The Prevention of Acute Renal Failure in the Rat by Long-Term Saline Loading: A Possible Role of the Renin-Angiotensin Axis* (33937)

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(Introduced by E. H. Sonnenblick)

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Renal micropuncture experiments have shown that a reduction in glomerular filtration rate is the primary cause of oliguria and azotemia in mercury-induced and glycerol-induced hemoglobinuric acute renal failure in the rat (1–4). Neither increased intratubular pressure nor leakage of inulin as a result of tubular disruption and passive backflow of filtrate could be incriminated as the principal cause of the decreased glomerular filtration rate. Recent micropuncture studies by Ruiz-Guiñzú and associates (5), Barenberg and associates (6) and Cirksena and Teschan (7) lend support to these conclusions in the mercury and methemoglobin models, respectively, although Bank *et al.* (8) found support for the passive backflow theory in mercury poisoned animals. Assuming that there is no major change in glomerular permeability, a primary hemodynamic factor must be implicated as the major cause of the observed acute renal failure.

Schnermann *et al.* (9) implicated the renin-angiotensin axis as a cause of decreased GFR following renal ischemia or hemorrhagic hypotension and, by extrapolation, suggested a similar role in acute renal failure. If this thesis applies to glycerol-induced acute renal failure in the rat, animals depleted of renin by chronic saline loading should be at least partially protected from the usual renal lesions. The present study established the protective role of chronic saline loading in glycerol-induced hemoglobinuric acute renal failure.

Methods. Female Sprague-Dawley rats weighing 150–175 g were provided a diet of Purina lab chow pellets (Na = 200 meq/kg; K = 185 meq/kg) and either tap water, 10% glucose solution, or 1% NaCl *ad libitum* for more than 2 months. At the end of this time, they weighed about 275 g. Animals in each group were placed in individual metabolic cages for two or more days before glycerol injection. Fluid intake, urine output and body weight were recorded daily for the 2 days after glycerol injection, after which time samples of blood were taken by cardiac puncture for BUN, Na, and K determination. Urine osmolalities were measured with an Advanced osmometer. Urine and plasma Na and K concentrations were determined using a Baird internal standard flame photometer. Blood urea nitrogen was measured by the method of Gentzkow (10) on 0.2-ml aliquots of whole blood.

Hemoglobinuria was induced in each group of animals by the intramuscular injection of 10 ml/kg of body weight of 50% glycerol in tap water. The animals were not dehydrated prior to this injection, and the appropriate drinking fluid was allowed *ad libitum* immediately after the injection.

Plasma volumes of rats in each group were measured during the control period and 4–6 hr after glycerol injection. Human serum albumin labeled with ^{125}I (Squibb Albumotope) was used as previously described (11). In order to investigate the possibility that blood volume expansion alone accounted for

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the protection of the saline-drinking animals, a group of normal water-drinking animals was transfused with rat blood. A volume equal to the measured mean control blood volume difference between the two groups, 1.4 ml/100 g of body weight, was given. Blood was drawn by cardiac puncture as rapidly as possible from donor animals into heparinized syringes and refrigerated until infused less than 2 hr later. The recipient water-drinking animals were given light ether anesthesia, and blood was infused into the femoral vein at a constant speed of 0.2 ml/min using a Braun infusion pump. The femoral incision was closed and the animals allowed to awaken. These rats were given the standard glycerol dose within 30 min after the tranfusion and were handled in the same manner as rats in the other groups thereafter.

Results. The values for body weight, fluid intake, urine volume, osmolality, Na and K concentration, solute excretion, plasma volume, and blood hematocrit of the various experimental groups are shown in Table I. In the control period, saline loaded rats had a significantly larger plasma volume ($p<0.0005$), fluid intake ($p<0.001$), urine volume ($p<0.001$) and urine sodium concentration ($p<0.001$) than their water-drinking counterparts. Urine osmolality and potassium concentration were lower ($p<0.001$). Glucose-drinking rats had a plasma volume equivalent to that of water-drinking animals ($p<0.15$), but differences in fluid intake and urine volume, osmolality and potassium concentration were even greater than those observed in saline loaded rats (see Table I). The alteration in urine sodium concentration, however, was opposite in direction to that observed in the saline-drinking rats, and total solute excretion equaled that of water-drinking control animals.

On the day after glycerol injection, the BUN of saline-drinking rats rose to 43 ± 6 (SE) mg/100 ml a value significantly higher than the control value ($p<0.001$), but not so great as that observed in each of the other groups ($p<0.05$). The mean BUN of saline-drinking rats returned to normal in the succeeding 24 hr ($p<0.20$), while the mean BUN of the water-drinking rats had increased insignificantly ($p<0.20$).

Only the glucose-drinking rats developed oliguria after glycerol injection. In the first 24 hr, this, coupled with continued glucose ingestion, resulted in a 7.3% weight increase, while all other groups lost 1.7–4.2% of body weight. The fall in "plasma volume," seen 4–6 hr after injection and due to fluid sequestration at the glycerol injection site, was far less in glucose-drinking rats than that observed in the others ($p<0.0005$), presumably due to their large fluid intake and oliguria.

Discussion. A primary alteration in renal hemodynamics with near cessation of glomerular filtration has been shown to be the cause of renal insufficiency in glycerol-induced myohemoglobinuric acute renal failure (2). This effect occurs in the absence of hypotension and persists after restoration of plasma volume to normal (2). The renin–angiotensin axis has been implicated in the hemodynamic alterations observed in hemorrhagic shock (9). In view of the potent vascular effect of angiotensin and the proximity of the macula densa to the afferent arteriole, this hypothesis is attractive. The present study was undertaken to see whether renin depletion modifies the development of glycerol-induced acute renal failure in the rat.

Rats receiving saline in place of tap water for as little as 2 weeks have been shown to have a very low renin content both in kidney tissue and in peripheral blood (12). Those in this study were placed on the saline regimen for some 2 months to assure renin depletion. This treatment was effective, in that oliguria did not develop and the BUN 48 hr after glycerol injection was indistinguishable from normal. A transient, small BUN rise was apparent early, however, and an obligatory diuresis occurred despite acute weight loss persisting for at least 48 hr.

Other features associated with chronic saline loading might be considered to have played a role in the observed protection. First, the plasma volume of saline-drinking rats in the control state and after glycerol was some 25% greater than that of water-drinking rats, but transfusion of water-

TABLE I. Clinical Data.

	Time (hr)	Water-drinking rats ($N = 36$)[a]	Water-drinking rats given saline blood ($N = 17$)[a]	Saline-drinking rats ($N = 14$)[a]	Glucose-drinking rats ($N = 18$)[a]
BUN (mg/100 ml)	Control	13 ± 1 ($N = 65$)[b]		13 ± 1 ($N = 13$)	
	24	75 ± 14 ($N = 15$)		43 ± 6 ($N = 17$)	
	48	98 ± 14 ($N = 36$)	94 ± 4	16 ± 2 ($N = 14$)	112 ± 6 ($N = 17$)
Wt (g)	Control	272 ± 5	291 ± 5	283 ± 7	295 ± 6
	24	266 ± 6	279 ± 5	278 ± 7	316 ± 6
	48	256 ± 6	271 ± 5	270 ± 8	319 ± 7
Wt change in 1st 24 hr (%)		−2.6 ± 0.6	−4.2 ± 0.7	−1.7 ± 0.7	+7.3 ± 1.2
Fluid intake (ml/24 hr)	Control	49 ± 1	38 ± 3	70 ± 8	122 ± 8
	24	33 ± 3	34 ± 5	93 ± 11	40 ± 4
	48	45 ± 4	36 ± 5	127 ± 14	26 ± 5
Urine volume (ml/24 hr)	Control	10 ± 1	12 ± 2	47 ± 7	65 ± 6
	24	18 ± 2	24 ± 4	61 ± 8	1.9 ± 0.9
	48	32 ± 4	30 ± 5	121 ± 16	6 ± 3
Urine osmolality (mOsm/kg)	Control	1790 ± 64	1740 ±137	1002 ± 93	256 ± 27
	24	843 ± 49	858 ± 88	769 ±147	407 ± 9
	48	754 ± 66	757 ± 92	593 ± 49	600 ±139
Urine Na (meq/liter)	Control	139 ± 8	152 ± 14	248 ± 14	21 ± 2
	24	47 ± 4	48 ± 7	158 ± 6	82 ± 23
	48	47 ± 5	40 ± 7	188 ± 8	113 ± 22
Urine K (meq/liter)	Control	192 ± 10	232 ± 16	77 ± 11	32 ± 4
	24	99 ± 7	90 ± 8	41 ± 7	35 ± 3
	48	59 ± 7	67 ± 10	31 ± 4	57 ± 16
Solute excretion (mOsm/24 hr)	Control	17.4 ± 1.9	18.7 ± 1.8	40.8 ± 2.8	14.5 ± 1.0
Hematocrit (%)	Control	42 ± 1 ($N = 8$)		39 ± 1 ($N = 17$)	43 ± 1 ($N = 16$)
	48	38 ± 1 ($N = 36$)	44 ± 2 ($N = 17$)	40 ± 1 ($N = 14$)	32 ± 1 ($N = 17$)
Plasma volume (ml/100 g of body wt)	Control	3.42 ± 0.12 ($N = 8$)		4.21 ± 0.10 ($N = 9$)	3.28 ± 0.04 ($N = 8$)
	4–6 after glycerol	1.83 ± 0.06		2.22 ± 0.08	2.78 ± 0.05

[a] Number of studies of each parameter, except where indicated.

[b] All values represent mean ± 1 SEM.

drinking rats to a comparable blood volume just prior to glycerol injection was not protective. Second, the large fluid intake and urine volume of the saline-drinking rats were exceeded by the glucose-drinking controls and by rats with diabetes insipidus which are not spared from renal failure (4), so that these parameters would not appear to be important protective features. Third, the high urinary sodium concentration of the saline-drinking rats, some 240 meq/liter, might be suspected of being protective by the mechanism suggested by Schnermann *et al.* (9), yet this concentration is virtually identical with the 250 meq/liter urine value obtained in dehydrated rats that are singularly susceptible to glycerol-induced acute renal failure (13). Total sodium excretion and solute load were far higher in saline-drinking rats than in other groups, and may have played a protective role as postualted by Vander (14). Even here, however, the renin–angiotensin axis is implicated, a large sodium load reaching the macula densa supposedly inhibiting renin release.

The development of acute renal failure both in man and the experimental animal correlates well with circumstances in which renin release is affected. Dehydration, sepsis, hemorrhage, and hypotension predispose to the development of acute renal failure and are potent stimuli for renin release. Plasma renin titers (15) and angiotensin activity (16) in man have been markedly elevated in established acute renal failure. Hayes and associates (17, and personal communication), however, found that plasma renin titers in rats with glycerol-induced acute renal failure are elevated 6 and 12 hr after injection but normal by 24 hr. At no time was renal tissue renin titer augmented.

Although it is unproved that suppression of renin activity was responsible for the impressive degree of protection observed in the present study, the results lend support to that possibility. Elevated blood levels of renin and angiotensin alone can not be responsible for the physiologic events in the kidney with acute renal failure, however. If that were the case, any state in which plasma renin titers are comparably high, such as in malignant hypertension (15), would inevitably be associated with acute renal failure. Obviously, this does not occur. Instead, it is postualted that acute renal failure may be the result of several interacting mechanisms, only one of which is renin release. The results of the present experiment suggest that this latter mechanism, however, may be of importance to the development of myohemoglobinuric acute renal failure in the rat.

Summary. Substitution of 1% saline for drinking water largely prevents the development of established acute renal failure in rats given glycerol intramuscularly. Control experiments indicate that this protection from acute renal failure is not due to the blood volume expansion, polyuria, polydypsia, lower urine osmolality, or other parameters studied. It is suggested that the depletion of renin, associated with chronic saline-loading, may have played a protective role, and that the renin–angiotensin axis may be a potent factor in the development of glycerol-induced acute renal failure in the rat.

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Interaction of Lysosomes and Anti-inflammatory Drugs (33938)

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Recent experiments have resulted in a system for the detection of anti-inflammatory drugs based on the stabilization of cellular membranes (1, 2). Lysosomes have not been investigated in depth in this respect. The present study was conducted to establish optimal thermal-labilizing conditions for lysosomes, including pH studies and subsequently, the effect of anti-inflammatory drugs on lysosomal stability and lysosomal enzyme activity per se *in vitro*.

Methods. Preparation of lysosome-rich fractions. Modifications of methods described by Weissmann and Thomas (3) and by Dingle (4) were used for isolating lysosomes. Male, fasted (18 hr) rats (Harlan, Wistar strain, 140–200 g) were sacrificed by dislocation of cervical vertebrae. Livers from two animals were immediately removed, minced, and washed in cold (2°) 0.25 *M* sucrose until free of blood. A 10% (w/v) homogenate (H) was prepared in a Potter-type homogenizer (suspended in a 2° bath) fitted with a motor-driven Teflon pestle and centrifuged (1000*g*, 4°, 10 min) to remove unbroken cells and nuclear debris (N). The supernate (C) from (N) was decanted and recentrifuged (25,000*g*, 4°, 10 min) to obtain a lysosome-rich fraction (L). Aliquots of all fractions were maintained at 2° until assayed.

Initial labilization of lysosomes. In preliminary studies aliquots of (L) resuspended in 0.25 *M* sucrose to volume of (S),[2] were incubated at 45° for 0, 30, 60, 90, and 120 min to effect thermal enzyme release. Maximal release was determined by submitting a duplicate aliquot of (L) to hypotonic media (suspension in distilled water) or Triton X-100 (0.2%) for 30 min at room temperature. Centrifugation (25,000*g*, 10 min, 4°) yielded two fractions, supernate and residue (containing undisrupted lysosomes); the former aliquots exposed to hypotonic media were also incubated at 45° in 0.25 *M* sucrose for 0, 30, 60, 90, and 120 min to assess stability of the released enzymes.

Enzyme activity. Aliquots of each liver fraction (usually 0.1 ml) were assayed directly for acid phosphatase and β-glucuronidase activities. Enzyme activity represents increase in absorbance (OD) per milliliter after 30-min incubation at 37°. Acid phosphatase activity was measured in all studies as outlined in Sigma Technical Bulletin No. 104. The reaction mixture contained 2.0 mg of *p*-nitrophenyl phosphate, 0.5 ml of distilled water, 0.5 ml of 0.09 *M* citric acid, pH 4.8, and 0.1 ml of liver fraction. The β-glucuronidase activity was measured according to the procedure outlined in Sigma Technical Bulletin No. 105. The reaction mixture consisted of 0.1 ml of 0.01 *M* phenolphthalein glucuronic acid, 1.3 ml of 0.075 *M* phosphate buffer, pH 4.5, and 0.1-ml aliquot of liver fraction. Commercially available acid phosphatase (Worthington) and β-glucuronidase (Worthington) were assayed at three different levels in each study involving lysosomes. Results of 31 experiments with 0.125, 0.250, and 0.500 mg/ml acid phosphatase yielded absorbance values (± SE), respectively, of 0.151 ± 0.002, 0.279 ± 0.005, and 0.512 ± 0.009; 0.1 ml of standard enzyme solutions was used per assay. Results of 31 experi-

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