Intrarenal Hemodynamics in Glycerol-Induced Myohemoglobinuric Acute Renal Failure in the Rat

By G. Ayer, A. Grandchamp, T. Wyler, and B. Truniger

ABSTRACT

A modified $^{133}$Xe washout technique was used to study changes in intrarenal hemodynamics during glycerol-induced acute renal failure in the rat. Within 10 minutes after glycerol injection, the flow fraction supplied to component I of the washout curve ("cortex A") decreases, reaching minimum values 24 hours after glycerol (31% of control conditions). Seven days thereafter five of the rats, still uremic, showed cortical flow fractions less than 50% of control values. Nine others had recovered. Twenty-four hours after glycerol, renal blood flow was 27% and increased to 86% of control conditions in the recovered rats 7 days after glycerol. $^{85}$Kr autoradiographs and silicone rubber casts of the renal vasculature demonstrated a severe cortical hypoperfusion at the height of oliguria that gradually disappeared in parallel with the functional recovery of the kidney. These observations, in good agreement with micropuncture data, suggest that oliguria and renal insufficiency in this experimental model are the result of a primary decrease in glomerular filtration rate due to an increased preglomerular resistance.

KEY WORDS: distribution of flow, regional blood flow, oliguria, renal vascular resistance, inert gas washout, renal vasculature, renal cortical hypoperfusion.

Glycerol-induced myohemoglobinuria has been used with increasing frequency as a model to study the mechanisms responsible for acute renal failure. In its clinical course, reversibility, and histological findings, the myohemoglobinuric acute renal failure of the rat closely resembles the picture in patients with acute renal failure of different origin (1-7). Extensive studies of the excretory function of the kidney in this experimental model have led to the conclusion that a reduction in glomerular filtration rate, rather than an increase in intratubular pressure or an increase in absorption of a normal volume of filtrate is the primary cause of oliguria and renal insufficiency. If this decrease in glomerular filtration rate is mainly due to an increased preglomerular resistance, as has been suggested on the basis of micropuncture data (8), important changes in intrarenal hemodynamics could be expected.

Using a modification of the inert gas washout technique (9) recently developed in this laboratory (10), the intrarenal distribution of blood flow and local blood flow rates were investigated during the course of myohemoglobinuric acute renal failure in the rat. Our observations—a severe, progressive, and preferential cortical hypoperfusion during oliguria and a normalization of the intrarenal hemodynamics during functional recovery—offer the hemodynamic substrate for the conclusion that, in this model of acute renal failure, a primary decrease in glomerular filtration rate is the main cause of oliguria and renal failure (11).

Methods

Experiments were performed on male Wistar


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rats weighing between 270 and 450 g. The animals were fed a standard laboratory chow. To increase the incidence of renal failure, water was withheld for 24 hours before glycerol injection and, except for the rats in series A (see below), was freely available thereafter.

Myoglobinuric acute renal failure was induced by the injection of 50% glycerol (1 ml/100 g) into the muscles of the left hind limb. Animals were fed a standard laboratory chow. To increase the incidence of renal failure, water was withheld for 24 hours before glycerol injection and, except for the rats in series A (see below), was freely available thereafter.

Myoglobinuric acute renal failure was induced by the injection of 50% glycerol (1 ml/100 g) into the muscles of the left hind limb. At different times before and after the induction of acute renal failure, intrarenal hemodynamics were analyzed by the 133Xe washout technique, 85Kr autoradiography, and silicone rubber casting of the renal vascular tree. In series A (15 animals), the measurements were made under control conditions (i.e., 24 hours of dehydration), at 10 minutes, and at 2 hours after the injection of glycerol. The animals in series B (13 rats) were analyzed 24 hours and those in series C (14 rats) 7 days after glycerol injection.

The animals were anesthetized with pentobarbital sodium (10 mg/100 g ip in rats in series A; a reduced dose in the uremic animals in series B and C) and placed on a heated operating table. After tracheotomy the abdomen was opened, and the left kidney was exposed, gently mobilized, and put into a counting chamber as described previously (10). The right femoral artery was dissected and a polyethylene catheter (i.d., 0.28 mm; o.d., 0.61 mm) was introduced transfemorally into the aorta so that its tip was at the origin of the left renal artery. The accurate position of the catheter tip was achieved by external measurement and by repeated injections of small amounts of lissamine green (1%) under close inspection of the kidney and the abdomen. Saline, 0.9%, containing 20 U U.S.P. heparin/ml, was infused through the aortic catheter at a rate of 1 ml/hour. 30 minutes of stabilization time was allowed after the preparation of the animal and before the first measurements were started. The mean aortic blood pressure was measured intermittently by mercury manometry.

133Xe washout studies were performed according to the method described previously (10): 0.15-0.25 mc of 133Xe dissolved in 0.1-0.25 ml of 0.9% saline followed by 0.1-0.2 ml of saline were injected through a double-barreled needle and the aortic catheter. The radioactivity in the kidney excised and quick-frozen in Dry Ice-acetone. Contact radiographs were then developed as described previously (10).

Silicone rubber casts of the renal vascular tree were obtained in an additional group of rats under control conditions (12 animals), 2 hours after glycerol injection (four animals), and 24 hours (six animals) and 7 days (five animals) after the induction of acute renal failure. Immediately before the casts were made, the animals were given 5000 U U.S.P. heparin. A polyethylene catheter (i.d., 0.86 mm; o.d., 1.27 mm) was introduced into the abdominal aorta.
and advanced up to 2 mm distal to the origin of the left renal artery. The silicone rubber preparation was injected in vivo through the aortic catheter at a rate of 0.2–0.5 ml/sec. After 3 or 10 seconds, the renal vessels were tied, and the kidney was excised, "cured" overnight at room temperature, and passed through increasing concentrations of ethyl alcohol from 25% to 100%. The dehydrated organs were cut with a razor blade into slices 0.5–1.0 mm thick. These slices then were cleared and stored in methyl salicylate. In animals with myohemoglobinuric acute renal failure, the time course of the injection and the amounts injected had to be adapted to the severely impaired renal circulation.

At the end of each experiment blood was collected for measuring blood urea nitrogen (BUN) (autoanalyzer) and hematocrit; local blood flow rates, as calculated from the slopes of the components of the washout curve, were corrected for changes in hematocrit, as suggested by Andersen and Ladefoged (13). Urinary flow rates were measured in some animals by ureteral catheterization. Because of a severe bleeding tendency in uremic animals, the procedure had to be abandoned. The statistical analysis of the data was done on a CDC-3800 computer (Unité d'informatique, Hôpital Cantonal, Geneva).

**Results**

### GENERAL PICTURE

The clinical picture observed after glycerol injection in dehydrated animals resembled closely the one described by Thiel et al. (7). Frank myohemoglobinuria was observed within 1 hour, and most animals showed marked oliguria or anuria within 24 hours after glycerol injection. All rats developed a clearcut uremic syndrome with values for BUN averaging 147 mg/100 ml after 24 hours (SD 42 mg/100 ml). Seven days after initiation of myohemoglobinuric acute renal failure, the surviving animals could be divided into two groups according to their azotemia. Group 1 was still severely uremic, values for BUN ranging between 146 and 319 mg/100 ml (mean 267 mg/100 ml, SD 71 mg/100 ml). Seven days after glycerol injection the flow fraction supplied to component I (cortex A) decreased to 67.5% of total RBF (P = 0.055 compared with our control animals). Similarly the local cortical blood flow rate diminished slightly (P = 0.191).

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Two hours after glycerol the redistribution processes were quite marked, the differences in intrarenal distribution of blood flow compared with control conditions being significant (P < 0.001). The flow fraction supplied to component I decreased to 48.5% of RBF whereas the fraction supplied to component II showed an increase to 41.0% compared to 15.7% in control conditions. This increase is not only complementary but corresponds to a true in-
**TABLE 1**

*Intrarenal Distribution of Blood Flow, Local Blood Flow Rates, Renal Blood Flow (RBF) and Mean Aortic Blood Pressure (BP) during the Course of Myohemoglobinuric Acute Renal Failure in the Rat*

<table>
<thead>
<tr>
<th>Percent total RBF to Component</th>
<th>Local blood flow rate (ml/min⁻¹ g⁻¹) in Component</th>
<th>RBF (ml/min⁻¹ g⁻¹)</th>
<th>BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (15 Rats, 15 Measurements)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>78.8</td>
<td>15.7</td>
<td>5.5</td>
</tr>
<tr>
<td>sd</td>
<td>7.2</td>
<td>5.6</td>
<td>2.1</td>
</tr>
<tr>
<td>se</td>
<td>1.9</td>
<td>1.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

| Ten minutes after Glycerol (15 Rats, 15 Measurements) | | |
| Mean | 67.4 | 25.0 | 7.6 | 4.13 | 1.07 | 2.79 | 114 |
| sd | 20.6 | 17.8 | 3.8 | 0.66 | 0.32 | 1.30 | |
| se | 5.3 | 4.6 | 1.0 | 0.18 | 0.08 | 0.28 | |
| P | 0.055 | 0.064 | 0.065 | 0.191 | 0.689 | 0.070 | |

| Two Hours after Glycerol (14 Rats, 14 Measurements) | | |
| Mean | 48.5 | 41.0 | 10.5 | 3.99 | 1.08 | 1.92 | 130 |
| sd | 20.8 | 17.3 | 4.6 | 0.82 | 0.27 | 0.78 | |
| se | 5.6 | 4.6 | 1.2 | 0.22 | 0.07 | 0.20 | |
| P | <0.001 | <0.001 | <0.001 | 0.108 | 0.774 | 0.001 | |

| Twenty-Four Hours after Glycerol (15 Rats, 20 Measurements) | | |
| Mean | 24.4 | 43.9 | 31.7 | 4.28 | 0.80 | 0.92 | 113 |
| sd | 20.4 | 12.2 | 17.4 | 1.68 | 0.28 | 0.61 | |
| se | 4.6 | 4.6 | 1.2 | 0.22 | 0.07 | 0.20 | |
| P | <0.001 | <0.001 | <0.001 | 0.592 | 0.012 | <0.001 | |

| Seven Days after Glycerol (Group 1, Azotemic) (5 Rats, 10 Measurements) | | |
| Mean | 30.6 | 47.8 | 12.6 | 3.83 | 0.98 | 1.54 | 91 |
| sd | 18.1 | 14.6 | 5.1 | 1.93 | 0.32 | 0.14 | |
| se | 5.7 | 4.6 | 1.6 | 0.35 | 0.10 | 0.04 | |
| P | <0.001 | <0.001 | <0.001 | 0.103 | 0.401 | <0.001 | |

| Seven Days after Glycerol (Group 2, Recovered) (9 Rats, 17 Measurements) | | |
| Mean | 69.7 | 22.7 | 7.6 | 4.74 | 1.05 | 2.92 | 108 |
| sd | 11.4 | 11.2 | 1.5 | 2.14 | 0.25 | 0.63 | |
| se | 2.8 | 2.7 | 0.4 | 0.52 | 0.06 | 0.15 | |
| P | 0.013 | 0.036 | 0.003 | 0.752 | 0.564 | 0.040 | |

*P = significance of the difference from control values; Student's t-test.*

crease in blood flow supplied to component II, as calculated for a standard kidney weight of 1 g (0.70 ml/min vs. 0.53 ml/min in control conditions). Local blood flow rates did not change significantly.

Twenty-four hours after the induction of myohemoglobinuric acute renal failure, the changes in intrarenal hemodynamics were at their height: the flow fraction supplied to component I averaged 24.4%, whereas the percent of RBF supplied to component II had increased to 43.9%. Despite this increase, the true blood flow supplied to component II was reduced to 0.38 ml/min, as calculated for a standard kidney weight of 1 g. Accordingly, the local blood flow rate of component II was significantly reduced to 0.70 ml min⁻¹ g⁻¹ vs. 1.12 ml min⁻¹ g⁻¹ in control conditions.

Seven days after glycerol injection group 1 still showed severely disturbed, although somewhat improved intrarenal hemodynamics. The flow fraction supplied to cortex A averaged 39.6%, whereas the percent of RBF supplied to component II was 47.8%. In contrast, group 2 (BUN 28 mg/100 ml) showed an important improvement in intrarenal hemodynamics. The cortical flow fraction, although still significantly different from...
control conditions, was back to 69.7% of RBF; the fraction supplied to component II was 22.7% (significantly different from control conditions \( P = 0.036 \)), and the local blood flow rates of components I and II did not differ from control values.

Except for the animals in group 1 the mean aortic blood pressure during the analysis of intrarenal hemodynamics was normal or slightly elevated (Table 1).

\[^{85}\text{Kr}  \]

 Autoradiographs demonstrated characteristic changes in intrarenal distribution of the radioactive indicator gas (Fig. 1). Two hours after glycerol the cortical filling showed the first signs of a patchy hypoperfusion. Except for these inhomogeneities the relationship between the time course of the washout curve and the indicator filling of the different zones of the kidney was the same as in normal animals (10). Twenty-four hours after the induction of myohemoglobinuric acute renal failure, severe changes in indicator distribution became visible (Fig. 1B): 10 seconds after the bolus injection of \[^{85}\text{Kr}  \] there was a weak and patchy filling of the renal cortex, irregular areas of faint radioactivity alternating in a mosaic pattern with zones of near normal staining. One minute after \[^{85}\text{Kr}  \] injection the cortical filling was considerably stronger but still inhomogeneous. Two minutes after slug injection \[^{85}\text{Kr}  \] persisted in the cortical area in an almost homogeneous fashion. In contrast, in normal control kidneys the radioactivity was washed out from the renal cortex by this time (Fig. 1A). Similarly, the washin and washout of \[^{85}\text{Kr}  \] into and from the renal medulla was considerably delayed compared with control kidneys. In many kidneys the indicator gas persisted within patchy areas of the cortex for more than 10 minutes after bolus injection. Therefore, in contrast to normal control kidneys, a clearcut correlation between the components of the washout curve and the anatomical zones of the kidney (cortex, outer and inner medulla and hilar fat) was no longer possible. Seven days after glycerol injection the autoradiographic picture in group 1 still corresponded to that described for animals 24 hours after induction of acute renal failure. Autoradiographs from group 2 were almost indistinguishable from normal control kidneys, except for a slight delay in cortical filling (Fig. 1C).

\section*{Silicone Rubber Casts of the Renal Vascular Tree}

Normal kidneys show a complete filling of the renal vasculature up to the peritubular capillaries and to the subcapsular layers of the renal cortex (Fig. 2, top). During the early stage of acute renal failure, i.e., 3 hours after glycerol injection, small subcapsular filling defects developed and gradually increased in size and depth, resulting in an extremely reduced cortical filling by 24 hours after induction.
Silicone rubber casts of the renal vascular tree in control conditions (top), 24 hours (middle) and 7 days after glycerol injection (bottom). Note the lack of cortical filling at the height of oliguria 24 hours after injection and the almost complete recovery with a few subcapsular areas of incomplete filling only (Fig. 2, bottom). Since the viscosity of the silicone compound is somewhat higher than that of whole blood (15-20 cP), lack of vascular filling does not necessarily indicate a complete ischemia of the area.

Discussion

On the basis of their clinical observations and micropuncture data, Oken and collaborators (1, 7, 11, 14, 15) concluded that oliguria and renal insufficiency of myohemoglobinuric renal failure of the rat are the result of a primary decrease in glomerular filtration rate. Since the latter does not seem to be due to tubular obstruction with casts and since there is no evidence, in this model, for an increased absorption of a normal volume of filtrate, it was suggested that the abnormality in glomerular function may be the result of changes in afferent arteriolar tone (11). Unless these changes were balanced by opposite variations in efferent arteriolar tone, one would expect to find important hemodynamic alterations in terms of intrarenal distribution of blood flow and/or local blood flow rates of the kidney in acute renal failure. So far there has been no way of measuring these parameters in the rat. Measurements of total renal blood flow in patients with acute renal failure showed a reduction to approximately one third (16, 17) to 50% of normal (18). Recent observations by Hollenberg et al. (19) indicate that the reduction in total RBF in patients with acute renal failure is due mainly to a diffuse, preferential reduction in renal cortical perfusion.

Our modification of the inert gas washout technique allows analysis of the intrarenal hemodynamics during the course of the myohemoglobinuric acute renal failure of the rat. In the present studies, within 10 minutes
after glycerol injection, renal blood flow began to decrease, together with a reduction in the fraction supplied to component I (cortex A). Two hours thereafter, at a time when marked myohemoglobinuria was present, mean renal blood flow dropped to 56% of control conditions. A significant reduction in flow fraction supplied to cortex A corresponds to a patchy hypoperfusion of the renal cortex, as demonstrated by silicone rubber casts (the pictures are nearly the same as the one shown in Fig. 2, bottom). While cortex A was still perfused with the usual high local blood flow rates, it seems that the hypoperfused areas (cortex B) maintain blood flow rates indistinguishable from the slower components (II or III + IV) of the washout curve. These hemodynamic changes reached their maximum 24 hours after glycerol injection. At this stage of acute renal failure, all animals were severely oliguric and uremic. Mean renal blood flow was 27% of control values, the flow fraction supplied to component I being reduced to an average of 30% of control. Again, cortex A maintained its normal blood flow rate. In good agreement with these findings, autoradiographs indicate that there was a severe and patchy reduction in cortical perfusion with only small cortical areas of apparently near-normal blood flow. Although the pictures obtained by silicone rubber casting of the renal vascular tree cannot be taken as indicating a complete cortical ischemia, they confirm the important cortical hypoperfusion (Fig. 2, middle). Within 1 week after induction of acute renal failure an overall improvement in intrarenal hemodynamics resulted in near normalization of intrarenal distribution of blood flow and renal blood flow, along with the functional recovery of the kidneys in some animals (group 2). In contrast, rats in group 1, still markedly uremic, showed only partial recovery, resulting in a small increase in RBF and cortical flow fraction. Autoradiographs and silastic vascular casts confirm the marked improvement in cortical perfusion of group 2.

These hemodynamic changes require important variations in renal vascular resistance. If the increased resistances leading to hypoperfusion are mainly preglomerular, as has been suggested recently by Ruiz-Guinauz et al. (8), they will readily account for the primary decrease in glomerular filtration rate postulated on the basis of the micropuncture data. To reduce total RBF to 22% of normal conditions, as seen during severe oliguria, the total renal vascular resistance would have to increase by a factor of 4.6. Assuming constant postglomerular resistances and using the data of Gertz et al. (20) for normal glomerular capillary pressure, it can be calculated that with a constant aortic blood pressure of 110 mm Hg the preglomerular resistance would have to increase by a factor of 18.9. This again would lower the glomerular capillary pressure to 19.3 mm Hg, a value below the range of oncotic pressure of the plasma. Since in our experiments, at the height of oliguria there were still some areas of cortex A, it seems likely that these segments account for the remaining urine flow. These observations are in good agreement with previous findings of a functional heterogeneity of the nephrons in terms of proximal tubular fluid flow rate during glycerol-induced acute renal failure (1). Thus the present experiments seem to present a satisfactory hemodynamic substrate for the earlier conclusion that a primary decrease in glomerular filtration rate due to an increase in afferent arteriolar tone, is mainly responsible for the renal insufficiency and oliguria of myohemoglobinuric acute renal failure in the rat.

The mechanisms responsible for the afferent arteriolar constriction are still not well understood. Further studies will have to examine the relative role of the renin-angiotensin system, renal nerves, circulating catecholamines, and other vasoactive mediators. Recent observations in patients with acute renal failure of different origin suggest changes in intrarenal hemodynamics similar to those demonstrated in the present experiments (19, 21). In analogy to the rat model, it can be assumed that in the various forms of human acute renal failure, an increase in afferent arteriolar tone plays a similar, important role for the pathogenesis of the persisting oliguria.
This assumption, however, does not rule out the contribution of additional mechanisms to renal insufficiency. Depending on the original cause, tubular obstruction or tubular disruption with increased absorption of tubular fluid could be cofactors in the pathogenesis of oliguria and acute renal failure in man.

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References

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