Intrarenal Vascular Resistance in Glycerol-Induced Acute Renal Failure in the Rat

CHEN H. HSU, THEODORE W. KURTZ, AND CHRISTINE E. SANDS

SUMMARY We measured mean afferent arteriolar diameter and renal blood flow with microsphere techniques in awake rats 24 hours after induction of acute renal failure by glycerol injection. Renal blood flow, mean arterial pressure, and total renal vascular resistance in rats with acute renal failure were not different from control levels, despite significantly elevated serum urea nitrogen. Mean afferent arteriolar diameter was decreased in rats with acute renal failure compared to controls (21.1 ± 0.10 vs. 22.0 ± 0.16 μm, mean ± SE, n = 5, respectively, P < 0.01). The percentage of renal intravascular microspheres (mean diameter of spheres = 22.3 ± 2.1 μm) entering the glomeruli also was decreased in rats with acute renal failure compared to control (75.9 ± 1.4 vs. 85.7 ± 0.8%, n = 7, respectively, P < 0.001). Blood viscosity values of rats injected with glycerol and measured at shear rates of 61.8/sec (4.36 ± 0.06 centipoise (cP, n = 6) and 123.6/sec [3.78 ± 0.05 cP, n = 6] were significantly higher than those of controls (3.84 ± 0.08 cP, n = 6, P < 0.005, and 3.36 ± 0.09 cP, n = 6, P < 0.005). Therefore, preglomerular resistance is increased during glycerol-induced acute renal failure due to a combination of afferent arteriolar vasoconstriction and increased blood viscosity. The increase in preglomerular resistance will cause a reduced glomerular capillary hydrostatic pressure in glycerol-induced acute renal failure. Circ Res 45: 583-587, 1979

A SUBSTANTIAL number of studies have demonstrated that a decreased glomerular filtration rate (GFR) is not necessarily associated with impaired renal perfusion in acute renal failure (Churchill et al., 1977; Eisenbach et al., 1974; Hsu et al., 1977; Kurtz et al., 1976; Stein et al., 1975). These studies contrast sharply with earlier reports documenting a relationship between decreased GFR and decreased renal blood flow (RBF) in acute renal failure (Ayer et al., 1971; Chedru et al., 1972; Flamenbaum et al., 1972; Flamenbaum et al., 1974). Initially, preglomerular vasoconstriction was suggested to be the hemodynamic mechanism responsible for the impaired GFR (Chedru et al., 1972; Ruiz-Guinazu et al., 1975). The recent demonstration of normal renal blood flow in acute renal failure, however, does not preclude this concept, provided that postglomerular resistance decreases simultaneously with an increase in afferent arteriolar resistance. Thus, preglomerular vasoconstriction is still a viable hemodynamic explanation for the decreased GFR of acute renal failure. To test this hypothesis, we measured afferent arteriolar diameter in glycerol-induced acute renal failure, using a recently developed microsphere method (Chenitz et al., 1976; Ishikawa and Hollenberg, 1977; Ofstad et al., 1975).

Methods

Male Sprague-Dawley rats, weighing 200-240 g, were given Purina rat chow and tap water ad libitum. Acute renal failure was induced by hindlimb intramuscular injection of 50% glycerol, 1 ml/100 g, after 15 hours of dehydration. Water was freely available thereafter. Controls were injected with equivalent volumes of saline.

Renal blood flow was measured 24 hours after glycerol injection and in normal control rats. The rats were weighed and lightly anesthetized with ether. The femoral artery was cannulated with polyethylene tubing (PE 10) for blood collection, and the left ventricle was cannulated via the carotid artery for injection of microspheres. After surgery, rats were placed in restraining cages and allowed to recover for at least 1 hour prior to injection. Approximately 0.1 ml of a 10% dextran solution, containing 85Sr microspheres (15 ± 5 μm) at a concentration of 2 mg/ml, was injected into the carotid catheter within 10-15 seconds. The femoral catheter was opened simultaneously, and blood flowed freely into a preweighed tube for exactly 1 minute. About 0.1-0.2 ml of blood was collected from the femoral artery in this fashion as a reference blood sample. Just prior to injection, the spheres were sonically disrupted for 10 minutes and vigorously agitated with a vortex mixer for at least 5-10 minutes. Approximately 60,000 microspheres were administered in each injection. The details of the procedure have been described previously (Hsu et al., 1975). Prior to the injection of the microspheres, mean arterial pressure (MAP) was measured through the femoral artery catheter using a Gilson
solution of alcian blue for 15 minutes. The restained slice was soaked in xylene to remove the paraplast, hydrated, and restained by immersion in a 2% terioles are blocked by microspheres, each tissue glomerular capillaries or vessel walls when the ar-

Kidneys were removed, decapsulated, and counted for radioactivity (Packard y Counter). Blood samples collected after the injection of microspheres also were counted and weighed. The blood volume was calculated by dividing the weight of the blood sample by the specific gravity of rat blood, which previously had been determined to be 1.107 ± 0.001 g/ml. RBF, which represents the combined RBF of both kidneys, was calculated as follows: RBF = [Two kidneys (counts/min)]/[Femoral blood (counts/min)] × Femoral blood flow rate (ml/min per 100 g body weight). Renal vascular resistance (RVR) was estimated by: RVR = MAP/RBF.

Afferent arteriolar diameter was assessed with microspheres in separate groups of rats, 24 hours after glycerol injection and in controls. The method had been described previously (Chenitz et al., 1976; Ishikawa and Hollenberg, 1977; Ofstad et al., 1975). In theory, any microsphere with a diameter smaller than that of an afferent arteriole will pass through the vessel and be trapped in a glomerulus. Conversely, if the sphere is larger than the arteriole, it will be lodged outside the glomerulus. Thus, the frequency distribution of the various sizes of microspheres trapped within the afferent arterioles can be used to estimate afferent arteriolar diameter.

The left ventricle of each animal was cannulated through the right carotid artery. The rats were allowed to recover as previously described. Approximately 100,000 nonradioactive microspheres [22.3 ± 2.1 μm (SD) in diameter, 3M Company] in 0.15 ml of a 10% dextran solution were injected within 15 seconds through the carotid catheter. After withdrawal of 2 ml of blood for measurement of SUN, 0.5 ml of 2% alcian blue was infused slowly over several minutes. The rats were killed immediately with saturated KCl solution, administered iv, and the kidneys were removed, decapsulated, serially exposed to increasing concentrations of ethanol, and cleared with methylsalicylate. The kidneys then were embedded in Paraplast (Sherwood Medical, Inc.) prior to slicing with a microtome (American Optical) was used to measure the diameters of at least 150 microspheres trapped within clearly identifiable afferent arterioles in each kidney. Microspheres trapped at the junctions of afferent arterioles with glomerular capillary loops (hilar position) were included as well, since data from the study of Morkrid et al. (1978) have shown that these spheres have similar diameters to spheres identified proximal to hilar locations. With these data, a histogram was constructed which depicted the diameter distribution of the trapped microspheres for each rat (Fig. 1). Mean afferent arteriolar diameter then was calculated by using these diameter distributions according to the method of Ofstad et al. (1975). This method is based on a mathematical model which assumes that three main factors determine the diameter distribution of microspheres trapped within afferent arterioles.

A micrometer eyepiece with an accuracy of 0.2 μm (American Optical) was used to measure the diameters of at least 150 microspheres trapped within clearly identifiable afferent arterioles in each kidney. Microspheres trapped at the junctions of afferent arterioles with glomerular capillary loops (hilar position) were included as well, since data from the study of Morkrid et al. (1978) have shown that these spheres have similar diameters to spheres identified proximal to hilar locations. With these data, a histogram was constructed which depicted the diameter distribution of the trapped microspheres for each rat (Fig. 1). Mean afferent arteriolar diameter then was calculated by using these diameter distributions according to the method of Ofstad et al. (1975). This method is based on a mathematical model which assumes that three main factors determine the diameter distribution of microspheres trapped within afferent arterioles.

RVR = MAP/RBF.

FIGURE 1 The frequency distribution of various sizes of microspheres within the afferent arterioles of two representative rats. ARF = acute renal failure.
These factors are (1) the diameter distribution of the injected microsphere population, (2) the diameter distribution of the afferent arterioles, and (3) afferent arteriolar blood flows which are proportional to the fourth power of the afferent arteriolar diameters. Since the diameter distribution of the injected microspheres is known a priori and the diameter distribution of trapped microspheres is determined experimentally, one can derive the diameter distribution of the afferent arterioles and hence, mean afferent arteriolar diameter. The mathematical details of this approach have been outlined by Ofstad et al. (1975).

Since blood viscosity is also a major determinant of intravascular resistance, a cone plate viscometer (Brookfield Engineering Lab) was used to measure blood viscosity in additional controls and in rats with acute renal failure. Blood was tested at shear rates of 61.8/sec and 123.6/sec, since a high shear rate will approximate more closely the condition of blood flow in small blood vessels (Rosenblum, 1977). The viscosity measurements were conducted at a heating jacket temperature of 37°C. Body temperatures of rats with glycerol-induced acute renal failure and controls were not different and ranged from 37°C to 38°C. Other factors that influence viscosity also were measured, including hematocrit, total plasma protein (Biuret method), albumin, serum protein electrophoresis (cellulose acetate method), and triglycerides (Kessler and Lederer, 1965). In addition, the blood of rats with glycerol-induced acute renal failure was tested for the presence of glycerol with a gas chromatograph-mass spectrometer (5840 A gas chromatograph, Hewlett-Packard). All data are expressed as mean ± SEM. Student’s t-test was used for statistical analysis.

**Results**

Values for RBF, MAP, RVR, and SUN of controls and of rats with acute renal failure are summarized in Table 1. Acute renal failure was evident in glycerol-injected animals, as SUN levels were significantly elevated compared to controls. RBF and MAP decreased slightly and calculated RVR increased slightly, but these were not statistically significant changes from the levels in the control group.

The percentage of microspheres entering the glomeruli and estimated mean afferent arteriolar diameters are presented in Table 2.

**Table 2 Percentage of Microspheres Entering Glomeruli, Mean Afferent Arteriolar Diameter, and SUN 24 Hours after Glycerol Injection**

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal</th>
<th>Glycerol-ARF</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of microspheres</td>
<td>n = 7</td>
<td>85.7 ± 0.8, P &lt; 0.001</td>
</tr>
<tr>
<td>entering glomeruli</td>
<td></td>
<td>75.9 ± 1.4</td>
</tr>
<tr>
<td>Mean afferent arteriolar diameters (μm)</td>
<td>n = 5</td>
<td>22.0 ± 0.16, P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.1 ± 0.10</td>
</tr>
<tr>
<td>SUN (mg/100 ml)</td>
<td>n = 7</td>
<td>19.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>83.4 ± 21.6, P &lt; 0.001</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

*P < 0.001; †P < 0.01.

The percentage of microspheres entering glomeruli for rats with glycerol-induced acute renal failure were significantly lower than those of normal controls. The mean afferent arteriolar diameter of those with glycerol-induced acute renal failure (21.1 ± 0.10 μm, n = 5) also was significantly smaller than the control value (22.0 ± 0.16, P < 0.01, n = 5).

Blood viscosity, total serum protein, triglyceride, and hematocrit levels 24 hours after glycerol injection are presented in Table 3. The mean blood viscosity values (centipoise, cP) of rats with glycerol-induced acute renal failure at shear rates of 61.8/sec (4.36 ± 0.06 cP, n = 6) and 123.6/sec (3.78 ± 0.05 cP, n = 6) were significantly higher than those of controls (61.8/sec, 3.84 ± 0.08 cP, n = 6, P < 0.005, and 123.6/sec, 3.36 ± 0.09 cP, n = 6, P < 0.005). This occurred despite the fact that the mean hematocrit of rats injected with glycerol (35.3 ± 1.2, n = 6) was significantly lower than that of controls (42.2 ± 0.6, n = 6, P < 0.005). The total serum protein of rats with acute renal failure (5.9 ± 0.11 g/100 ml, n = 6) was slightly higher than that of controls (5.4 ± 0.07 g/100 ml, n = 6, P < 0.01), and this may have contributed slightly to the difference in blood viscosity. The increase in protein was due primarily to increases in globulin fractions, since albumin concentrations were similar in both groups (control, 2.75 g/100 ml; acute renal failure, 2.74 g/100 ml). The cause of the elevated viscosity in rats with acute renal failure could not be attributed to circulating glycerol, since none was detected by chromatographic analysis. Serum triglyceride values were not different between controls (102.0 ± 12.1 mg/100 ml) and experimental rats (97.7 ± 11.4 mg/100 ml).

**Discussion**

Tubular obstruction, decreased glomerular ultrafiltration coefficient (Blantz, 1975), tubular leakage,
and alterations in renal hemodynamics all have been implicated as possible causes of the oliguria in acute renal failure. Of these, the hemodynamic theories have received the most attention, particularly since early studies demonstrated impaired renal perfusion during acute renal failure. With the advent of more accurate and reliable methods for measurement of renal blood flow, however, it has become apparent that total renal blood flow may be dissociated from changes in glomerular filtration in many forms of acute renal failure (Churchill et al., 1977; Eisenbach et al., 1974; Hsu et al., 1977; Stein et al., 1975). An intrarenal hemodynamic mechanism that could account for decreased glomerular filtration in the presence of normal renal perfusion involves opposite changes in afferent and efferent arteriolar resistances. Assuming constancy of the other factors affecting filtration, postglomerular vasoconstriction, with an attendant fall in glomerular capillary hydrostatic pressure and filtration without decreasing RBF. The observation of collapsed tubules and low efferent arteriolar pressures in methemoglobin-induced acute renal failure (Ruiz-Gui-azu et al., 1975) is compatible with this hypothesis. In addition, Reubi et al. (1973) have indicated also that the lack of correlation of GFR with relatively well preserved RBF in humans with acute renal failure may be due to a combination of afferent vasoconstriction and efferent vasodilation.

To test this hypothesis more directly, we used a recently developed microsphere technique to assess in vivo afferent arteriolar diameter in a model of acute myohemoglobinuric renal failure, characterized by normal renal blood flow (Churchill et al., 1977; Kurtz et al., 1976). We employed a slightly modified version of the microsphere technique reported by Ishikawa and Hollenberg (1977) in conjunction with the mathematical approach to calculating afferent arteriolar diameter developed by Ofstad et al. (1975). The mean afferent arteriolar diameter of normal rats in our study (22.0 μm) was fairly close to that obtained by Ishikawa and Hollenberg (1977) in rats (23.7 μm) but substantially higher than that reported by Ofstad et al. (1975) for the dog (16.3 μm). This discrepancy probably is due to differences in method. Our tissue preparation technique was different in that section thickness in our study was 120 μm, whereas 35-μm thick sections were made by Ofstad’s group. It is likely that such a thin tissue slicing technique will result in sectioning of the microspheres as well, thereby shifting the visible sphere diameters toward lower values. In addition, the number of microspheres examined by Ofstad et al. (1975) is substantially less than ours, and the accuracy of the diameter measurement increases with the number of microspheres examined. As mentioned, the results of this study are quite similar to those obtained by Ishikawa and Hollenberg (1977) in the rat using similar methods but a different mathematical approach to estimating afferent arteriolar diameter. The calculation of Ishikawa and Hollenberg does not correct for the diameter distribution of the injected microspheres or for variations in afferent arteriolar blood flow within the kidney. When our data are treated according to their method, a value for afferent arteriolar diameter of 23.5 μm is obtained, which is nearly identical to the figure of 23.7 μm that they have reported (Ishikawa and Hollenberg, 1977).

Our results demonstrate that the afferent arteriolar diameters of rats with glycerol-induced acute renal failure are significantly lower than those of control rats. In addition, the percentages of microspheres entering glomeruli in normal rats are significantly greater than in those with acute renal failure. Therefore, one can conclude that preglomerular vasoconstriction occurs in this model of acute renal failure. To relate the alteration in vessel diameter to the impaired glomerular filtration in glycerol-induced acute renal failure, evaluation of segmental vascular resistance changes and their influence on glomerular capillary hydrostatic pressure is required. Assuming that intrarenal blood flow is laminar and that vessel length is constant, the increase in preglomerular resistance can be estimated using the Poiseuille equation \[ R_a = \frac{8 V}{\pi r^4} \] , where \( R_a \) = afferent arteriolar resistance, \( V \) = blood viscosity, \( L \) = vessel length, and \( r \) = afferent arteriolar radius. Since vessel resistance is related to the fourth power of the radius, the small absolute decrease in afferent arteriolar diameter of rats with glycerol-induced acute renal failure is sufficient by itself to increase preglomerular resistance by 15%. A quantitative estimate of the increase in vessel resistance caused by increased blood viscosity in glycerol-induced acute renal failure is not possible if based only on whole blood viscosity measurements. This is due to a myriad of complicating factors (Cokelet, 1976) including the fact that, as vessel diameter approaches red cell diameter, the deformation of cells will alter blood viscosity. In addition, viscosity will vary within a blood vessel since hematocrit varies with radial position in vessels smaller than 300 μm. Finally, the Fähræus-Lindquist effect of decreasing viscosity with decreasing vessel size also exists, although the small absolute difference in vessel diameters between

### Table 3: Blood Viscosity, Total Serum Protein, Triglyceride, and Hematocrit, 24 Hours after Glycerol Injection

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal</th>
<th>Glycerol ARF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood viscosity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shear rate 61.8/sec (cP)</td>
<td>3.84 ± 0.08</td>
<td>4.36 ± 0.06*</td>
</tr>
<tr>
<td>Shear rate 123.6/sec (cP)</td>
<td>3.36 ± 0.09</td>
<td>3.84 ± 0.08*</td>
</tr>
<tr>
<td>Total serum protein (g/100 ml)</td>
<td>5.4 ± 0.07</td>
<td>5.9 ± 0.11f</td>
</tr>
<tr>
<td>Triglyceride (mg/100 ml)</td>
<td>102.0 ± 12.1</td>
<td>97.7 ± 11.4</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.2 ± 0.6</td>
<td>35.3 ± 1.2*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE. *P < 0.005, †P < 0.01.
controls and experimental animals probably would produce little difference in viscosity by this mechanism. Nevertheless, the finding of increased whole blood viscosity during glycerol-induced acute renal failure is probably an accurate indication of a qualitative difference in arteriolar blood viscosity between normal rats and those injected with glycerol. The combination of decreased afferent arteriolar diameter and increased blood viscosity will produce elevated preglomerular vascular resistance in glycerol-induced acute renal failure. Therefore, it appears that the increase in preglomerular resistance, either alone or in association with a simultaneous decrease in postglomerular resistance, will effect a decrease in glomerular capillary pressure. However, the extent of decrease in the net driving force for glomerular filtration cannot be assessed without simultaneous measurements of other factors influencing GFR, such as glomerular capillary oncotic pressure, Ks, and tubular pressure.

In summary, preglomerular vasoconstriction has been demonstrated in glycerol-induced acute renal failure on the basis of the findings of (1) decreased percentage of microspheres entering glomeruli and (2) decreased mean arteriolar diameter. Preglomerular resistance is increased due to a combination of afferent vasoconstriction and increased blood viscosity in glycerol-induced acute renal failure.

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