonly noted 96 hours after uranyl nitrate administration.

**Discussion**

Acute renal failure induced by uranyl nitrate in the dog is characterized by a diminished inulin clearance, a progressively falling urine volume, an initial increase in urine sodium concentration, a decreased urine osmolality, rising blood urea nitrogen concentration, and altered renal hemodynamics. Early in the course of acute renal failure induced by uranyl nitrate, at 6 hours, inulin clearance was 25% of control; whereas renal blood flow was 52% of control. The decrease in inulin clearance out of proportion to the fall in renal blood flow could result from either a redistribution of renal perfusion to areas of cortical nephrons with lower inherent glomerular filtration rates (33) or a marked increase in preglomerular resistance, as has been previously demonstrated in experimental acute renal failure (6). Since the filtration fraction, determined from inulin clearance and mean renal blood flow measured by 133Xenon washout, decreased from 0.15 ± 0.02/100 g kidney weight to 0.07 ± 0.02/100 g kidney weight (P < 0.005), increased preglomerular resistance could account for the disproportionate reduction in inulin clearance. It seems improbable that the severe depression in inulin clearance is attributable to passive backflow of filtrate across necrotic tubular epithelium or intratubular obstruction, since 6 hours after uranyl nitrate administration microscopic examination revealed minimal tubular epithelial necrosis and a lack of tubular dilatation or cast formation.

Our data are consistent with previous reports describing inhibition of renal tubular function after uranium administration (34-37). The initially well-maintained urine volume, relative to renal blood flow and inulin clearance, is indicative of a functional rather than a structural alteration in renal tubular epithelium. In addition, the persistent urine volume of low osmolality and the increased urine sodium concentration appear to be a reflection of the markedly diminished glomerular perfusion of nephrons with severely limited ability to reabsorb sodium or to concentrate tubular fluid; or both. An increase in medullary blood flow with washout of the cortical-medullary osmotic gradient could have a role in the decreased urine osmolality (38), but this possibility cannot be evaluated without measuring urinary concentrating and diluting capacities or tissue solute gradients or anatomically localizing compartment flow to the medulla.

A role for either tubular obstruction or passive backflow cannot be eliminated later in the course of acute renal failure induced by uranyl nitrate. Histopathological examination of the kidney 24 hours after uranyl nitrate administration did not reveal a marked increase in tubular damage as compared with that 6 hours after uranyl nitrate was given. Without a sequential study between 24 and 96 hours after uranyl nitrate administration, no comment as to the relation between serial changes in structure and function can be made. At 96 hours after uranyl nitrate administration microscopic examination revealed minimal tubular epithelial necrosis and a lack of tubular dilatation or cast formation.

**Figure 6**

Kidney cortex after uranyl nitrate poisoning. A: Six hours after administration, most tubules are open with no apparent epithelial loss or obvious necrosis. Some tubules are collapsed (arrow). Paraffin-embedded, hematoxylin and eosin stain. Calibration 50μ. B: Six hours after administration. Higher magnification showing a tubule containing amorphous material (a) and a tubule with necrotic epithelial alteration (arrow). Capillaries (c) are open, and little interstitial edema is present. Resin-embedded, toluidine blue stain. Calibration 25μ. C: Ninety-six hours after administration. A variety of tubular alterations are present. A variety of tubular alterations are present. Some tubules contain amorphous debris (a). Many tubules have shed their epithelium (e) or still contain the necrotic remains of their epithelium (p). A shrunken macula densa segment also contains amorphous debris (arrow). A few tubules are patent and show minimal architectural alteration (p). Paraffin-embedded, hematoxylin and eosin stain. Calibration 50μ.
administration, well after the appearance of altered renal hemodynamics and the impairment of overall renal function, severe tubular necrosis and intratubular cast formation were apparent. Consequently, although neither passive backflow nor intratubular obstruction was responsible for the initiation of renal dysfunction, they could have contributed to the persistent impairment in overall renal function. Indeed, Eisner and co-workers (39) have presented evidence, using the distribution volumes of inulin and sodium, for increased tubular permeability to inulin 2–4 days after a lower dose of uranyl nitrate.

The marked renal hemodynamic alterations in acute renal failure induced by uranyl nitrate in the dog are characterized by a prompt and persistent diminution in overall renal perfusion, which is stable from 3–96 hours after uranyl nitrate administration. Total renal blood flow decreased to 40–50% of control, which is consistent with a direct measurement of renal venous effluent by others (39) in this model of acute renal failure in the dog. Concomitant with the decrease in overall renal blood flow was a significant alteration in the intrarenal distribution of blood flow. This change appeared 3 hours after uranyl nitrate was given; a decrease in the rapid, or cortical, flow compartment of renal xenon washout and an increase in the second compartment, or outer medullary flow, occurred. Subsequently, from 6–96 hours after uranium administration, compartments I and II merged into a single monoexponential function which was quantitatively greater than compartment II and less than compartment I in the control period. It has been previously suggested that “fusion” of compartments I and II results from a decrease in compartment-II half-time and an increase in compartment-I half-time such that they are no longer separable by curve stripping (9). The demonstration of significant outer medullary radioactivity and of a marked decrease in and a heterogeneity of cortical radioactivity in autoradiographs obtained 5 seconds after renal arterial injection of 85krypton support this interpretation. The reduction in cortical perfusion was confirmed by radiomicrosphere distribution 6 and 96 hours after uranyl nitrate administration; this procedure demonstrated a very marked depression in outer cortical perfusion in association with maintenance of inner cortical flow.

The site(s) of increased vascular resistance responsible for the renal hemodynamic alterations in acute renal failure induced by uranyl nitrate is not directly apparent from our data. If the site of increased vascular resistance is preglomerular, as has been suggested in studies of both human and experimental acute renal failure (6, 9, 11), then the decrease in cortical perfusion demonstrated by our data is sufficient to account for the marked diminution in glomerular filtration in this model of acute renal failure. The appearance of the autoradiographs suggests that the alteration in renal vascular resistance is not uniform, since there were cortical areas with grossly normal amounts of radioactivity. The persistent blood flow in these areas could be the result of postglomerular arteriolar dilatation, which also would diminish effective glomerular filtration pressure. Either preglomerular constriction or postglomerular dilatation, or a combination of both, would account for a severe enough depression in glomerular perfusion pressure to cause the alterations in glomerular filtration demonstrated in acute renal failure induced by uranyl nitrate without postulating either tubular obstruction or disruption. This finding is consistent with the conclusions of others that altered renal hemodynamics with a secondary decrease in glomerular filtration are the cause of oliguria and renal insufficiency in a variety of models of experimental acute renal failure (5-8, 40-46).

Either 133xenon- or 85krypton-washout curves similar to those observed after uranyl nitrate administration in the dog have been reported in human acute renal failure of diverse etiologies (9, 10), in experimental acute renal failure (11), and after renal arterial infusions of angiotensin II (47). The mean fusion-curve flow rates reported by Hollenberg and co-workers (10) in patients after nephrotoxin- or shock-induced acute
renal failure were 132 and 141 ml/100 g, respectively. These values are considerably lower than the values we observed after uranyl nitrate administration in the dog. The apparent discrepancy could be related to the presence of a faster compartment I in the normal dog as compared with that in man (10). As a result of this inherent difference, similar reductions in flow rate would result in a larger fusion flow rate in the dog. In addition, variations in the degree of redistribution of intrarenal blood flow could account for the species difference. Carriere and Friborg (47) reported fusion curves in dogs receiving renal intra-arterial infusions of angiotensin. They observed the same heterogeneity in the distribution of cortical hypoperfusion. Our autoradiographs, however, demonstrated less severe alterations in cortical perfusion and an earlier appearance of outer medullary radioactivity. Similar nonuniform cortical hypoperfusion has been observed after intrarenal epinephrine infusion (48) or hemorrhagic hypotension (49).

The mechanisms responsible for the initiation, or maintenance, or both, of renal ischemia and altered renal hemodynamics in the dog after uranyl nitrate administration are obscure. The similarity of hemodynamic findings in human acute renal failure of diverse etiologies and in various models of experimental acute renal failure suggests a common mechanism. A significant role for the renin-angiotensin system in the development of acute renal failure has been postulated because of the demonstration of elevated levels of plasma renin activity in acute renal failure in man (50–52) and the amelioration of experimental acute renal failure after saline-induced renin depression (5, 8, 43). In contrast to studies of plasma renin activity in man, which have demonstrated persistent elevations in plasma renin activity throughout the oliguric phase of acute renal failure, previous studies of plasma renin activity in experimental acute renal failure have revealed only an early, transient increase in plasma renin activity (53, 54). Our data offer no explanation for the maintained elevation of plasma renin activity in the dog after uranyl nitrate administration. It would not appear to be the selection of the dog as the experimental animal since Ruiz-Guinazu (54) observed prompt diminution in plasma renin activity during methemoglobin-induced acute renal failure in the dog. Previous studies of plasma renin activity in experimental acute renal failure have involved models with less severe tubular injury, such as glycerol-induced myohemoglobinuric (53) or methemoglobin-induced acute renal failure (54). The more severe tubular epithelial dysfunction associated with uranyl nitrate poisoning, as manifested by increased urine sodium concentration and decreased urine osmolality, could explain the maintenance of increased plasma renin activity in this model of acute renal failure. The macula densa would be presented with tubular fluid of increased sodium concentration, which is a proposed stimulus for renal renin release (55). This fact, in association with the amelioration of myohemoglobinuric (43), mercuric chloride-induced (8), and dichromate-induced (5) experimental acute renal failure by saline loading suggests a significant role for the renin-angiotensin system in the initial development of acute renal failure regardless of the role it may have in maintaining renal ischemia.

The elevations in plasma renin activity 3 and 6 hours after uranyl nitrate administration in the dog are relatively modest compared with the dramatic reductions in inulin clearance and the alterations in renal blood flow. This finding suggests that, if the renin-angiotensin system participates in the initiation of acute renal failure, intrarenal renin, rather than circulating renin, levels may be the vasoactive component of the system. The demonstration that the enzymes required for the formation of angiotensin exist on the nephron level in the juxtaglomerular apparatus supports this hypothesis (56). In this regard, the primary site of decreased cortical perfusion after uranyl nitrate administration is in the outer cortex, an area observed to have glomeruli with the highest renin content (57, 58). The observation that myohemoglobinuric
acute renal failure can occur in animals with low plasma renin activity but normal renal renin concentration is consistent with the importance of renal, rather than peripheral, renin in the pathogenesis of acute renal failure (59).

For the renin-angiotensin system to be involved in the pathogenesis of the renal hemodynamic alterations in acute renal failure, an initial event which results in renin release must occur. Although the initial event is obvious in those instances of acute renal failure associated with hypotension, it is obscure in nephrotoxin-induced acute renal failure without systemic alterations in blood pressure. Preliminary observations (unpublished) of blood pressure and renal blood flow in dogs for up to 3 hours after uranyl nitrate administration indicate that renal blood flow decreases without a concomitant depression in systemic blood pressure. Several alternative mechanisms are possible. There is no evidence excluding an effect of uranyl nitrate or of other nephrotoxins directly on the renal vasculature or indirectly through circulating catecholamines, the sympathetic nervous system, or other vasoactive mediators. In addition, a direct stimulation of renal renin release by uranyl nitrate cannot be excluded.

Acknowledgment

The authors gratefully acknowledge the technical assistance of Mr. Melvin Routh, Mr. Douglas Walters, and Mr. John Farrar and the secretarial assistance of Miss Linda Bosak.

References


EXPERIMENTAL ACUTE RENAL FAILURE

Experimental Acute Renal Failure Induced by Uranyl Nitrate in the Dog
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Circ Res. 1972;31:682-698
doi: 10.1161/01.RES.31.5.682

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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