Pathogenesis of Acute Renal Failure following Temporary Renal Ischemia in the Rat

By William J. Arendshorst, William F. Finn, and Carl W. Gottschalk

ABSTRACT

In this study, we characterized the sequence of several intrarenal events and evaluated their relative importance in the pathogenesis of unilateral oliguric acute renal failure induced experimentally in rats by complete occlusion of a renal artery for 1 hour. Kidneys were studied prior to occlusion and 1-3 hours and 22-26 hours after release of the temporary occlusion. Renal blood flow measured by an electromagnetic flow transducer was reduced to 40-50% of control during both postocclusion periods. Flow of tubular fluid was markedly reduced, and the damaged kidneys were oliguric. Proximal and distal convolutions were filled with fluid and dilated 1-3 hours after occlusion; their pressures were greatly heterogeneous and were elevated, on the average, to 31 and 16 mm Hg, respectively. Glomerular capillary pressure at this time was normal or slightly increased. Histological sections showed extensive tubular obstruction. We conclude that initially the oliguria is primarily due to intraluminal obstruction in the absence of predominant increases in preglomerular vascular resistance. Observations at 22-26 hours after occlusion indicated acute tubular necrosis. Moreover, the combined involvement of preglomerular vasoconstriction, persisting tubular obstruction, and passive backflow of tubular fluid appeared to be important in the maintenance of the oliguria. Glomerular capillary, proximal intratubular, and peritubular capillary hydrostatic pressures were reduced below control values. After acute volume expansion, the reduced pressures and renal blood flow were reversed, yet the experimental kidneys remained oliguric. Thus, it is clear that tubular obstruction is a significant factor responsible for both the genesis and the maintenance of oliguria in this experimental model of ischemia-induced acute renal failure.

A controversy concerning the nature and sequence of events involved in the development and maintenance of oliguria in acute renal failure persists despite the application of a number of investigative techniques in a variety of experimental models. Studies of acute renal failure have been performed in an attempt to determine the relative importance of the following etiologic factors: (1) primary failure to filter fluid across glomerular membranes which might result from arteriolar vasomotor changes lowering glomerular capillary pressure or plasma flow or from reductions in the glomerular ultrafiltration coefficient, (2) obstruction of the flow of tubular fluid, and (3) passive backflow of tubular fluid across damaged tubular epithelium. Although evidence implicating each of these factors has been published, most data have been interpreted as demonstrating that the principal, common cause of oliguric acute renal failure is a nearly complete cessation of glomerular filtration due to preglomerular vasoconstriction (1, 2). Some investigators have taken the position that there is no single cause for the anuria and that all three mechanisms may be involved in its genesis, their relative importance varying with the situation and the stage of evolution of the disease process (3).

In the present study, we characterized the sequence of several intrarenal events responsible for the pathogenesis of oliguric acute renal failure immediately and 24 hours following temporary complete unilateral renal artery occlusion in rats. Micropuncture techniques were used to study single nephron function, and a new noncannulating flowmeter was used to measure kidney blood flow.

Methods

Observations are reported on a total of 52 male Wistar and mutant-Wistar rats that were deprived of their standard rat pellet diet (Purina) but were allowed free...
access to water overnight prior to study. The rats were anesthetized with sodium pentobarbital (50 mg/kg body weight, ip) and placed on a heating table; the left kidney was then exposed through an abdominal incision for micropuncture as previously described (4). The renal capsule, the covering peritoneum, and the perirenal fat were left undisturbed. Both ureters were usually cathe-terized with PE-10 polyethylene tubing; PE-50 tubing was used in some instances, and similar results were obtained. Femoral arterial blood pressure was recorded continuously with a Statham P23Db pressure transducer connected to a recorder.

After control (preocclusion) micropuncture and blood flow observations had been made, unilateral acute renal failure was induced by a 1-hour period of complete ischemia. The left renal artery was isolated, 10-15 units/100 g body weight of heparin (1,000 units/ml, The Upjohn Co., Inc.) was injected intravenously, and a small smooth-surfaced tension clamp (Schwartz 1-inch clip, Roboz Surgical Instrument Co., Inc.) was positioned on the artery so that it was completely occluded. A piece of saline-soaked cotton covered the exposed kidney surface during the period of ischemia. The clamp was removed after 1 hour.

Studies commenced again 1 hour after removal of the clamp and continued for 2 hours, i.e., 1-3 hours after occlusion of the left renal artery. Saline (0.85% NaCl) was infused intravenously at 40 μliters/min before and after occlusion; the rate of infusion during the ischemic period was reduced to 20 μliters/min.

In other rats, observations were made 22-26 hours after restoration of blood flow. In these rats, the left kidney was exposed through a small subcostal incision. After 1 hour of complete ischemia, the incision was closed and the rats were returned to their cages. The preparation for micropuncture on the next day was the same as that described earlier, and 0.85% NaCl was infused into a femoral vein at 40 μliters/min. After initial observations at 24 hours, four rats were acutely expanded by a rapid intravenous infusion of fresh plasma from donor rats at 400 μliters/min for 5 minutes and then maintained at 200 μliters/min. Two other rats were acutely expanded with isotonic saline at 1 ml/min for 5 minutes and then maintained at 400 μliters/min for the duration of the experiment. Postexpansion observations commenced 30 minutes after administration of the priming solution and continued for 1 hour.

Hydrostatic pressure in random surface proximal and distal convolutions and postglomerular vessels was measured using sharpened glass pipettes (3-7μ, o.d.) filled with 2m NaCl and a continuously recording, electronic servonulling apparatus. Sharpened glass pipettes with outer tip diameters of 1-3μ were also used for direct measurements of glomerular capillary pressure and hydrostatic pressure in Bowman’s space in superficial glomeruli of Munich-Wistar rats. Penetration into Bowman’s space and entry into individual glomerular capillaries were performed under stereomicroscopic control (250x magnification). Output from the pressure transducer of the electronic servonulling device was monitored by a continuously writing recorder and also displayed on the screen of a dual-channel oscilloscope. Direct measurements of glomerular capillary pressure were accepted as technically satisfactory when the recording was pulsatile and synchronous with femoral arterial blood pressure, stable for at least 90 seconds, and free of mechanical noise due to kidney movement resulting from respiration (Fig. 1). We rarely observed more than three glomeruli located in areas accessible to reliable micropuncture on the ventral surface of an individual kidney; several of the rats did not possess any superficial glomeruli.

Afferent effective filtration pressure (AEFPa) was calculated from these direct values as AEFPa = GCPa - (BSP + μa), where GCPa is directly measured glomerular capillary pressure, BSP is hydrostatic pressure in Bowman’s space, and μa is the afferent colloidal osmotic pressure and is assumed to equal systemic plasma oncotic pressure. Glomerular capillary pressure in glomeruli of superficial nephrons not accessible to direct micropuncture was estimated in Wistar and Munich-Wistar rats from the sum of stop-flow hydrostatic pressure (SFP), which was measured in the earliest accessible loop of a proximal tubule as previously described (5, 6), and μa. From these pressures afferent effective filtration pressure was estimated as AEFPa = SFP - PITP where PITP is proximal intratubular pressure. These calculations of afferent effective filtration pressure assume that the colloidal osmotic pressure of the filtrate in Bowman’s space is insignificant.

Transit times of the appearance of tubular fluid dye

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FIGURE 1
Recorder tracing of glomerular capillary pressure measured directly in a superficial glomerulus using an electronic servonulling device. Femoral arterial blood pressure recorded simultaneously is also shown.

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columns in segments of proximal and distal convolutions were determined after intravenous injections of 0.05 ml of 5% buffered solution of F, D, and C no. 3 dye (Keystone Aniline and Chemical Co., Chicago, Illinois). Qualitative estimates of the rate of proximal tubular flow were also made by observing the progress down the nephron of small microinjected volumes of dye or droplets of Sudan black-stained castor oil. In an attempt to visually detect major changes in tubular permeability, small volumes of F, D, and C no. 3 dye were microinjected slowly into proximal convolutions at controlled pressure in a few rats. Intraluminal pressure upstream to the injection pipette was monitored concurrently in the same convolution by another pipette connected to an electronic servonulling device.

Renal blood flow was recorded continuously before and after 1 hour of complete ischemia using a small-diameter noncannulating electromagnetic flow transducer (EP model 401.5 with a lumen 1.5 mm in circumference) connected to a square-wave flowmeter (model 501, Carolina Medical Electronics). The calibration of this system and the reliability and accuracy of measuring renal blood flow in the anesthetized rat in our laboratory have been recently described (7). Appropriate small corrections were made for the decrease in hematocrit during the volume expansion studies at 24 hours after occlusion. Intrarenal vascular resistance was calculated from the arteriovenous pressure difference and the blood flow expressed in ml/min. Renal venous pressure was assumed to be constant and assigned a value of 5 mm Hg in the calculations.

Blood samples were obtained periodically from the tip of the tail during most experiments, and hematocrit was measured in heparinized capillary tubes. Plasma protein concentration was determined using rat plasma total protein standards2 by an adaptation of the Lowry technique (8), and colloidal osmotic pressure3 was calculated using the Landis-Pappenheimer equation (9). At the end of the experiment, both kidneys were excised, cleared of perirenal fat, decapsulated, and weighed immediately. Several kidneys from each observation period were fixed in Helly’s solution for histological examination.

Values calculated from the mean per rat are presented as means ± 1 SD (number of rats is given in parentheses). Paired and unpaired Student’s t-tests were employed for the evaluation of statistical significance. A P value greater than 0.05 was considered not statistically significant (ns).

Results

1-3 Hours After Occlusion

During the hour of renal artery occlusion, the kidneys decreased in size and became pale and then cyanotic. Coincident with these changes, all surface proximal convolutions collapsed, and peritubular capillary blood flow and urine flow ceased. Following release of the clamp, the kidneys resumed their normal color as circulation rapidly returned (Fig. 2), and they increased in size as the tubules filled with filtered fluid; the tubules were distended and translucent 1 hour after the clamp was released. Mean renal blood flow returned to approximately 50% of the flow before occlusion, and renal vascular resistance increased almost twofold (Table 1). Restoration of renal blood flow after the ischemic period was observed in almost all experiments. On the rare occasions when it did not occur, the failure was restricted to segmental areas of the kidney. Functional studies were not performed on these kidneys. Intravenously injected dye appeared in the vasculature as a fairly uniform colored flush; it was filtered into the early proximal convolutions, but its transit along the proximal tubules was delayed. The appearance of dye in the distal convolutions was seen only occasionally; in these cases it was delayed and its intensity was less than normal. At times, dye-stained intraluminal debris was observed in the distal convolutions.

Histological sections showed that the glomeruli were normal with red blood cells present in capillary loops. The tubules in the cortex were patent, and a moderate number were dilated. Tubular epithelial cells were swollen. Proteinaceous material was present in the tubular lumens, particularly in the medullary portion of the nephrons. No inflammatory cell infiltrate was seen in the interstitium. These changes were most marked at the corticomedullary border and the outer medulla. On sectioning of the fresh kidney, this area appeared grossly congested.

Urine flow rate was markedly decreased 1–3 hours after occlusion (Table 2). The weight of the kidneys 3 hours after occlusion was significantly greater than that of either the contralateral undisturbed kidneys or the left kidneys of rats not subjected to the ischemic insult (Table 2).

Hydrostatic pressure in proximal and distal convolutions, vascular stars, and peritubular capillaries was significantly elevated above preocclusion values 1–3 hours after occlusion (Table 2). Mean proximal and distal intratubular pressures rose about threefold, and mean vessel pressures increased roughly 1.5-fold. Thus, the proximal intratubular-peritubular capillary hydrostatic pressure difference increased markedly, the mean rising from +2.2 to +18 mm Hg. The distal intratubular-peritubular capillary hydrostatic pressure gradient was reversed from −4 to +3 mm Hg, on the average.
SCHEMIA-INDUCED ACUTE RENAL FAILURE

Frequency distributions of proximal intratubular hydrostatic pressures during the observation periods before and after occlusion are shown in Figure 3. Proximal intratubular pressure in kidneys studied during the periods after occlusion was in general predictable. Dilated tubules had higher pressures; lower pressures were recorded in tubules with narrow lumens. In an attempt to minimize any investigator bias, proximal convolutions were punctured in several different areas on the kidney surface in a random fashion irrespective of lumen diameter, a condition inherently difficult to attain when one employs the Landis technique but less difficult with the electronic servonulling device. Although proximal intratubular pressures were considerably more heterogeneous 1-3 hours after occlusion, a vast majority of the measurements, 90%, was above the range observed under preocclusion conditions. Figure 3 does not show six (2.2%) proximal intratubular pressure values between 52 and 60 mm Hg.

Glomerular capillary pressure was measured directly (Fig. 1) and estimated by the stop-flow technique in Munich-Wistar rats before and after renal ischemia. By paired analysis of the means per rat, estimated glomerular capillary pressure was not statistically different from the direct determinations made during the control period and in the

| TABLE 1 |

Renal Blood Flow and Renal Vascular Resistance before and after 1 Hour of Unilateral Renal Artery Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Before occlusion</th>
<th>0.5–1.5 hours after occlusion</th>
<th>22–26 hours after occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal blood flow (ml/min)</td>
<td>5.0 ± 1.0</td>
<td>2.6 ± 0.7*</td>
<td>2.3 ± 1.0*</td>
</tr>
<tr>
<td>Renal vascular resistance (mm Hg/ml min⁻¹)</td>
<td>22 ± 6</td>
<td>40 ± 11*</td>
<td>58 ± 24*</td>
</tr>
<tr>
<td>Femoral arterial blood pressure (mm Hg)</td>
<td>111 ± 17</td>
<td>103 ± 15</td>
<td>120 ± 18†</td>
</tr>
</tbody>
</table>

N = 8

Values are means ± 1 sd. Unless a P value is indicated, there is no significant difference between values either 0.5–1.5 or 22–26 hours after occlusion and control values or between values 22–26 and 0.5–1.5 hours after occlusion. N = number of rats tested.

* Significantly different from control, P < 0.001.
† Significantly different from value 0.5–1.5 hours after occlusion.

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Effect of 1 Hour of Unilateral Renal Artery Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Before occlusion</th>
<th>P&lt;0.001</th>
<th>1–3 hours after occlusion</th>
<th>P&lt;0.025</th>
<th>22–26 hours after occlusion</th>
<th>P&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow, left kidney (µliters/min)</td>
<td>3.1 ± 1.2 (16)</td>
<td></td>
<td>0.3 ± 0.4 (9)</td>
<td></td>
<td>0.05 ± 0.68 (19)</td>
<td></td>
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<tr>
<td>Glomerular capillary pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Direct</td>
<td>45.2 ± 4.2 (9)</td>
<td>NS</td>
<td>48.3 ± 6.2 (4)</td>
<td>&lt;0.001</td>
<td>22.4 ± 6.2 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estimated (paired with direct)</td>
<td>44.5 ± 2.7 (9)</td>
<td>&lt;0.005</td>
<td>52.7 ± 5.9 (4)</td>
<td>&lt;0.001</td>
<td>25.3 ± 4.2 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estimated (all rats)</td>
<td>46.5 ± 4.1 (17)</td>
<td>&lt;0.025</td>
<td>52.1 ± 4.9 (6)</td>
<td>&lt;0.001</td>
<td>31.1 ± 5.9 (18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Afferent effective filtration pressure (mm Hg)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Direct</td>
<td>21.8 ± 4.9 (9)</td>
<td>&lt;0.025</td>
<td>11.2 ± 8.2 (4)</td>
<td>NS</td>
<td>3.1 ± 3.5 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estimated</td>
<td>23.2 ± 3.2 (22)</td>
<td>&lt;0.001</td>
<td>9.8 ± 5.1 (11)</td>
<td>NS</td>
<td>9.5 ± 3.7 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stop-flow pressure (mm Hg)</td>
<td>34.7 ± 3.5 (22)</td>
<td>&lt;0.001</td>
<td>40.2 ± 4.2 (11)</td>
<td>&lt;0.001</td>
<td>18.8 ± 5.1 (19)</td>
<td>&lt;0.001</td>
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<tr>
<td>Proximal intratubular pressure (mm Hg)</td>
<td>11.5 ± 1.6 (22)</td>
<td>&lt;0.001</td>
<td>31.2 ± 5.3 (11)</td>
<td>&lt;0.001</td>
<td>9.2 ± 2.5 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distal intratubular pressure (mm Hg)</td>
<td>5.3 ± 1.4 (4)</td>
<td>&lt;0.005</td>
<td>16.4 ± 5.9 (9)</td>
<td>&lt;0.001</td>
<td>7.8 ± 2.3 (9)</td>
<td>NS</td>
</tr>
<tr>
<td>Star vessel pressure (mm Hg)</td>
<td>13.9 ± 2.8 (7)</td>
<td>&lt;0.01</td>
<td>20.5 ± 5.6 (11)</td>
<td>&lt;0.001</td>
<td>8.6 ± 2.5 (15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peritubular capillary pressure (mm Hg)</td>
<td>9.3 ± 2.8 (4)</td>
<td>&lt;0.025</td>
<td>13.5 ± 2.1 (8)</td>
<td>&lt;0.001</td>
<td>6.0 ± 1.1 (8)</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Femoral arterial blood pressure (mm Hg)</td>
<td>111 ± 11 (22)</td>
<td>&lt;0.025</td>
<td>100 ± 11 (11)</td>
<td>&lt;0.005</td>
<td>116 ± 12 (19)</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit (ml/100 ml)</td>
<td>55 ± 3 (17)</td>
<td>NS</td>
<td>54 ± 3 (7)</td>
<td>&lt;0.001</td>
<td>47 ± 4 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
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<tr>
<td>Left</td>
<td>0.91 ± 0.18 (10)</td>
<td>&lt;0.025</td>
<td>1.16 ± 0.24 (10)</td>
<td>NS</td>
<td>1.04 ± 0.17 (15)</td>
<td>NS</td>
</tr>
<tr>
<td>Right</td>
<td>0.95 ± 0.18 (10)</td>
<td>NS</td>
<td>0.93 ± 0.18 (10)</td>
<td>NS</td>
<td>1.03 ± 0.18 (15)</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>244 ± 42 (22)</td>
<td>NS</td>
<td>242 ± 45 (11)</td>
<td>NS</td>
<td>250 ± 46 (19)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± 1 sd. The number of rats tested is given in parentheses. P< indicates the significance of comparisons between values 1–3 hours after occlusion and control values, P< indicates the significance of comparisons between values 1–3 hours after occlusion and those 22–26 hours after occlusion, and P< indicates the significance of comparisons between values 22–26 hours after occlusion and control values. NS = not significant.

rats studied 1–3 hours after occlusion (Table 2). Use of the indirect method enabled us to sample a larger population of nephrons and to estimate glomerular capillary pressure in rats whose kidneys did not possess glomeruli accessible to micropuncture. Directly measured glomerular capillary pressure 1–3 hours after restoration of renal blood flow was not statistically different from the control mean, 45 ± 4 mm Hg, although estimated glomerular capillary pressure and stop-flow hydrostatic pressure were slightly higher in a larger group of rats. Thus, glomerular capillary pressure was essentially unchanged or slightly elevated at a time when renal vascular resistance was doubled, suggesting that afferent and efferent arteriolar vascular resistances increased in parallel. Based on the hydrostatic pressure in the surface peritubular capillaries, there was also an increase in resistance in the intrarenal venous system. Afferent effective filtration pressure was markedly reduced as a consequence of elevated hydrostatic pressure in proximal convolutions and in Bowman’s space. In the presence of an unchanged or slightly higher glomerular capillary pressure, sluggish flow of tubular fluid and a pronounced increase in hydrostatic pressure in proximal convolutions and Bowman’s space clearly indicate enhanced resistance to tubular fluid outflow.

22–26 HOURS AFTER OCCLUSION

The kidney surface viewed microscopically in vivo 22–26 hours after occlusion was appreciably changed; it appeared pale with a marked reduction in peritubular capillary perfusion. Renal blood flow measured by an electromagnetic flow transducer was approximately 45% of preocclusion flow, and renal vascular resistance had increased about threefold (Table 1). Under low magnification (64x), the surface tubules often appeared opaque and "collapsed," without readily discernible lumens. Under higher magnification (160x), however, these convolutions had narrow, slitlike lumens. The tubular epithelial cells were swollen and appeared otherwise normal. Few of the tubu-
lar lumens were dilated. The tubular epithelium was homogeneous and necrotic; this change was widespread and diffuse throughout the cortex. Most tubule lumens were occluded by debris from desquamated epithelial cells. The epithelium that remained intact displayed a great deal of vacuolization. There was general congestion of the vasculature, and some red blood cells were noted in tubules. Marked interstitial inflammatory cell infiltrate was evident.

Urine flow from the experimental kidney was extremely reduced and was statistically lower than that recorded 1–3 hours after occlusion (Table 2). Kidney weight was not different from that for the experimental kidney during the control period or 1–3 hours after occlusion or from that for the contralateral kidney at any of the observation times.

Proximal intratubular and postglomerular vessel hydrostatic pressures had fallen appreciably from the elevated levels observed 1–3 hours after occlusion to values below the means before occlusion (Table 2). In contrast, distal intratubular pressure was less than the level 1–3 hours after occlusion but slightly above that found in the period before occlusion; it approximated proximal intratubular pressure. Most of the values for proximal intratubular pressure, although more heterogeneous, were grouped below and within the control range (Fig. 3), but they were considerably lower than those at 1–3 hours. Even though some overlap at the extremes does exist, it is readily apparent that the population of proximal intratubular pressure values measured 22–26 hours after occlusion was distinctly different from that observed at 1–3 hours.

The summarized results of intrarenal pressure measurements for this group are presented in Table 2. In comparison with the control means, substantial reductions were observed in glomerular capillary pressure, stop-flow pressure, and afferent effective filtration pressure. Glomerular capillary pressure and stop-flow hydrostatic pressure were also significantly less than those recorded 1–3 hours after occlusion, whereas afferent effective filtration pressure was similar to the value 1–3 hours after occlusion. The decline in glomerular capillary pressure was the principal factor responsible for the pronounced and maintained reduction in afferent effective filtration pressure at 22–26 hours, since proximal intratubular pressure and hydrostatic pressure in Bowman’s space were no longer elevated. At this time, they had fallen below the control level. The abnormally low glomerular capillary pressure and renal blood flow at a time when arterial blood pressure was normal indicates enhanced preglomerular vascular resistance, at least in the superficial nephrons.

Following microinjections of small volumes of dye solution into proximal convolutions, performed without increasing intraluminal pressure more than 2 mm Hg, some of the dye was observed to pass radially from the lumen, creating a “halo” effect as it was rapidly swept away by the peritubular circulation.

To determine the relative importance of increased renal vascular resistance, reduced glomerular capillary pressure, and intratubular obstruction in the maintenance phase of the oliguria at 24 hours, a group of rats was acutely volume expanded after the base-line observations had been made. Coincident with the progressive improvement in

* We have previously shown (6) that subjecting rats to anesthesia and surgery comparable to that used on the rats studied 22–26 hours after renal artery occlusion has no detectable effect 24 hours later on arterial or intrarenal hydrostatic pressures. These results serve as time-control data for the present experiments. They indicate that the changes observed 24 hours after renal artery occlusion are a consequence of the period of complete renal ischemia and do not represent the delayed effects of the previous anesthesia, surgery, etc.
renal blood flow during the 30-minute equilibration period, peritubular capillary perfusion became more rapid and more fluid was filtered into proximal convolutions so that they became dilated in contrast to the narrow lumens observed before expansion. The kidneys increased in size and became more tense. Oil droplets injected into the lumens of proximal convolutions flowed downstream at rates roughly proportional to the measured afferent effective filtration pressure.

The acute volume expansion produced impressive increases in renal blood flow and estimated glomerular capillary pressure (Table 3), reflecting a significant reversal in the overall and the preglomerular vascular resistance. Glomerular capillary pressure was restored to normal, and renal blood flow rose above the control mean. Even though proximal intratubular pressure increased twofold and rose above normal, the damaged kidneys failed to increase urine output, remaining severely oliguric. In contrast, the undisturbed contralateral kidneys became diuretic. There were also significant increases in stop-flow, afferent effective filtration, and peritubular capillary hydrostatic pressures.

**Discussion**

We believe that the factors responsible for the oliguria of acute renal failure should be categorized under two headings: those dealing with its generation and those involved in its maintenance. For this reason, we conducted a series of experiments 1-3 hours and 22-26 hours after a 1-hour episode of complete ischemia. In addition, in the latter group we studied the effect of acute volume expansion to better define the importance of the several simultaneously operative mechanisms responsible for the oliguria.

Immediately after removal of the occluding clamp, renal blood flow rapidly returned to about 50% of normal and the previously collapsed proximal convolutions filled with fluid, indicating the resumption of glomerular filtration. At 1–3 hours after release of the renal artery occlusion, most of the proximal convolutions were grossly dilated; microinjected oil droplets and filtered dye flowed very slowly through the proximal lumens. Proximal intratubular pressure was elevated and was more heterogeneous than normal, averaging 31 ± 5 mm Hg, and distal intratubular pressure was also higher and more variable than normal, averaging 16 ± 6 mm Hg. These observations demonstrate increased resistance to tubular outflow at the loop of Henle and more distal sites. In view of histological evidence of intratubular casts and debris and an increased pressure gradient from the proximal lumen to the peritubular capillary, we conclude that this increased resistance was due to intraluminal obstruction and not to tubular compression. The fact that such a high intratubular pressure developed suggests that abnormal tubular permeability was not a major cause of the oliguria at 1–3 hours.

Total renal vascular resistance was doubled at 1–3 hours, and there were increases in both pre- and postglomerular resistances of superficial nephrons, since glomerular capillary pressure was unchanged. Thus, tubular obstruction in the presence of normal glomerular capillary pressure was responsible for the observed decrease in afferent filtration.

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
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<tbody>
<tr>
<td><strong>Effect of Acute Volume Expansion on Renal Blood Flow, Intrarenal Hydrostatic Pressures, and Urine Flow at 22–26 Hours after Occlusion</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Before expansion</th>
<th>Volume expansion</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Renal blood flow (ml/min)</strong></td>
<td>2.6 ± 0.7</td>
<td>7.3 ± 2.8</td>
<td>6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Renal vascular resistance (mm Hg/ml min</strong></td>
<td>48.6 ± 12.1</td>
<td>18.5 ± 8.7</td>
<td>6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Estimated glomerular capillary pressure (mm Hg)</strong></td>
<td>30.2 ± 3.3</td>
<td>45.1 ± 8.5</td>
<td>6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Stop-flow pressure (mm Hg)</strong></td>
<td>18.0 ± 3.3</td>
<td>31.3 ± 5.9</td>
<td>6</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td><strong>Proximal intratubular pressure (mm Hg)</strong></td>
<td>9.2 ± 2.6</td>
<td>18.1 ± 6.0</td>
<td>6</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td><strong>Afferent effective filtration pressure (mm Hg)</strong></td>
<td>9.5 ± 1.6</td>
<td>15.3 ± 3.6</td>
<td>6</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td><strong>Urine flow, left kidney (μlitters/min)</strong></td>
<td>0.05 ± 0.11</td>
<td>0.12 ± 0.14</td>
<td>6</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Urine flow, right kidney (μlitters/min)</strong></td>
<td>4.6 ± 0.3</td>
<td>58.7 ± 21.0</td>
<td>3</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td><strong>Star vessel pressure (mm Hg)</strong></td>
<td>8.5 ± 1.5</td>
<td>14.3 ± 5.2</td>
<td>4</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Peritubular capillary pressure (mm Hg)</strong></td>
<td>6.7 ± 0.8</td>
<td>13.8 ± 1.7</td>
<td>3</td>
<td>&lt; 0.06</td>
</tr>
<tr>
<td><strong>Femoral arterial blood pressure (mm Hg)</strong></td>
<td>126 ± 7</td>
<td>119 ± 11</td>
<td>6</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Hematocrit (ml/100 ml)</strong></td>
<td>48 ± 4</td>
<td>34 ± 5</td>
<td>6</td>
<td>&lt; 0.005</td>
</tr>
</tbody>
</table>

Values are means ± 1 sd. Body weight averaged 243 ± 23 g (N = 6). N = number of rats tested, and ns = not significant.
effective filtration pressure. Although we made no attempt to quantify superficial single nephron glomerular filtration rate because of technical problems (the difficulty of collecting tubular fluid without lowering the elevated pressure in Bowman’s space of obstructed tubules and the possibility of transtubular inulin leakage), the very sluggish flow of tubular fluid suggests that it was significantly decreased. Continued filtration at some undetermined rate in all regions of the kidney is indicated by the presence of Prussian blue precipitate in all microdissected proximal convoluted tubules of superficial, midcortical, and juxtamedullary nephrons after intra-aortic injections of sodium ferrocyanide (C. R. Morris, unpublished observations).

The findings were strikingly different 22–26 hours after the release of the renal artery clamp. Renal blood flow remained approximately 50% of normal and severe oliguria continued, but the measured intrarenal hydrostatic pressures had fallen to normal or below normal. The surface convolutions were collapsed with narrow lumens, and intraluminal debris was frequently observed. Glomerular capillary pressure had fallen to half of the normal value. Proximal intratubular and peritubular vascular pressures were also less than normal, whereas distal intratubular pressure had returned to the normal range. These findings indicate failure of filtration due to marked preglomerular vasoconstriction.

The only overt evidence of tubular obstruction at this time was the relatively high distal intratubular pressure and the luminal casts and debris evident in vivo and histologically. The latter might be considered secondary to a primary failure of filtration were it not for the clear-cut evidence of functional tubular obstruction as a primary disturbance immediately following the release of the renal artery clamp. The findings after extracellular fluid volume expansion, however, clearly demonstrate the functional significance of the persisting tubular obstruction. With acute volume expansion, there was a rapid reversal of the intrarenal vasoconstriction. Renal blood flow and glomerular capillary pressure returned to normal, and most surface convolutions again filled with tubular fluid and became dilated; yet, the experimental kidneys remained severely oliguric. Proximal intratubular pressure increased above control values but was not as high as it was at 1–3 hours. We suspect that the increase in intratubular pressure was limited by abnormal tubular permeability, passive back diffusion, and the inability to develop and maintain a large transtubular pressure gradient across the obviously necrotic tubular epithelium with high hydraulic conductivity. Although we cannot quantify its importance as a cause of the oliguria, the tubular epithelium was abnormally permeable: dye microinjected with little or no increase in intratubular pressure passed across the tubular epithelium. This phenomenon never occurs normally. A decrease in glomerular permeability with a consequent reduction in glomerular filtration rate could also limit the rise in intratubular pressure even in obstructed tubules. Parsimony, however, leads us to conclude at this time that a large increase in tubular and not a decrease in glomerular permeability was probably the major cause.

Cox et al. (10) have recently suggested that alterations in glomerular permeability following prolonged administration of norepinephrine may cause oliguria in the presence of normal renal blood flow. Moreover, Blantz (11) has reported a decrease in the ultrafiltration coefficient (Kf) as well as extensive tubular leakage of inulin in rats after administration of uranyl nitrate. Daugherty et al. (12), also using direct methodology, found no evidence of a change in Kf after 3 hours of nearly complete occlusion of the renal artery in Munich-Wistar rats, and the tubules remained impermeable to inulin. Glomerular filtration pressure equilibrium continued after ischemic injury, and single nephron glomerular filtration rate was reduced in proportion to glomerular plasma flow. Glomerular capillary pressure was unchanged, indicating proportionate increases in afferent and efferent arteriolar resistance, as appeared to be the case in our studies 1–3 hours postocclusion.

Although we made no measurements of Kf in our rats, it seems quite clear that a decrease in glomerular permeability could not be the major cause of their oliguria. Decreased Kf may or may not have been present as an additional factor. Even if Kf were decreased at 1–3 hours, this decrease could not have been the primary cause of the oliguria at that time since the obstructed tubules were distended with filtered fluid. Nor is it credible that it was the sole cause at 24 hours, when glomerular capillary pressure was low and renal vascular resistance was quite high in the absence of extracellular fluid volume expansion. After acute volume expansion, the tubular lumens again became distended with fluid as renal blood flow and glomerular capillary pressure increased, indicating tubular obstruction and not failure of filtration. Recognizing that glomerular filtration rate can be plasma flow limited under normal circumstances, in our experiments the glomerular filtration rate was limited primarily by increased pressure in Bow-
man's space 1–3 hours after the period of ischemia and by decreased pressure in the glomerular capillaries at 24 hours before volume expansion.

Tanner et al. (13) have also reported tubular obstruction with high proximal intratubular pressure in rats immediately after 1 hour of total renal artery occlusion. They thought that tubular leakiness, demonstrated by microinjections of inulin, was the other major cause of the acute renal failure so produced. The measured superficial nephron glomerular filtration rate was 70% of normal, but as the authors state this value may "exaggerate" the filtration rate prior to collection of tubular fluid from tubules with increased proximal intratubular pressure. Urine flow from their damaged kidneys was increased, not decreased as it was in our experiments. Since the experimental protocols were so similar, the reason for this difference is not apparent.

The so-called "no reflow" phenomenon, the failure of the circulation to return to normal after an episode of temporary vascular obstruction, has been investigated, mainly with morphological techniques, in several studies of the rat kidney (14–16). Flores et al. (15) have postulated that after removal of the initial cause of the ischemia a self-sustaining cycle is established of swelling of ischemia-anoxia-damaged endothelial cells of small renal vessels leading to compromise of the vascular lumen with a decrease in renal blood flow. Administration of hypertonic mannitol solution is beneficial under these circumstances (15, 16), a fact used in support of the hypothesis. In addition, or alternatively, it appears to us that the beneficial effect of hypertonic mannitol might result from a vasodilator action on larger vessels or a shrinkage of tubular epithelial cells. In contrast to the morphological findings of Flores et al. (15), our functional data at 24 hours demonstrate that acute volume expansion with isotonic or isoncotic solutions produces impressive reversals in renal blood flow, renal vascular resistances, and intrarenal hydrostatic pressures by some mechanism(s) other than an osmotic effect.

Failure of filtration due to preglomerular vasoconstriction has been reported most often as the major cause of oliguria in clinical and experimental acute renal failure, leading Oken (17) to propose the name "vasomotor nephropathy." Nevertheless, tubular obstruction has been reported in studies of other models of acute renal failure. Jaenike (18) has found that tubular obstruction with increased proximal intratubular pressure is a prominent feature 24 and 48 hours after intravenous injection.

**FIGURE 4**

Schematic representation of the basic mechanisms thought to be involved in the pathogenesis of oliguric acute renal failure induced experimentally by 1 hour of complete ischemia. This synthesis of our observations at 1–3 and 22–26 hours after occlusion is an attempt to pictorially correlate the sequence of intrarenal events and their possible interaction and relative importance in the genesis and maintenance of oliguria.
of methemoglobin. In some nephrons tubular pressure and flow are low, suggesting a failure of filtration, and in still others both obstruction and failure of filtration appear to coexist. Schmidt and his colleagues (19) have reported that oliguria in acute renal failure induced by the administration of folic acid to rats results from tubular obstruction and that intrarenal hemodynamics are unchanged. Evidence of tubular obstruction with normal, rather than elevated, intratubular pressure in rats 24 hours after low-dose mercury poisoning was found by Flamenbaum et al. (20), suggesting to them that effective filtration pressure was probably grossly reduced. Yet, despite slow flow in the indisturbed nephron, tubular fluid could be collected from proximal convolutions at a normal rate, and single nephron glomerular filtration rate was measured to be approximately 80% of normal. The later observation and the sustained normal single nephron glomerular filtration rate and free tubular flow in some nephrons after the tubular obstruction had been relieved by washout of debris suggested to these investigators "a feedback mechanism between impairment of tubular flow and intraglomerular filtration pressure."

The cause of the delayed development of a predominant increase in pregglomerular vascular resistance in our experiments remains obscure. Of great interest in this regard, in addition to the observations of Flamenbaum et al. (20), are our observations (6) on obstructed but otherwise normal kidneys and nephrons. Complete unilateral ureteral ligation for 24 hours caused a reduction in estimated glomerular capillary pressure of surface nephrons in the obstructed kidney. In kidneys without ureteral ligation, a similar response occurred in individual nephrons obstructed with viscous oil for 24 hours, but nearby unblocked tubules were not affected. Thus, this delayed response to tubular obstruction occurs on an individual nephron basis and presumably results from obstruction of individual afferent arterioles. The responsible mechanism has not been identified, but it may well be the same as that in the present experiments on kidneys with ischemic acute renal failure. One or more mechanisms may be brought into play by prolonged obstruction to tubular flow. Activating a tubuloarteriolar feedback system, per- mitting increased renal vascular resistance in the ischemia model may be related to the presence of a vasoconstrictor, the absence of a vasodilator, or combination of both. The resulting constriction of afferent arterioles might initiate a secondary cycle of reduced renal blood flow and glomerular capillary pressure leading to a further reduction in distal delivery.

From the present study of postischemic acute renal failure at two different times after 1 hour of unilateral renal artery occlusion, it is evident that tubular obstruction is a pivotal event and plays a key role in both the generation and the maintenance of oliguria in this model of acute renal failure. Immediately after release of the occluding clamp, tubular obstruction is an obvious feature, since the tubules become dilated and develop high intratubular pressures. The severe oliguria 24 hours after the insult results from a combination of persisting luminal obstruction, intrarenal vasomotor changes, and passive backflow. Although not systematically evaluated, other factors may also contribute. Whatever the precise mechanism(s) involved, we believe the sequence of events outlined schematically in Figure 4 presents our observations in a clarifying and unifying manner; namely, the figure illustrates that the relative importance of the factors responsible for the development and maintenance of oliguria depends on the evolution of the underlying disease process. Indeed, whether a similar sequence of events may have gone unrecognized in other experimental models of oliguric acute renal failure remains to be determined.

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