PART II

EDITORIAL
PATHOPHYSIOLOGY OF ACUTE RENAL FAILURE

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The pathophysiology of acute renal failure is not known as yet, despite the many experimental studies carried out in recent years. Three main pathophysiological mechanisms have been proposed and thoroughly investigated up to now: (1) passive backflow of filtrate, due to tubular epithelial necrosis resulting from a direct toxic effect or from an ischaemic event; (2) tubular obstruction, due to intratubular accumulation of debris and cast formation and/or tubular compression by interstitial oedema; (3) alteration in renal haemodynamics with loss of effective glomerular filtration pressure.

Passive Backflow of Filtrate

According to this theory the direct effect of toxic factors or the indirect effect of an ischaemic event are responsible for the tubular epithelial necrosis in acute renal failure; this tubular lesion allows the passive diffusion of filtrate from the tubular lumen toward the renal interstitium where it is reabsorbed into the blood stream. According to such a theory, therefore, renal blood flow and glomerular filtration rate are normal in acute renal failure, the anuria being explained by the backflow of filtrate.

This theory appeared to receive additional support from recent micropuncture experiments in rats with experimental acute renal failure secondary to mercuric chloride injection. Bank et al measured glomerular filtration rate in single nephrons (SNGFR) by micropuncture techniques in rats 24 to 48 hours after the subcutaneous injection of mercuric chloride, using inulin as marker. We know that the clearance of inulin in normal subjects corresponds to the glomerular filtration rate (GFR).

The same is valid for the measurement of the clearance of inulin in a single nephron by micropuncture techniques. If we puncture a superficial tubule of an exposed kidney of a normal rat and perform a timed complete collection of tubular fluid, the filtered load of inulin (SNGFR x Pinulin) must be equal to the inulin collected at the puncture site.
\[ \text{SNGFR} \times P_{\text{Inulin}} = TF_{\text{Inulin}} \times V \]

where \(TF_{\text{Inulin}}\) is the concentration of inulin in the tubular fluid sample and \(V\) is the volume of fluid collected in one minute.

Thus:
\[ \text{SNGFR} = \frac{TF_{\text{Inulin}} \times V}{P_{\text{Inulin}}} \]

Hence SNGFR must be constant whatever the puncture site along the nephron\(^6\). In rats with anuria secondary to \(\text{HgCl}_2\) injection, SNGFR measured as clearance of inulin was not constant along the nephron but decreased with the distance from the glomerulus, being low when the puncture site was located in the last superficial loops of the proximal tubules and even lower when the puncture site was in the distal tubule\(^4\). This observation was explained as the result of a leakage of inulin from the tubular lumen because of damage to the tubular wall.

Additional evidence came from studies of Lissamine Green injections. Lissamine Green is a dye which is immediately filtered (and neither secreted nor reabsorbed) by the kidney after injection into the jugular vein of a normal rat. After the injection of the dye, the latter soon appears in the superficial loops and the kidney surface becomes more and more deeply green as dye proceeds from the first loops of the proximal tubule to the distal tubule, because of the progressive concentration from water reabsorption. In rats with experimental anuria due to \(\text{HgCl}_2\) injection, the intensity of colour on the kidney surface after injection of Lissamine Green decreased as the dye progressed along the proximal tubule; furthermore the dye did not appear at all in the distal tubule\(^4\). These results suggest that there is a leakage of Lissamine Green, also, through the damaged tubular wall in mercury-poisoned rats, and support the conclusion that anuria in rats treated with \(\text{HgCl}_2\) is accounted for by a passive backflow of filtrate.

Steinhausen et al\(^5\) gave further support to this interpretation by showing that intratubular injection of \(^{14}\text{C}\) inulin in rats treated with \(\text{HgCl}_2\) was followed by the appearance of radioactivity in urine collected from the contralateral kidney, suggesting an unusual tubular reabsorption of inulin.

The studies of Bank et al\(^4\), however, were not confirmed by Barendberg et al\(^7\) and Flamenbaum et al\(^8\) who found a constant SNGFR measured by inulin clearance whatever the tubular puncture site in rats treated with \(\text{HgCl}_2\).

Furthermore, shrinking-drop experiments showed practically no reabsorption in the proximal tubules of mercury-poisoned rats\(^9\).

More recent studies\(^10\) have demonstrated that SNGFR is identical when measured by proximal or distal micropuncture in rats with uranyl nitrate-induced acute renal failure; furthermore, Lissamine Green injection into the jugular vein of these rats was followed by the appearance of dye not only in the proximal but also in the distal tubules.

The best recent evidence against a primary role of passive backflow of filtrate in the pathophysiology of acute renal failure has been given by Mason and Thurau\(^11\). These authors have carried out micropuncture experiments in which known quantities of sodium ferrocyanide or inulin were microinjected...
into early proximal tubules of normal rats and of rats with various models of acute renal failure; a complete collection was made from the late proximal tubule, the distal tubule or the final urine. The proximal recovery of sodium ferrocyanide was 97% in controls, 94% in rats treated with methaemoglobin, 91% in rats after 60 minutes of ischaemia, 94% in uranyl nitrate-poisoned rats and 93% in mercuric chloride-poisoned rats. The distal recovery of inulin was 95% in controls and 88% after 45 minutes of ischaemia. The urinary inulin recovery was 95% in controls, 82% after 45 minutes of ischaemia and 88% in uranyl nitrate-poisoned rats. Complete urinary recovery of inulin microinjected into the proximal tubules was previously obtained by others in rats with experimental acute renal failure.\(^8,12\).

On the basis of these results it can be concluded that even if passive backflow of filtrate takes place it cannot account for the anuria in acute renal failure, because it is not constant and, when present, is insignificant\(^10,11\).

**Tubule Obstruction**

According to this theory, the main pathogenetic factor in acute renal failure is tubule obstruction by intratubular accumulation of debris and cast formation\(^13–20\), and/or tubular compression by interstitial oedema\(^21,22\).

The driving force for glomerular filtration is the balance of hydrostatic and oncotic pressures across the glomerular membrane and the glomerular effective filtration pressure (EFP) is given by

\[
\text{EFP} = \text{P}_G - \text{P}_T - \pi_G
\]

where \(\text{P}_G\) is the glomerular hydrostatic pressure, \(\text{P}_T\) the hydrostatic intratubular pressure and \(\pi_G\) the oncotic pressure of plasma in the glomerular capillaries\(^23,24,25(*)\). Recent micropuncture studies have demonstrated that \(\text{P}_G\) is of the order of 45–50 mmHg\(^26–34\). If we use as \(\pi_G\) the arithmetic mean of oncotic pressures of blood entering and leaving the glomerulus, the mean EFP will be approximately 10 mmHg\(^30,33,35\).

Andreucci et al\(^35\) have demonstrated that partial ureteric obstruction in rats is followed by a fall in mean EFP from 10 mmHg to 7 mmHg with a proportional fall in SNGFR. It is therefore possible that intratubular accumulation of debris and cast formation, or tubular compression by interstitial oedema can reduce and then completely abolish EFP (and, therefore, glomerular filtration) because of the increase in \(\text{P}_T\). We may check this hypothesis by measuring \(\text{P}_T\) by renal micropuncture in rats with experimental acute renal failure.

These measurements have been carried out by several authors with different results. Thus Oken et al\(^36\) found a fall (rather than a rise) of \(\text{P}_T\) in rats with

\(^(*)\) Actually, the correct equation for calculating EFP is as follows:

\[
\text{EFP} = (\text{P}_G - \text{P}_T) - (\pi_G - \pi_T)
\]

where \(\pi_T\) is the oncotic pressure of tubular fluid due to filtered proteins. However, since protein concentration in the filtrate is quite low, \(\pi_T\) is trivial and may be omitted\(^6\).
glycerol-induced acute renal failure. Cirksena\textsuperscript{37} demonstrated a fall in PT between 25% and 50% in rats treated with methaemoglobin-ferrocyanide solution despite the intratubular appearance of pigment casts. Ruiz-Guinazu et al\textsuperscript{38} observed an initial rise in PT in methaemoglobin-treated rats followed by a significant decrease in PT. Flamenbaum\textsuperscript{10} found normal values of PT in uranyl nitrate-poisoned rats. Furthermore, it was observed that intratubular casts moved downstream when PT was increased by micropuncture to a value not sufficient to abolish EFP in rats with acute renal failure secondary to treatment with mercuric chloride, globin, dichromate or glycerol\textsuperscript{8,36,39}.

Tanner et al\textsuperscript{19,20} have recently found that in rats with ischaemia-induced acute renal failure most tubules were obstructed and very high pressures were needed to flush out the obstruction. Nevertheless, this factor was not considered, by the authors, as the sole reason for renal insufficiency.

These observations suggest that intratubular accumulation of debris and/or casts is not the cause of acute renal failure.

**Alteration in Renal Haemodynamics**

A few years ago a hypothesis was presented that ischaemic swelling of endothelial cells may occlude small blood vessels in the kidney so that the circulation is not restored even after removal of the initial cause of the ischaemia. In favour of this ‘no-reflow’ hypothesis was the efficacy of mannitol in shrinking swollen cells, thus permitting reflow of blood to the kidney\textsuperscript{40}.

Besides this interesting hypothesis, which is limited to ischaemia-induced renal failure, several authors have suggested and supported a peculiar vascular mechanism as responsible for acute renal failure\textsuperscript{9,36,41,42,43}. Many studies have provided evidence that a redistribution in intrarenal blood flow, with cortical ischaemia, takes place in uranyl nitrate or glycerol-induced acute renal failure in dogs and in rats\textsuperscript{44–47}, and in human acute renal failure, both in post-surgical and nephrotoxic cases\textsuperscript{48,49}. Many micropuncture studies have shown a fall in SNGFR in superficial nephrons of rats with different models of acute renal failure\textsuperscript{7,8,9,12,36,39,47,50}. These observations have suggested that an alteration in renal haemodynamics is responsible both for toxic and ischaemic acute renal failure. The mechanism responsible for such an alteration, however, is as yet not known.

**The Renin-Angiotensin System**

Goormaghtigh\textsuperscript{41} first suggested that the renin-angiotensin system has an important role in the pathophysiology of acute renal failure. This author demonstrated a distinct hypertrophy of cells in the juxtaglomerular apparatus of patients with post-traumatic renal failure; he postulated a release of a vasoressor substance from the juxtaglomerular apparatus resulting in a fall of renal blood flow and GFR. The measurement of plasma renin activity, however, in human acute renal failure and in different models of experimental acute renal failure led to conflicting results\textsuperscript{10,51–58}. In favour of an important role for the renin-angiotensin system in the pathogenesis of acute renal failure is the
demonstration that the i.v. infusion of angiotensin II in the rabbit resulted in renal functional and structural alterations similar to those usually observed in acute renal failure\textsuperscript{52,59}; furthermore \textsuperscript{133}Xe washout curves analogous to those obtained in acute renal failure were demonstrated after angiotensin II injection into the renal artery\textsuperscript{60}. On the other hand the cortical ischaemia in acute renal failure takes place in the outer cortex, i.e. in an area in which the nephrons have the greatest renin content\textsuperscript{61,62}.

The main factors regulating renin secretion in the normal kidney are: (a) wall tension in the renal afferent arterioles\textsuperscript{63,64,65}; (b) availability of sodium chloride at the macula densa level\textsuperscript{66–70}; (c) sympathetic nervous system activity or catecholamine release into the circulation\textsuperscript{71–79}; (d) potassium balance\textsuperscript{80–85}.

We have recently underlined the important role of renin suggested by Thurau\textsuperscript{66} in the intranephron regulation of salt excretion\textsuperscript{23}; this was based on the clear demonstration that all elements necessary for angiotensin II production (i.e. renin substrate, renin, converting enzyme) are located within the kidney. Hence when a rise in glomerular filtration takes place, more NaCl is delivered to the macula densa; this represents the signal for local production of renin; angiotensin I is thus formed which is converted into angiotensin II by the converting enzyme; a constriction of the afferent arterioles by angiotensin II will take place; thus glomerular filtration will be lowered, so abolishing the first step (i.e. rise in glomerular filtration) which was responsible for the signal (i.e. high NaCl delivery to the macula densa). According to Thurau, therefore, a tubular-glomerular feedback mechanism is continuously functioning in the normal kidney in order to prevent salt depletion by adjusting glomerular filtration to the reabsorption capacity of the proximal tubule\textsuperscript{24}.

Mason and Thurau\textsuperscript{11} have now suggested that this mechanism of tubular-glomerular feedback also functions when proximal tubular reabsorption is reduced because of tubular damage; the increased delivery of NaCl to the macula densa will be the signal for the intrarenal activation of the renin-angiotensin system; the constriction of afferent arterioles by angiotensin II will account for the abolition of glomerular filtration and, consequently, for the anuria.

However, for this mechanism to work, it should be demonstrated that in acute renal failure the following conditions are fulfilled: (a) sodium chloride concentration at the macula densa level is higher than normal; (b) the renin-angiotensin system can be activated as in normal conditions, and (c) the tubular-glomerular feedback is still working\textsuperscript{11}.

Schnermann et al\textsuperscript{43} have demonstrated that sodium chloride concentration in tubular fluid samples collected by micropuncture from the first superficial loop of distal tubules (i.e. near the macula densa) is higher than normal in rats with post-ischaemic acute renal failure. Similar results have been obtained by Flamenbaum et al\textsuperscript{85} in rats with uranyl nitrate-induced acute renal failure. Furthermore in both post-ischaemic and uranyl nitrate models of acute renal failure an increased juxtaglomerular renin activity has been shown\textsuperscript{11,86}.

These results clearly show that in experimental acute renal failure the signal is present at the macula densa level and that the renin-angiotensin system is
capable of being activated by this signal.

Mason and Thurau\textsuperscript{11} have recently given evidence that the tubular-glomerular feedback works normally in rats with experimental acute renal failure. The microperfusion of Henle’s loops (including the macula densa) with mannitol (ie without NaCl) and with isotonic NaCl, while measuring SNGFR by proximal micropuncture, clearly showed that SNGFR is higher during mannitol microperfusion (ie when the signal NaCl is absent) than during NaCl microperfusion (ie when the signal is present). In addition, when furosemide was added to the isotonic sodium chloride solution used for microperfusion, SNGFR rose to the values obtained with mannitol microperfusion\textsuperscript{11,87}; this is accounted for by the effect of furosemide in reducing NaCl reabsorption at the level of the macula densa, thus interfering with the ability of the macula densa to detect the increased sodium chloride concentration within the tubular lumen\textsuperscript{87}.

Further evidence in favour of the important role of the renin-angiotensin system in the pathogenesis of acute renal failure is the protective effect of salt loading against the development of acute renal failure.

Thus rats drinking 1\% sodium chloride rather than water for three weeks did not develop acute renal failure after an intramuscular injection of 50\% glycerol; acute renal failure was, however, induced with the same injection in water-drinking rats\textsuperscript{88,89}. Similar results were obtained by others in rats treated with dichromate\textsuperscript{39}, mercuric chloride\textsuperscript{90} and uranyl nitrate\textsuperscript{86}. Particularly interesting is the observation of normal renal function in salt-loaded rats despite a tubular necrosis similar to the one observed in ‘non protected’ rats, ie in water-drinking rats with acute renal failure\textsuperscript{26,90}. High salt diets are known to suppress the renin-angiotensin system through an inhibition of renin synthesis\textsuperscript{91}. Hence the conclusion that salt loading protects rats against experimental acute renal failure through renin depletion. This is in favour of the hypothesis that the pathogenetic mechanism of acute renal failure relies on a normally operating renin-angiotensin system.

Flamenbaum et al\textsuperscript{47} have given the best evidence that the renin-angiotensin system participates in the pathogenesis of experimental acute renal failure via local intrarenal renin rather than circulating plasma renin. Potassium chloride loading (known to inhibit renal renin synthesis and release) partially protected rats against acute renal failure secondary to mercuric chloride. KCl, however, was less effective in protecting the animals than NaCl, despite a comparable degree of plasma renin activity suppression. Since the suppression of renal renin content was less marked with KCl than with NaCl, the difference in protection appeared to be related to the minor fall in intrarenal renin during KCl loading. Further support for this conclusion is given by the observation that both the administration of desoxycorticosterone acetate (DOCA) plus NaCl and immunisation against renin, do not modify renal renin content nor the degree of acute renal failure in glycerol-poisoned rats, despite a significant fall in plasma renin activity\textsuperscript{46,47}.

The Prostaglandins

Increasing evidence has been provided during the past few years that lipid
hormones synthetised by the interstitial cells in the renal medulla play an important role in regulating intrarenal haemodynamics by opposing the effects of the activity of the renin-angiotensin system. In particular the prostaglandins, which are most potent vasodilators, increase renal blood flow, especially in the renal cortex, as well as sodium and water excretion; their inhibition, in fact, results in a significant reduction in total renal blood flow. Kotchen and Miller have recently given evidence of an 'in vitro' inhibition of angiotensin generation by prostaglandins. Hence prostaglandins may counteract the renin-angiotensin system both by inhibiting angiotensin generation and by antagonising the renal cortical vasoconstriction caused by angiotensin.

Prostaglandins are synthesised mainly in the renal medulla but have their effects on the cortical circulation. Only small amounts of prostaglandins are synthesised in the cortex which is rich in 15-hydroxydehydrogenase, the main metabolising enzyme for prostaglandins. Because of this enzyme it seems unlikely that prostaglandins synthesised in the medulla reach the cortex by diffusion; if so they would be enzymatically degraded before reaching their site of action.

Williams et al. have given evidence for the renal origin of urinary prostaglandins in dogs; renal prostaglandins would enter the urine at the distal nephron (possibly at the loop of Henle). This observation suggests that Henle's loop is the pathway through which prostaglandins travel from the medulla to the cortex.

According to Oken prostaglandins arrive in the tubular fluid at the macula densa level where they antagonise the vasoconstriction of glomerular arterioles. If this is the case, prostaglandins would play a key role in autoregulation. Any time a constriction of efferent arterioles takes place, in fact, the fall in SNGFR will reduce the tubular flow along Henle's loop; the slow flow and the increased prostaglandin release during renal ischaemia would increase prostaglandin concentration in the tubular fluid at the macula densa level. This will allow a greater antagonising effect on afferent arteriole constriction thus normalising SNGFR. In favour of this important role of prostaglandins is the demonstration that autoregulation in isolated perfused kidneys is diminished or even eliminated by blocking prostaglandin synthesis with indomethacin.

These results suggest that regulation of intrarenal haemodynamics results from the activity of two systems, the cortical vasoconstrictive renin-angiotensin system and the medullary vasodilator prostaglandin system.

A few years ago Fine suggested that haemodynamic changes in acute renal failure were secondary to disturbed prostaglandin secretion. More recently Torres et al. have produced evidence that renal prostaglandins protect against the development of the circulatory but not the nephrotoxic type of acute renal failure in rabbits. Indomethacin (a blocking agent of prostaglandin synthesis) enhanced the incidence and severity of glycerol-induced acute renal failure (a circulatory type of renal failure) but failed to aggravate that produced by mercuric chloride (a nephrotoxic type of renal failure).

Even more interesting are the experiments of Held et al. in which
the role of prostaglandins in an ischaemic model of acute renal failure was tested not by inhibiting their synthesis, but by preserving their activity. Thus mononephrectomised rabbits in which renomedullary homogenate (containing interstitial cells with the characteristic lipid droplets) was autotransplanted, were partially protected against temporary (90 minutes) renal ischaemia. All rabbits in the control group, in fact, died of uraemia, while the mortality of autotransplanted rabbits was limited to 50%, and renal function in surviving animals was preserved. These experiments suggest that post-ischaemic acute renal failure may be the result of an imbalance between the cortical vasoconstrictive renin-angiotensin system and the medullary vasodilator prostaglandin system, leading to vasoconstriction

If this hypothesis is correct the question follows: why is the activity of the prostaglandin system deficient in acute renal failure? According to Oken if prostaglandins travel from the medulla to the cortex via Henle’s loop, cessation of glomerular filtration will prevent prostaglandin migration to the cortex by stopping flow through the loop. Thus the activity of the renin-angiotensin system at the level of the afferent arterioles will not be counterbalanced by prostaglandins. In favour of this is the observation that non-filtering nephrons of mercuric chloride poisoned rats began to filter again after microinjection of isotonic saline into the proximal tubules at physiological hydrostatic pressure, possibly because of restoration of the pathway through which prostaglandins travel from the medulla to the macula densa.

Author’s Hypothesis

Oken’s hypothesis does not suggest the mechanism by which glomerular filtration is stopped. It is possible, however, that cessation of prostaglandin delivery to the cortex is important only in maintaining acute renal failure. The author suggests the following hypothesis (Figure 1). Because of the direct effect of toxins, or following prolonged renal ischaemia, proximal tubular damage/dysfunction may take place; this will induce a fall in proximal reabsorption with increased NaCl delivery to the macula densa cells; NaCl intake by the latter will represent the signal for intrarenal production of renin which will induce local production of angiotensin I and then of angiotensin II under the effect of renal converting enzyme; angiotensin II will constrict the afferent arteriole; thus glomerular filtration will fall, accounting for the oliguria. The decrease in tubular flow along Henle’s loop will impair prostaglandin delivery to the cortex with the following consequences: (a) greater production of angiotensin I (because renal renin is not inhibited), and (b) greater constriction of afferent arterioles (because the vasoconstrictor effect of angiotensin is not antagonised). These effects will maintain marked oliguria.

In this hypothesis treatment with mannitol, with ethacrynic acid and with furosemide is effective only in the first steps, by inhibiting NaCl intake by the macula densa cells; without the ‘signal’ the local overproduction of renin and angiotensin will not take place. The high dosage of diuretics required is accounted for by the need for a high concentration in the tubular fluid for the inhibition of NaCl reabsorption at the macula densa level.
PATHOGENESIS OF ACUTE RENAL FAILURE

INITIATING EVENT
(TOXIN, SHOCK, UNKNOWN)

PROXIMAL TUBULAR
DAMAGE / DYSFUNCTION

PROXIMAL TUBULAR
REABSORPTION

MANNITOL
Furosemide

Na Cl
MACULA Densa

DELIVERY OF
PROSTAGLANDINS
TO THE CORTEX

RENAL
RENIN

RENAL ANGIOTENSIN I

RENAL ANGIOTENSIN II

GLOMERULAR
FILTRATION RATE

TUBULAR FLOW
(incl. HENLE'S LOOP)

OLIGURIA

AFFERENT
ARTERIOLAR
CONSTRICITION

Figure 1
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