

# Micropuncture Studies of the Recovery Phase of Myohemoglobinuric Acute Renal Failure in the Rat

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**ABSTRACT** Micropuncture studies of the recovery phase of glycerol-induced myohemoglobinuric acute renal failure were performed in rats whose blood urea nitrogen (BUN) had fallen at least 20% below its peak value. The glomerular filtration rate (GFR) of individual nephrons in a single kidney in the recovery period generally either was in the normal range or minimal. Each animal's BUN concentration at the time of the study was inversely related to the proportion of functioning surface nephrons, but did not correlate with individual nephron GFR values. Proximal tubule fractional water absorption was significantly depressed as manifested by both depressed inulin (TF/P) values and supernormal volumes of collections, a finding which, in the absence of a urea-induced osmotic diuresis, suggests impaired sodium transport by the damaged nephron. The mean proximal tubule hydrostatic pressure in recovery was normal and there was little variation in pressure among functioning nephrons. It is concluded that recovery from this model of acute renal failure reflects the progressive recruitment of increasing numbers of functioning nephrons. The recovery of individual nephron glomerular filtration, once begun, was rapid and complete. No evidence could be adduced that the gradual return of renal function towards normal reflects a slow release of tubular obstruction or repair of disrupted tubular epithelium. Rather, recovery appeared to be directly attributable to the return of an adequate effective glomerular filtration pressure. Significant limitation in proximal tubule water absorption persisted after individual nephron GFR had returned to normal or supernormal values in this model of experimental acute renal

failure in the rat, a finding which readily accounts for the diuresis associated with the recovery phase of this syndrome.

## INTRODUCTION

Recovery from acute renal failure is an intriguingly slow process which has been attributed to the gradual healing of disrupted tubular epithelium or the relief of tubular obstruction. Previous micropuncture studies of the development stage and of the period of sustained renal insufficiency in glycerol induced myohemoglobinuric renal failure in the rat have shown almost complete cessation of glomerular filtration (1-3). Because of this, the majority of surface nephrons were collapsed and, although there was no evidence for passive back-flow of filtrate, the tubular epithelial integrity of the most severely affected nephrons could not be assessed directly. The present study was undertaken in an attempt to characterize the pathophysiological events associated with recovery from this model of acute renal failure in the rat, and to evaluate any possible role of tubular obstruction or disruption which might relate to the characteristic slowness of the recovery process.

## METHODS

Female Sprague-Dawley rats weighing 170-230 g were kept in individual cages and allowed free access to food throughout the experimental period. Water was withheld for 1 hr just before glycerol injection to increase the incidence of renal failure (4), but was freely available thereafter. Myohemoglobinuric acute renal failure was produced by the intramuscular injection of 10 ml/kg of 50% glycerol in water (1). 48 hr later, each animal's BUN concentration was measured by a modification of the method of Gentzkow (5) on 0.2 ml blood samples obtained from the tail. BUN values were determined every 1 or 2 days thereafter and on completion of the micropuncture experiments. Micropuncture studies were performed when each animal's BUN concentration had fallen 20% or more below its peak value. The severity of azotemia varies from rat to rat in this

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model of acute renal failure, recovery usually occurring later in those animals that are most azotemic on the 2nd day after injection. The cause of this variation is unknown, but appears to relate to intrinsic renal mechanisms (4). Studies were done 2–6 days after glycerol injections, therefore, according to the time of the beginning of recovery. Previous experience has shown that the majority of rats whose BUN concentration is less than 80 mg/100 ml on the 2nd day after glycerol injection have already entered the recovery phase. Some animals with BUN concentrations below 80 mg/100 ml were subjected to micropuncture study immediately after the BUN results were returned on the 2nd day (some 6–8 hr later). BUN determinations repeated at the end of each micropuncture study confirmed that these animals were in the recovery phase with mean BUN's 25% lower than the 48-hr values (49 mg/100 ml vs. 65 mg/100 ml (Fig. 1).

The animals were anesthetized with sodium pentobarbital, 40 mg/kg of body weight, and placed on a heated table. They were prepared and micropuncture performed in the manner described earlier (1). In brief, proximal tubule fluid flow rate was derived from the volume of fluid obtained in a timed collection period; individual nephron glomerular filtration rate was derived from the timed clearance of  $^{14}\text{C}$ -labeled inulin (New England Nuclear Corp., Boston, Mass.); proximal tubule water absorption was calculated from the tubule fluid/plasma inulin concentration ratio ( $\text{TF}/\text{P}_{\text{in}}$ ); intratubular pressure was measured by direct water manometry. Inulin- $^{14}\text{C}$  activity in tubule fluid and plasma was measured in a Nuclear-Chicago liquid scintillation counter<sup>1</sup> without correction for the water content of plasma. Localization of micropuncture sites, when determined, was achieved by intratubular injection of latex and subsequent nephron microdissection as described by Bott (6). Estimates of the proportion of open nephrons to collapsed nephrons on the kidney surface were made by count in at least three low power microscopic fields at widely different sites. Qualitative estimates of proximal tubule fluid free flow rate were made by injecting small droplets of Sudan black-stained mineral oil and observing their rate of flow along the nephron (7). Flow was assessed in functioning nephrons chosen as randomly as possible without regard to the appearance of the tubule (except for orientation of the tubule segments in the same axis as the micropipet); previous studies have shown good agreement between flow determined in this manner and that determined by quantitative collections (7). The blood pressure of each animal was monitored by arterial monometry during micropuncture studies. Those few animals whose mean blood pressure was lower than 85 mm of Hg were disqualified from further studies. The right (contralateral) kidney of 37 rats was excised at the termination of micropuncture, blotted, and weighed immediately on a Mettler balance.<sup>2</sup>

## RESULTS

The mean BUN concentration of 65 control rats was  $13.0 \pm 0.4$  (SE) mg/100 ml, and that of 47 experimental animals 48 hr after glycerol injection was  $85 \pm 5$  mg/100 ml. All rats studied by micropuncture on the 2nd through the 6th day after glycerol injection had a significant fall in BUN concentration below peak BUN values (Fig. 1), and were considered to be in the re-

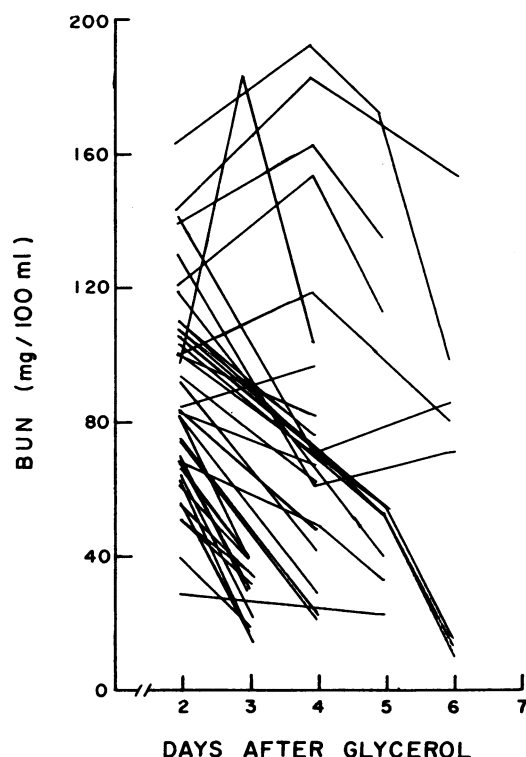


FIGURE 1 The time course of BUN change after glycerol injection. Values obtained in each animal are connected by lines; the last points are the study day BUN values.

covery phase of acute renal failure. 6 of the 47 animals (3 each at 72 and 144 hr) had a study day BUN concentration below 20 mg/100 ml. During recovery, the mean urine volume was 14.5 ml/24 hr compared with 7.5 ml/24 hr in the control period ( $P < 0.001$ ), despite a mean body weight loss of  $6.9 \pm 0.6$  (SE)/100 ml. Fluid intake did not change significantly ( $P < 0.25$ ).

**Appearance of the kidney.** The kidney surface had a generally mottled appearance which, on microscopic observation, was found to be due to the presence of collapsed tubules interspersed between tubules with slightly small, normal sized, or slightly large lumens. There was no readily discernible difference in the peritubular capillary circulation of these two populations of nephrons. Occasional tubules contained deeply stained red fluid. As can be seen from Fig. 2, an inverse relationship was found between each animal's BUN concentration and the proportion of open tubules on the kidney surface ( $r = -0.7$ ,  $P < 0.001$ ). To obtain a qualitative estimate of the rate of flow in a large number of nephrons, droplets of Sudan black-stained mineral oil were injected into individual proximal tubules and flow judged as poor, fair, or good according to their rate of passage along the tubules. Except for tubules containing red-tinted (hemoglobin-stained?) fluid, al-

<sup>1</sup> Nuclear-Chicago Corporation, Des Plaines, Ill.

<sup>2</sup> Mettler Instrument Corp., Princeton, N. J.

most all open nephrons into which oil droplets were introduced, a minimum of 10 tubules in each kidney, had flow which was considered "good." Collapsed tubules had no detectable flow.

The kidney of animals under study varied widely in size, none being small and many being massively enlarged (Fig. 4). In such kidneys, edema was so great as to raise the renal capsule considerably above the parenchymal surface. The kidney contralateral to that subjected to micropuncture was removed and weighed at the termination of 37 consecutive experiments. The weight of all 37 kidneys was more than 2 SD above the control value of  $0.46 \pm 0.02$  (SE)% body weight obtained in eight comparably sized normal rats (Fig. 4).

**Micropuncture studies.** The mean individual nephron GFR values measured in 40 nephrons of seven control rats was  $11.8 \pm 0.5$  (SE)  $\mu\text{l}/100$  g per min. Proximal tubule fluid flow rate,  $\text{TF}/P_{12}$ , and proximal tubule hydrostatic pressure values were comparable to those found in previous control studies, and are shown in Table I. The results obtained in micropuncture studies during recovery from acute renal failure are shown in the same table. Regardless of the time of study or the BUN concentration of the animal under study, mean GFR values of functioning nephrons in the recovery period were slightly but significantly higher than the control value ( $P < 0.01$ ). As may be seen from the

relatively narrow standard errors of the means, there was little variation in individual nephron function during recovery since most nephrons in which quantitative assessment of function could be made had achieved a normal or supernormal filtration rate. This is in sharp contrast with the finding in the developmental and sustained phases of this model of acute renal failure (1). Proximal tubule fluid flow rate was some 60% higher than that observed in the control period ( $P < 0.001$ ), primarily as the result of a markedly diminished proximal tubule fractional water absorption ( $P < 0.001$ ). Proximal tubule fractional water absorption is shown in Fig. 3 as a function of the percentage of proximal tubule length at which collections were made. Not only was water absorption in these nephrons considerably lower than that of controls, but, as may be seen, there was considerably more variation among nephrons than is usually seen in normal kidneys.

Proximal tubule hydrostatic pressure in recovery was little different from that found in control animals (Table I). Pressures in only 27 of 206 nephrons measured were greater than 20 cm of  $\text{H}_2\text{O}$ , eight values exceeding 25 cm of  $\text{H}_2\text{O}$ . Proximal intratubular pressure is a function of flow rate as well as resistance to outflow. In view of the supernormal proximal tubule fluid flow rates, evidence for significantly increased resistance to tubule fluid outflow could rarely be found. There was no cor-

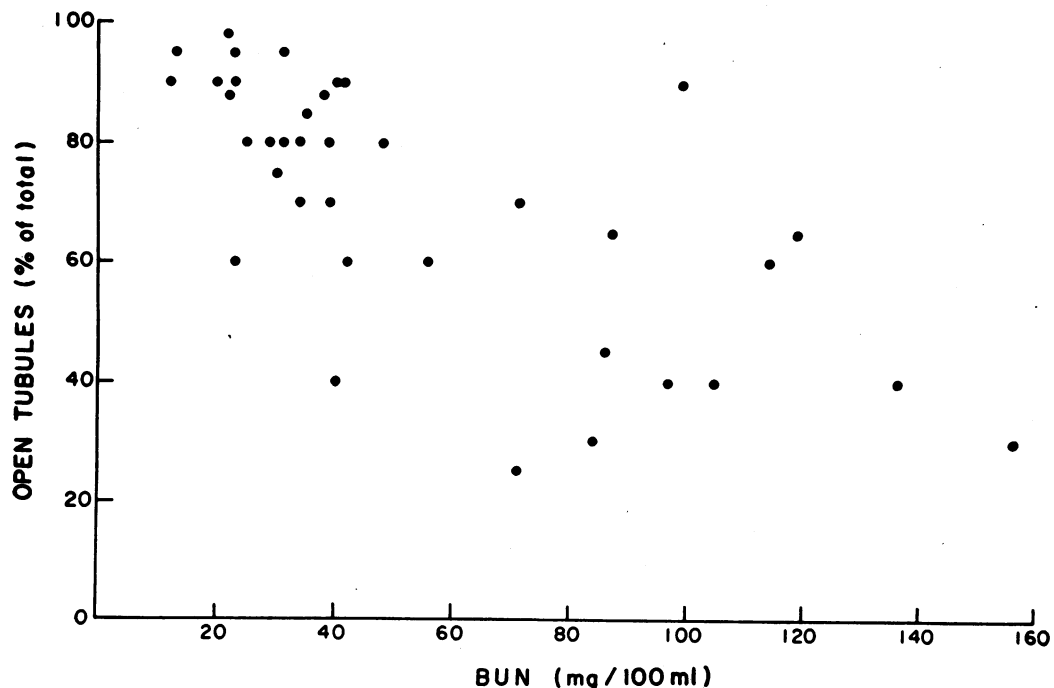


FIGURE 2 Graphic presentation of the relationship between the estimated proportion of functioning nephrons on the kidney surface to BUN concentration. ( $y = -0.4x + 93$ ;  $r = -0.7$ ;  $P < 0.001$ ).

TABLE I  
Study after Glycerol Injection

	Control	Day 2	Day 3	Day 4	Day 5	Day 6
Control BUN, mg/100 ml	13 ±0.4 (n = 65)					
48 hr BUN, mg/100 ml		65 ±10(SE)* (n = 8)	63 ±3 (n = 14)	90 ±3 (n = 12)	97 ±17 (n = 6)	129 ±9 (n = 7)
Study day BUN, mg/100 ml		50 ±8	31 ±2	64 ±9	67 ±17	65 ±19
Proximal tubule pressure, cm of H <sub>2</sub> O	15.5 ±0.1 (71/10)‡	15.7 ±0.8 (40/8)	16.9 ±0.5 (55/14)	14.5 ±0.6 (51/12)	14.3 ±0.8 (24/6)	14.5 ±0.9 (36/7)
Proximal tubule flow rate, μl/100 g per min	5.51 ±0.33 (40/7)	8.16 ±0.86 (20/7)	9.24 ±0.58 (36/12)	8.56 ±0.68 (35/10)	8.32 ±0.79 (21/5)	9.86 ±1.14 (23/6)
TF/P <sub>1a</sub>	2.27 ±0.08 (40/7)	1.65 ±0.06 (20/7)	1.54 ±0.03 (36/12)	1.61 ±0.05 (35/10)	1.64 ±0.07 (21/5)	1.57 ±0.04 (23/6)
GFR, μl/100 g per min	11.80 ±0.49 (40/7)	13.2 ±1.5 (20/7)	13.9 ±0.8 (36/12)	13.7 ±1.4 (35/10)	13.2 ±1.1 (21/5)	15.4 ±1.7 (23/6)

\* All values represent mean ±SEM.

‡ Numbers in parentheses signify the number of studies performed and the number of rats in each series.

relation between the lumen size (described as small, normal, or dilated) and the measured pressure value of 49 proximal tubules ( $P < 0.25$ ).

As may be seen from Table I and Fig. 1, the individual nephron function in recovery did not differ with the severity of acute renal failure initially produced, the degree of recovery, or the time after glycerol injection when studies were made. Renal weight at the time of micropuncture, on the other hand, was found to correlate with the degree of recovery achieved ( $P < 0.025$ ). Mean kidney weight of animals whose study day BUN concentration exceeded 80 mg/100 ml was  $0.77 \pm 0.04$  (SE)% of body weight while that of rats whose BUN concentration was less than 20 mg/100 ml was  $0.61 \pm 0.03\%$ . Between these extremes, however, there was no correlation between individual BUN values and kidney weight ( $P < 0.2$ , Fig. 4). As an example, the kidney weight of an animal whose BUN was 40 mg/100 ml (26% of the 48 hr value) on the 5th day after glycerol was 1.51% B.W., a value more than three times the normal mean.

## DISCUSSION

Acute renal failure in both humans and experimental animals persists long after the recognized initiating cause has disappeared. Previous studies have shown that a vasomotor phenomenon is primarily responsible for the marked impairment of glomerular filtration seen early in glycerol-induced myohemoglobinuric acute re-

nal failure in the rat (1). Passive backflow of filtrate across disrupted epithelium was not demonstrable, and increased resistance to outflow was detected in only occasional nephrons (1). In established acute renal failure, however, the overwhelming majority of nephrons have such a greatly reduced GFR that tubular obstruction or abnormal tubular permeability could well go undetected if they were present. Once the potential for adequate filtration is reestablished, these factors might play a covert role in delaying recovery of function, and thus account for the slow and gradual rate of recovery which typifies acute renal failure.

Recent studies by Bank, Mutz, and Aynedjian (8) and Steinhausen, Eisenbach, and Hemstädtter (9) have suggested that in low dose mercury poisoning, at least, increased tubular permeability is the chief determinant of acute renal failure. Disrupted tubular epithelium was described after nephrotoxic injury by Biber et al. (10), in support of earlier nephron microdissection studies by Oliver, MacDowell, and Tracy (11). As shown in Fig. 2, the severity of renal insufficiency at any time in the recovery phase of glycerol-induced acute renal failure correlated very well with the proportion of minimally functioning nephrons on the kidney surface. Since the surface tubules represent only a fraction of all nephrons within a kidney, this relationship probably would not exist unless recovery of glomerular function in the deep nephrons paralleled that of the superficial nephron population. Regardless of the completeness of the return of gross renal function during recovery, the filtration

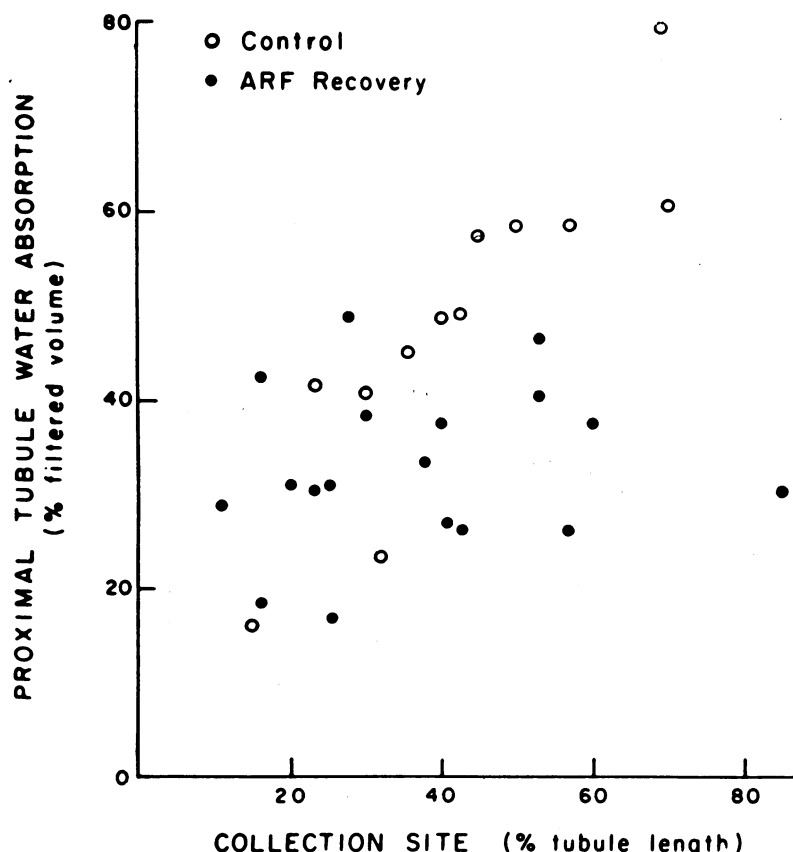


FIGURE 3 The relationship between proximal tubule water absorption and site of micropuncture in control rats and rats in the recovery phase of myohemoglobinuric acute renal failure (ARF).

rate of fluid-filled nephrons almost universally was found to be normal or supernormal, that of the remaining nephrons still being too low to permit quantitation. The wide spectrum of individual nephron glomerular dysfunction which is typical of the developmental and sustained phases of this model of acute renal failure was not seen. These findings indicate that recovery of renal function in this model of hemoglobinuric acute renal failure largely reflects the recruitment of increasing numbers of nephrons which had contributed little to over-all renal function in the phase of sustained renal insufficiency. The return of glomerular filtration of these nephrons to normal must be assumed to be quite rapid, since slow recovery should yield a wide range of glomerular filtration rates among individual nephrons in a given kidney. Such a rapid rate of recovery would not be expected if the apparent impairment of filtration found early were due to passive backflow of filtrate or were the factitious result of a loss of inulin across disrupted epithelium, the healing of renal tubular injury on histological studies being gradual and variable in extent from tubule to tubule (12).

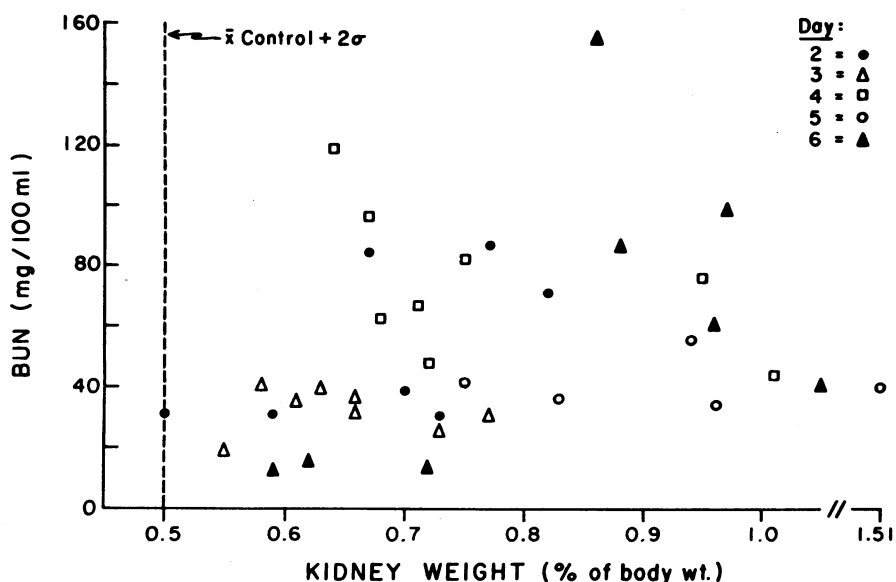
In the light of present knowledge, it is difficult to envisage a mechanism responsible for the phase of sustained acute renal failure which, when reversed, permits the rapid and complete return of glomerular filtration in increasing numbers of nephrons while filtration in the remainder stays minimally low or seemingly absent. The renin-angiotensin axis has been thought to play a key role in the pathogenesis of acute renal failure (13-17). Current concepts indicate that angiotensin conversion occurs extrarenally (18), however, so that the titer of angiotensin or of other known vasoactive agents reaching all nephrons should be the same. One must suggest, therefore, that factors inherent to individual glomeruli also play a significant role in this syndrome, even if circulating factors are involved. Since even normal glomerular control mechanisms remain poorly understood, however, any attempt to explain the findings in recovery from this model of acute renal failure in the rat must be highly speculative. Schnermann, Nagel, and Thureau have suggested that the sodium concentration of distal tubule fluid is important to the control of glomerular filtration in normal single neph-

rons (13). In view of the seemingly total collapse of nephrons in acute renal failure, however, there is little likelihood of fluid reaching the macula densa area where such control is said to be centered. Clearly, the cessation of filtration relates to a vasomotor phenomenon, but the effector mechanism cannot be considered to be adequately understood.

An element of tubular obstruction may be found early in the course of various models of acute renal failure in the rat. Flocculent material, presumably cell debris, can be seen to flow along the tubules of mercury-poisoned animals and, as flow progressively decreases, aggregate into structures which are potentially occlusive (7). Ruiz-Guiñazú, Coelho, and Paz (19) have reported that the proximal tubule hydrostatic pressure of dilated nephrons in rats subjected to methemoglobin-induced acute renal failure was some 5–7 mm of mercury higher than in controls, suggesting that the outflow resistance of these tubules was increased. The majority of the nephrons in that study, however, had grossly subnormal hydrostatic pressures (19). Jaenike (20) and Henry, Lane, and Kashgarian (21) have reported similar findings, leading them to postulate that obstruction and failure of filtration might both be operative. Occasional tubules have been found to have elevated hydrostatic pressure at all times during the period of sustained renal insufficiency in this model of renal failure also (2). Significantly elevated proximal tubule hydrostatic pressure was found very infrequently in the present study, however. If obstructive material significantly had delayed the return of glomerular fil-

tration or could be dislodged only at grossly supernormal intratubular pressure, a wide variation of intratubular pressure with many elevated values should have been found in these experiments. Such was not the case. Instead, those nephrons which were not totally collapsed had a remarkably uniform intratubular pressure, and only eight of 206 hydrostatic pressure values exceeded 25 cm of water. Any element of obstruction which may have developed during the oliguric phase must be assumed to have been rapidly and completely reversed, therefore, once intraglomerular filtration pressure achieved a meaningful value and not to have greatly influenced the return of glomerular function to normal.

Interstitial edema has been thought to increase intrarenal pressure and play an important role in the pathophysiology of acute renal failure. Previous studies have shown that edema is not causally related to the development of renal failure (1, 7) but Merrill has suggested on theoretic grounds that interstitial edema might impair glomerular filtration in the recovery phase of this syndrome (22). A significant degree of renal edema was indeed found in the present study, its extent increasing with time after the initiation of renal insufficiency and decreasing as the BUN became normal. Interstitial pressure is equal throughout the superficial aspect of the kidney (23), however, and is not expected to cause the virtual absence of function in some surface nephrons while causing supernormal filtration rates in others. Intratubular hydrostatic pressure, an index of interstitial pressure in a semiclosed compart-



ment (23), was not demonstrably increased in this study, and recovery was shown to occur in the presence of massive interstitial edema. It is unlikely, therefore, that interstitial edema is an important determinant of recovery in this model of acute renal failure in the rat.

The cause of interstitial edema in acute renal failure is unknown, but has been suggested to be either the result of increased tubular fluid absorption or tissue reaction to the content of the absorbate (11). The latter suggestion has little to recommend it, since the composition of the glomerular filtrate and proximal tubular absorbate is essentially the same as that of plasma. In the present study, tubule fluid absorption based on estimates of single nephron water transport was greatly subnormal, not increased as had been proposed. Nonetheless, renal lymph flow has been shown to be very high in acute renal failure (24). If this high lymph flow rate is not the result of increased transtubular absorption, it must reflect alterations in transcapillary fluid fluxes. Such alterations could manifest an increased permeability of the capillary bed to serum proteins, a concomitant of injury to any tissue (25). Whatever its cause, however, interstitial edema in this model of acute renal failure should not be attributed to the commonly cited theory of supernormal tubular fluid absorption.

In the present study impairment of proximal tubule sodium transport and low fractional water absorption were the most striking abnormalities found in the population of functioning nephrons. In view of the grossly supernormal volumes of collections obtained during the recovery period, there is no reason to believe that the low  $TF/P_{15}$  values obtained were an artifactual result of inulin leakage. Osmotic diuresis due to an elevated plasma urea concentration alone cannot have been responsible for the low fractional water absorption values, since the same degree of impairment in water absorption was seen in 21 tubules of six animals with BUN values below 20 mg/100 ml [ $TF/P_{15}$   $1.57 \pm 0.05$  (SE)] as was seen in 27 nephrons of the most severely azotemic rats with study day BUN values above 80 mg/100 ml ( $TF/P_{15}$   $1.57 \pm 0.05$ ). Extracellular fluid volume expansion which has been reported to result in decreased proximal tubule  $TF/P_{15}$  values in normal rats (26) cannot be incriminated because the animals under study spontaneously experienced a mean weight loss of  $6.9 \pm 0.6$  (SE)% below control weight while in renal failure. The same low  $TF/P_{15}$  values were found equally among nephrons with a low normal GFR and those whose filtration was supernormal ( $P < 0.25$ ), indicating that impaired fractional water absorption was not merely a response to augmented glomerular filtration in the recovery phase of this model of acute renal failure. It appears likely, therefore, that reduced

proximal tubule sodium transport and low fractional water absorption reflect incomplete repair of tubular injury which persists after the reversal of the glomerular filtration deficit. The degree of tubular impairment apparently was quite variable from nephron to nephron and the customary increase in inulin  $TF/P$  values at proximal tubule sites more distant from the glomerulus was lost. While this might represent impairment of sodium transport which is greater in distal than in proximal portions of the proximal tubule, the wide spread of values throughout the length of the proximal tubule available for micropuncture also might contribute to fractional water absorption and puncture site.

The increased proximal tubule water rejection fraction and urine output seen in these experiments may have a counterpart in man and explain the sometimes massive diuresis which occurs in the recovery phase of human acute renal failure. If such a diuresis derives from a small proportion of functioning nephrons, the rapid return of glomerular filtration in the remainder might result in a diuresis of such magnitude that it places the subject in jeopardy of rapid, life-threatening volume depletion. The grossly diminished glomerular filtration rate associated with acute renal failure, therefore, may have significant survival value.

#### ACKNOWLEDGMENTS

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