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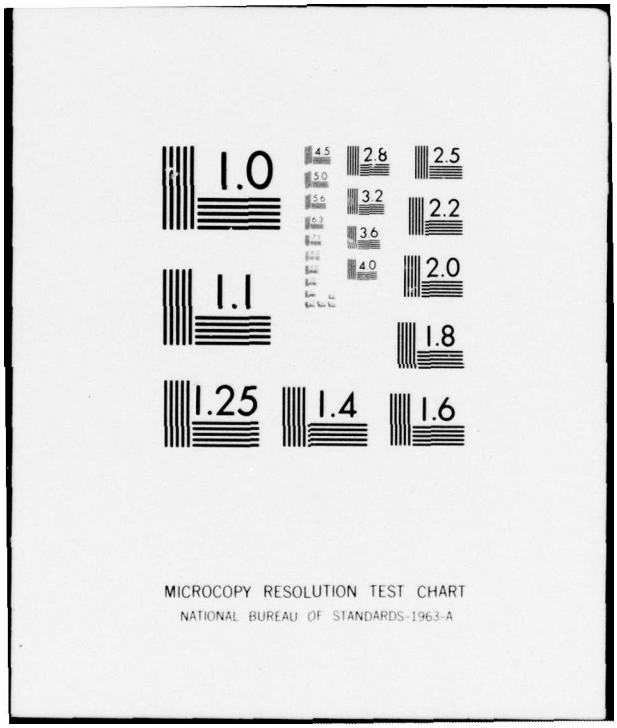
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INTRARENAL REGULATING MECHANISMS FOR RENAL HEMODYNAMICS DURING SHOCK

Final Technical Report

By

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20 ABSTRACT

Several models of experimental acute renal failure were used to study the leakiness of the tubular epithelium to inulin, the change in intratubular hydrostatic pressure and the operation of the tubulo-glomerular feedback mechanism.

The results show that (1) leakage of the acutely damaged tubular epithelium cannot be the causal factor for low inulin clearance, (2) the variability of the intratubular pressure in the acutely damaged kidney precludes the possibility that increased pressure is always associated with acute renal failure and (3) the operation of the tubulo-glomerular feedback mechanism is maintained in acute renal failure and preserves body fluids by glomerular shutdown, with resulting oliguria.

1.0 Summary

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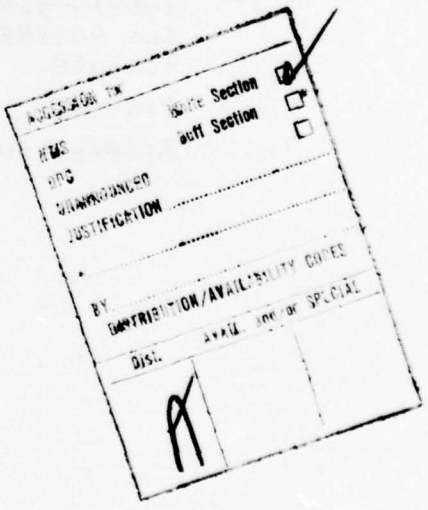

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3.0 Introduction

The pathophysiology of the impairment in the renal function during experimental acute renal failure has been studied intensively using various animal models. It is clear from this work that at the present time no single unifying hypothesis is available to explain the sequence of changes in renal function occuring during acute renal failure in man. However, as our knowledge of normal renal function has increased considerably during the last years, mainly because of the availability of micropuncture data, the rational approach to the pathophysiology of acute renal failure has incorporated new aspects which, at present, are under experimental study in many laboratories throughout the world (1).

As indicated in the grant application, the main purpose of this study was to investigate during experimental acute renal failure the mechanism of tubolo-glomerular feedback, which in the normal kidney adjusts filtration rate to the reabsorption capacity of the nephron.

There is increasing experimental evidence to show that the feedback control of glomerular filtration rate(GFR), operating through the function of the juxtaglomerular apparatus (JGA), participates in the establishment of tubulo-glomerular balance.

Two lines of evidence suggest that the adjustment of the glomerular filtration dynamics to the reabsorptive capacity of the tubular epithelium occurs by a vasomotor adjustment of the filtration dynamics, induced by a local formation and function of varying amounts of vasoactive angiotensin II in the area of the JGA. Firstly, in the normal rat kidney, it was demonstrated that an increase in NaCl concentration at the macula densa segment, produced by retrograde perfusion of the loop of Henle, increases the renin activity of that JGA belonging to the same nephron unit (2). Secondly, using the single nephron stop-flow pressure technique from which the glomerular capillary pressure can be derived, Schnermann (3.5) had shown that increasing NaCl signal to the macula densa leads to a decrease in glomerular capillary pressure, a result consistent with a vasomotor-induced decrease in GFR.

Because injury to the tubular cells is reflected in a reduction of their most important function, that of salt and water reabsorption, the low GFR may indicate the maintenance of a tubulo-glomerular balance to minimize loss of volume. Were this the case, the renin-angiotensin system would be expected to be activated during experimental acute renal failure and the feedback sensitivity to be intact.

4.0 Methods

The experiments were performed on Sprague-Dawley rats (150 to 250 g in body wt, maintained on a standard diet Altromin) with free access to water. The rats were anesthetized with thiobarbital (Inactin, 100 mg/kg of body wt i.p.

4.3 Intratubular Pressure.

Proximal tubules were punctured randomly and intratubular pressure was measured with the Landis technique, using a Gauer manometer and shortshafted micropipettes, 9-11u OD, filled with 0.5 or 1.0% lissamine green solution

4.4 Tubulo-glomerular Feedback.

To test the viability of the feedback mechanism after the initiation of experimental acute renal failure, the feedback response of heme pigment, ischemic and nephrotoxic damaged kidneys of the rat was investigated by altering the macula densa fluid composition by microperfusion techniques and evaluating the change in glomerular function. Normal animals, which received no noxious substance or direct renal damage, served as controls. Temporary renal ischemia was produced in two groups, by clamping the renal artery 45 or 75 min. Nephrotoxic acute renal failure was produced in two groups either by administration of uranyl nitrate, 10 mg/kg s.c. 6 to 18 hr before anesthesia, or by adminstration of mercuric chloride, 4.7 mg/kg s.c. 1 to 4 hr prior to anesthesia. Acute renal failure was induced in one group by administering methaemoglobin, 0.5 g/kg i.v. after 36 hr dehydration, 1 to 2 hr before anesthesia.

The macula densa sodium chloride concentration was changed directly to its theoretical minimum of zero by retrograde perfusion with isotonic Riner's or mannitol solution and the glomerular filtration rate was determined simultaneously.

Renin activity in single JGA's was assessed as follows: Microfil (Canton Biomedical Products) was injected into the renal circulation via a catheter placed in the carotid artery until the microscopic observation of the renal surface showed filling of the superficial glomerular capillaries.

At that moment, both renal artery and vein were occluded and the kidney was removed and frozen by immersion into a mixture of acetone and dry ice. Thereafter, the kidneys were freeze-dried at 0^{o} C for 24 to 36 hr.

The renin assay has been described by Dahlheim et al.(4) and, in short, is based on the determination of the enzyme kinetics within 15 to 60 min of incubation. The JGA's were homogenized in 0.1 ml of substrate solution by ultrasound and the incubation was performed at 37^{o}C in 150 mM phosphate buffer at pH 6.5. The angiotensin formed in the incubate was quantified using the rat blood pressure assay (injected volume, 0.01 to 0.05 ml).

5.0 Results and Conclusions

5.1 Reliability of inulin as a glomerular marker:

Proximal recovery of sodium ferrocyanide was $97\pm2.0\%$ in controls, $94\pm4\%$ after 0.75 g/kg methaemoglobin, $91\pm7\%$ after 60 min ischaemia, $94\pm5\%$ after 15 mg/kg uranyl nitrate and $93\pm5\%$ after 6.0 mg/kg mercuric chloride. Distal recovery of inulin was $95\pm2\%$ in controls and $88\pm10\%$ after 45 min ischaemia. Urinary inulin recovery was $95\pm4\%$ in controls, $82\pm4\%$ in controls, $82\pm11\%$ after 45 min ischaemia and $88\pm5\%$ after 10 mg/kg uranyl nitrate. These data which indicate that significant leakage of glomerular markers need not be associated with acute renal failure, are at discrepance with those obtained by others. Although most investigators agree that proximal and distal leaks do not occur, significant losses of inulin, when recovered from the final urine, have been reported. Nevertheless, some workers find, in agreement with us, that urinary inulin recovery is almost complete and leak is not apparent (5). The conclusion must therefore remain that although leakage may accompany acute renal failure, it cannot be the causal factor for the symptoms, for it is not universally present.

Arterial blood pressure was measured continuously through a polyethylene catheter in the carotid artery by means of a strain gauge and body temperature was maintained at 37.5°C.

The ureters of both kidneys were cannulated for the collection of urine. Glomerular filtration rate was estimated from the inulin clearance, inulin concentration in blood and urine being determined by the anthrone method.

4.1 Models of experimental acute renal failure:

The acute renal failure experiments were performed on ten groups of animals, comparible in body weight and ranging from 198-318 g. The nature of the noxa and the dose it was administered were as following:

methaemoglobin, 0.50 and 0.75 g/kg
uranyl nitrate, 10 and 15 mg/kg
mercuric chloride, 4.7 and 6.0 mg/kg
post-ischaemic kidneys, 45,60 and 75
renal artery clamping

4.2 Recovery of inulin in tubular fluid:

To test the leakiness of the proximal convolution to a low molecular weight marker, sodium ferrocyanide, of the distal tubule and whole nephron to a higher molecular weight substance inulin, the following experiments were performed in various models of acute renal failure: known quantities of these substances were microinjected into the early proximal tubule and quantitative collection was made either from the late proximal tubule, the distal tubule or the final urine.

5.2 Intratubular hydrostatic pressure during experimental acute renal failure.

The individual values of proximal intratubular pressure in normal and acute failure kidneys, presented as a population frequency distribution, are depicted in fig. 1 and 2.

In the 0.5g/kg methaemoglobin kidneys pressure was significantly raised by 61%. However, in approximately one third of the population, pressure was in the normal range. In the 0.75g/kg methaemoglobin kidneys, pressure was statistically higher than that of the controls, but only by 7%. In the 45 min ischaemic kidneys, pressure was not significantly different from the control values. In the 60min ischaemic kidneys, pressure was significantly higher than the controls, the increase representing 50%. In the 75min ischaemic kidneys, pressure in the patent tubules was signifcantly higher than the controls, the rise representing 64%.

In the 10mg/kg uranyl nitrate model, pressure was significantly lower than the controls and in the 15mg/kg uranyl nitrate kidneys, pressure was not siginifcantly different from the controls.

In the 4.7mg/kg mercuric chloride kidneys, pressure was not significantly different from controls and in 6.0mg/kg mercuric chloride kidneys, pressure was significantly higher than in the corresponding controls, the increase representing 37%.

Examination of the values of intratubular pressure obtained in acute renal failure kidneys revealed them to be either elevated, unchanged or reduced.

5.3 Tubulo-glomerular feedback, its significance for the decrease in GFR during experimental acute renal failure

There is now evidence to support the concept that glomerular filtration is confined to a level which matches the ability of the tubular epithelium to reabsorb. This tubuloglomerular feedback functions to keep filtration and reabsorption in balance so that excessive filtrate is not delivered to an epithelium unable to reabsorb it. The signal which causes the filtration rate to fall is not the increased flow itself, but the sodium chloride concentration at the top of the loop of Henle, at the macula densa. Under normal circumstances the concentration in this macula densa segment is hypotonic, but rieses with flow rate through the loop of Henle. It can be shown that the same flow-dependent signal which causes filtration rate to reduce, namely a rise in macula densa sodium chloride concentration, causes an increase in the renin activity of the juxtaglomerular apparatus of that nephron (Table 1 and 2). The implication of these experiments is that the increased macula densa sodium chloride concentration causes the reduction in filtration rate by activating the renin angiotensin system, stimulating local formation of angiotensin II and producing constriction of the glomerular arterioles.

The question then arises as to whether such a renin angiotensin mediated control of GFR could be responsible for the reduced clearance in acute renal failure. The same balance which occurs in normal kidneys, to stabilise filtration to reabsorptive capacity would be all the more useful in the damaged kidney in which tubular reabsorption is known to be impaired. This tubuloglomerular feedback could play an essential role in stopping the glomerulus supplying normal amounts of filtrate to an epithelium completely unable to reabsorb it. However, for the feedback mechanism to work,

sodium chloride conconcentration at the macula densa must be higher than normal, the renin angiotensin system must be capable of being activated and the existence of tubuloglomerular feedback in acute renal failure kidneys must be demonstrated.

Indication that the macula densa signal necessary to reduce GFR is available in post-ischaemic and uranyl nitrate models has been obtained by measuring increased sodium chloride concentration at the closest site, the early distal tubule (6). The increased juxtaglomerular renin activity which would be expected to accompany a raised macula densa sodium chloride concentration has also been found in ischaemic and uranyl nitrate damaged kidneys (fig. 1) (6), a fact illustrating that the renin angiotension system can respond to the macula densa signal.

The demonstration that the action of such a feedback mechanism is feasible in models of acute renal failure was obtained by proving that the mechanism is still operational in acute renal failure, that the signal could still be perceived and the glomerular vessels could still respond.

In each type of acute renal failure investigated, the macula densa sodium chloride signal was altered maximally from zero to isotonicity by perfusion with isomotic mannitol and isotonic Ringer's solution, and the single nephron filtration rate was determined. It can be seen (Fig. 3) that for each type of acute renal failure examined, filtration rate is higher during sodium chloride free perfusion than during Ringer's perfusion, indicating that receptor mechanism and response have all survived renal damage.

In acute renal failure , therefore, the feedback mechanism described and schematically illustrated in Figure 4, provides a means to maintain tubuloglomerular balance by reducing the glomerular filtration rate to a level compatible with the attenuated reabsorptive capacity. Both the sensing and response elements of the feedback mechanism have been found to be functionally intact in various models of acute renal failure (7).

Reduction in glomerular filtration rate to conform to diminished reabsorptive capacity means that the glomeruli have taken over the volume-conserving function normally exercised by the tubules. The importance of this reduction in glomerular filtration rate is made clearer by considering what might happen if the glomerular filtration rate remained normal and the tubular reabsorption were reduced by 50 per cent. Urinary volume and sodium excretion would be half the filtered load. In man, the result would be a urinary volume loss of approximately 60 ml/min and, unless salt and water loss could be replaced, the organism would lose its last sodium ion within a few hours. Volume conservation by reversible glomerular shutdown is immediately effective and therefore life-saving. It has the disadvantage of being indiscriminate; consequently nitrogenous wastes and other materials regularly cleared by filtration are retained. The immediate threat of death by hypovolemia is averted at the expense of the regulation of body fluid composition. This decision allows the organism time to repair structure and function of the damaged tubules.

Nephron damage in acute renal failure is of quite variable severity. Many instances of tubular injury undoubtedly occur in which the reduction in glomerular filtration rate is moderate and retention insufficient to come to the clinician's attention. It is only when reabsorptive function is severely reduced that depression of the glomerular filtration rate leads to a degree of retention that is clinically relevant. The degree of retention in some measure, therefore, reflects the extent of tubular reabsorptive insufficiency.

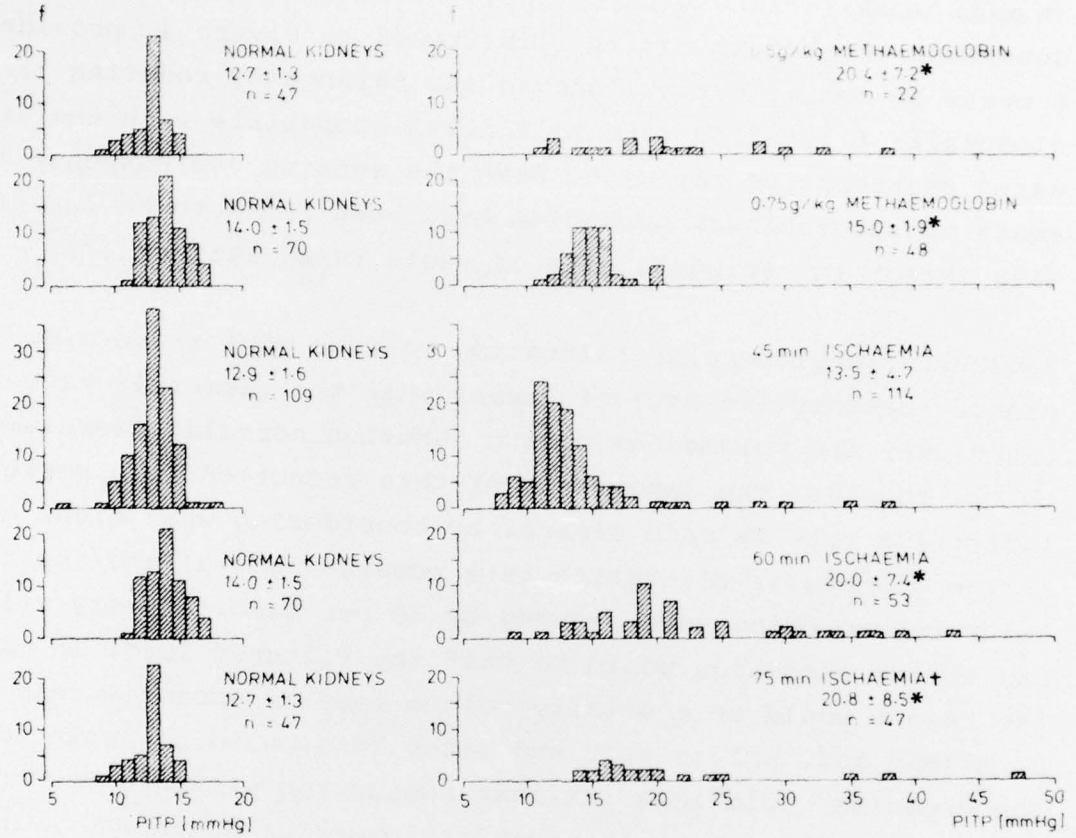

fig. 1. Proximal intratubular pressure, PITP, plotted as a frequency population histogram, for normal kidneys and methaemoglobin and ischaemic models of acute renal failure. For each group, the mean $\pm$ SD and number of measurements is given. * denotes statistically significant difference from the corresponding control values, given on the left.

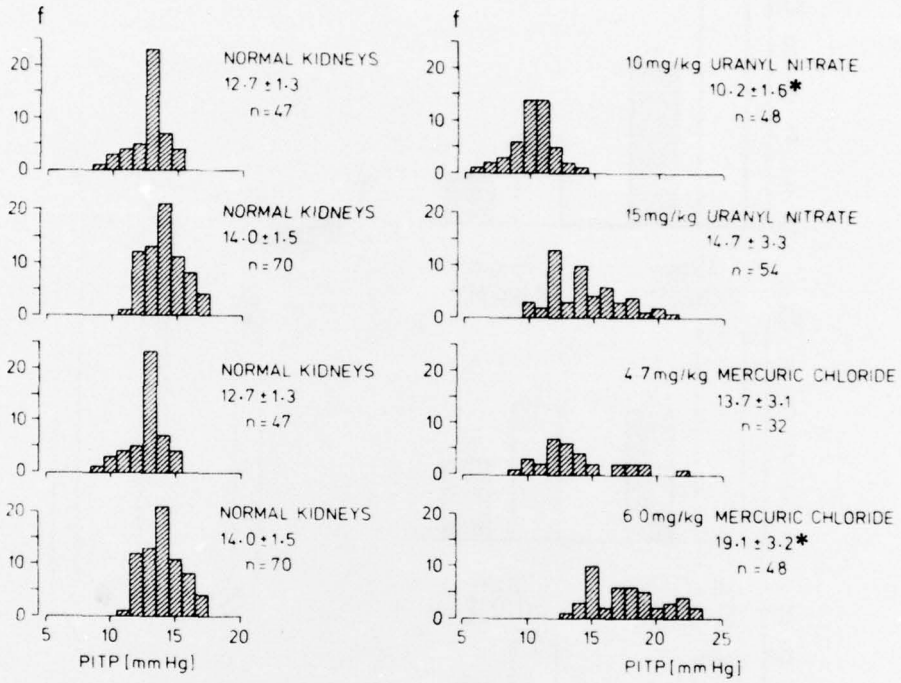

fig. 2. Proximal intratubular pressure, PITP, plotted as a frequency population histogram, for normal kidneys and uranyl nitrate and mercuric chloride models of acute renal failure. For each group, the mean $\pm$ SD and number measurements is given. * denotes statistically significant difference from the corresponding control values, given on the left.

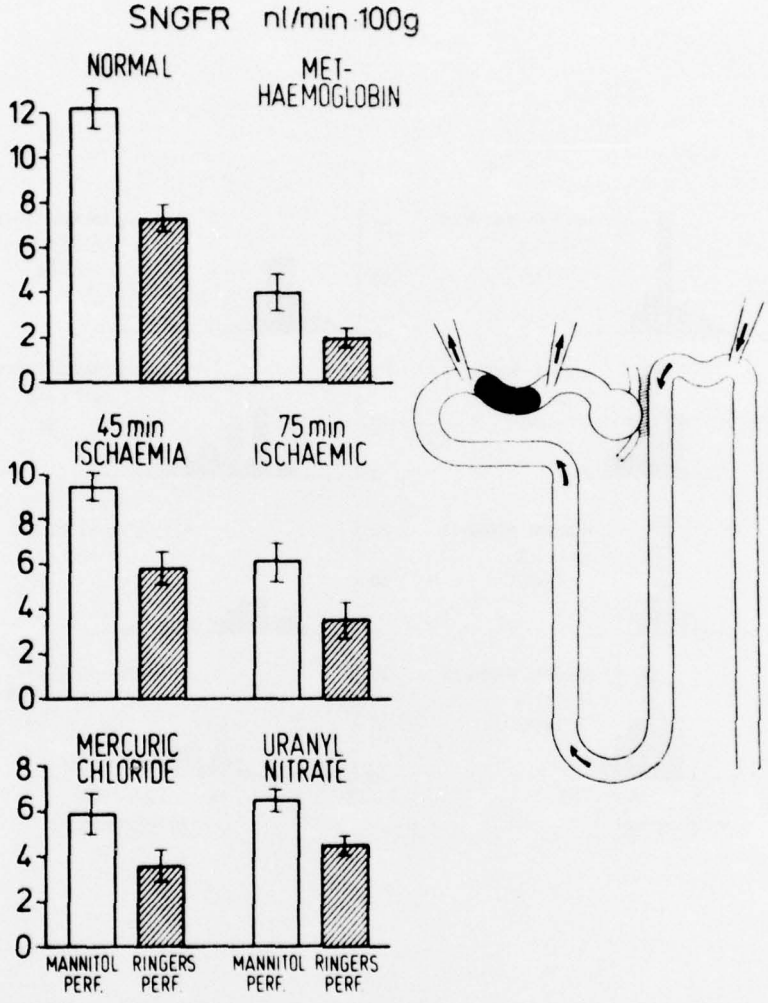

Fig. 3 The effect of retrograde perfusion of the macula densa segment with sodium chloride free mannitol or isotonic Ringer's solution on nephron filtration rate in various models of acute renal failure. Data obtained in cooperation with Olbricht and Takabatake.

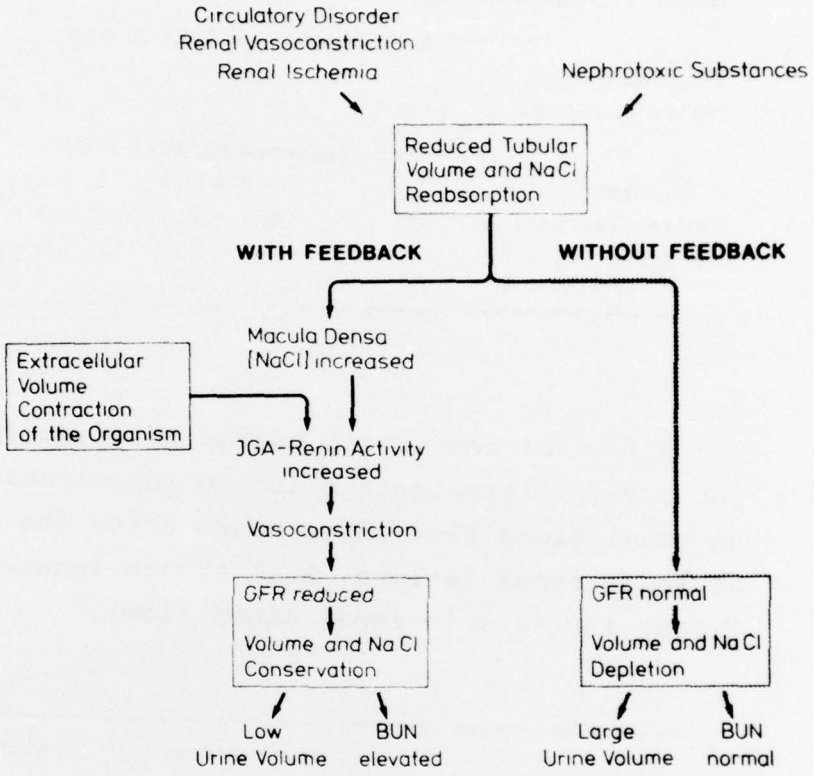

Fig. 4 Diagram illustrating the postulated effect of the presence or absence of tubuloglomerular feedback on urine volume and blood urea nitrogen in the inadequately reabsorbing tubules of an acute renal failure kidney.

	Left Kidney	Right Kidney
Control		
V (µl/min)	3.7 ± 0.7	3.5 ± 0.7
Inulin Clearance (ml/min)	0.8 ± 0.1	0.9 ± 0.4
During Ischaemia of Left Kidney		
V (µl/min)		3.4 ± 0.4
Inulin Clearance (ml/min)		0.6 ± 0.1
Following Ischaemia of Left Kidney		
V (µl/min)	1.0 ± 0.4	2.8 ± 0.5
Inulin Clearance (ml/min)	0.1 ± 0.1	0.4 ± 0.1
JGA Renin Activity (ng/0.1 ml·h)	6.2 ± 1.8	3.8 ± 1.1

table 1 The effect of converting enzyme blockade (SQ 20881) and a competitive antagonist to angiotensin (P113) on renal blood flow three hours after the induction of acute renal failure. Both agents induced a significant increase in renal blood flow.

	Left Kidney	Right Kidney
Control		
V (µl/min)	2.2 ± 0.2	2.3 ± 0.3
Inulin Clearance (ml/min)	1.0 ± 0.1	1.1 ± 0.1
During Ischaemia of Left Kidney		
V (µl/min)		2.9 ± 0.3
Inulin Clearance (ml/min)		1.1 ± 0.2
Following Ischaemia of Left Kidney		
V (µl/min)	0.8 ± 0.2	2.3 ± 0.4
Inulin Clearance (ml/min)	0.2 ± 0.1	0.9 ± 0.1
JGA Renin Activity (ng/0.1 ml·h)	11.0 ± 3.3	6.8 ± 2.1

table 2 The effect of graded doses of P113 on renal blood flow 24 hours after induction of acute renal failure. Note the significant increase in renal blood flow induced by 3 and 10 g/kg/min, despite a significant reduction in arterial blood pressure induced by P113 at this time.

7.0 Literature

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