FACTORS AFFECTING THE OUTCOME OF PATIENTS WITH ACUTE RENAL FAILURE

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Superficially it might be said that the function of the kidneys is to make urine, but in a more considered view one can say that the kidneys make the stuff of philosophy itself.

Let our kidneys fail for even a short time to fulfil their task, and our mental integrity, our personality, is destroyed.

Homer W Smith, 1940

Lectures on the Kidney

Abstract

The principle aim of this thesis is to examine the hypothesis that patients with acute renal failure (ARF) who are treated with high dose loop diuretics have a better prognosis in terms of survival, need for dialysis and speed of recovery than those who do not receive these drugs.

A prospective, double blind, randomised, placebo controlled study was carried out on 94 patients with ARF in Glasgow Royal Infirmary. The use of loop diuretics caused a significant increase in urine output and fractional excretion of sodium in the first 24 hours, but had no effect on the final outcome (recovery, dialysis or death) at day 21. Patients who became non-oliguric (with or without loop diuretics) had a better survival but were less ill (APACHE II score 17.2 v 20.6, $p=0.007$, non-oliguric v oliguric) and had less severe renal failure (creatinine clearance 14ml/min v 4.5ml/min, $p<0.0001$) than those who remained oliguric.

Loop diuretics can lower cytosolic calcium in normal individuals and in those with hypertension. Because cell death has been shown to be accompanied by a rise in intracellular calcium, I postulated that cytosolic calcium levels would be high in patients with ARF. Further, the use of loop diuretics might lower cytosolic calcium in patients with ARF and thereby exert a beneficial effect on renal tubule cells.

Intraplatelet calcium levels were high in patients with ARF compared to normal controls (109nm v 92.4nm, $p=0.004$). Administration of a loop diuretic had no effect on intraplatelet levels of calcium.

I hypothesised that this rise in intracellular calcium might be related to the severity of illness and thus correlate with the APACHE II score, an objective scoring system used to stratify patients according to prognosis. No correlation was found.

Finally, indirect calorimetry is an accurate, although painstaking, method of measuring energy expenditure in the critically ill patient. Metabolic rate may be related to clinical outcome. If the resting energy expenditure (REE) correlated with the APACHE II score, the latter, simpler measurement could be used as part of a formula to predict metabolic rate. My studies of REE in patients with ARF showed no correlation with the APACHE II score which should therefore not be used to predict energy expenditure in ARF. Nor was there any association between metabolic rate and the clinical outcome of the patient.

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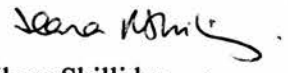
Declaration

I declare that the work presented in this thesis is my own and that I have composed the thesis.



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List of Abbreviations

$[Ca]_f$	free cytosolic calcium
$[Ca]_i$	free intracellular calcium
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
ANP	atrial natriuretic peptide
APACHE	acute physiology and chronic health evaluation
APIII	atriopeptin III
APIII-D	atriopeptin III plus dopamine
ARF	acute renal failure
ATP	adenosine triphosphate
AVP	arginine vasopressin
BEE	basal energy expenditure
BMR	basal metabolic rate
BSA	body surface area
cGMP	cyclic guanosine monophosphate
EGF	epidermal growth factor
EE	energy expenditure
EGTA	ethyleneglycolbis(aminoethylether)tetra-acetate
ET	endothelin
FeNa	fractional excretion of sodium
FiO_2	inspired oxygen concentration
GFR	glomerular filtration rate
HBREE	Harris-Benedict resting energy expenditure

List of Abbreviations (continued)

HGF	hepatocyte growth factor
ICAM	intercellular adhesion molecule
ICU	intensive care unit
IGF-1	insulin like growth factor-1
K_f	(glomerular) ultrafiltration coefficient
mTAL	medullary thick ascending limb
NA	noradrenalin
NO	nitric oxide
OFR	oxygen free radical
PAF	platelet activating factor
PCT	proximal convoluted tubule
PDGF	platelet derived growth factor
PG	prostaglandin
Pi	phosphorus
PMN	polymorphonuclear leukocyte
PRP	platelet rich plasma
RAC	renal artery clamp
RBC	red blood cell
RBF	renal blood flow
REE	resting energy expenditure
RGD	argenine-glycine-aspartic acid sequence
RQ	respiratory quotient
SOD	superoxide dismutase
TGF-β	transforming growth factor β

List of Abbreviations (continued)

TPN	total parenteral nutrition
VCO_2	carbon dioxide production
VO_2	oxygen consumption

1 Historical Perspective - before 1965

1:1 Pathophysiology of acute renal failure

1:1:1 Pathology

In 1912 Suzuki described the pathological appearance associated with a number of nephrotoxins.[1] The lesions were specifically located in the proximal tubules, but later these findings could not be confirmed by other pathologists. Renal impairment due to crushing injury had been noted by Frankenthal during the 1914 - 1918 war,[2] although the importance of the lesion was not emphasised until 1923.[3]

During the Second World War pathologists became particularly interested in patients with crushing injuries to skeletal muscles, but no direct damage to the kidneys. These victims died from uraemia and histological examination of the kidneys showed complete tubular disruption, especially in the distal tubules, with intratubular obstruction by casts.

In 1941 Professor Shaw-Dunn described the renal lesions in victims of the Glasgow air raids who died from crush syndrome, noting the severe lesions in the distal nephron.[4] Bywaters and Beal also published extensively and brought acute renal failure (ARF) into the limelight. They described the pathological appearance of the kidneys in patients dying from crushing injuries to skeletal muscle during World War II.[5-9] Only the distal tubules and the collecting ducts were believed to be affected.

Lucke coined the term 'lower nephron nephrosis' and stated that it was present in 10-20% of deaths resulting from battle wounds in World War II.[10] He showed that a variety of disorders, shock, trauma, transfusion reactions etc resulted in the same pathological appearance rather than specific appearances as previously believed. Changes were shown to be '...selectively restricted to the lower segments of the nephrons...'. He felt that several factors were involved in the pathogenesis, including degradation products of myoglobin and

haemoglobin, products of tissue breakdown, alteration of renal blood flow and changes in the chemical milieu of blood. He also showed that the renal lesion was recoverable and that if the patient survived complete regeneration of the renal tubules occurred.

The morphological picture which finally emerged from these early studies was as follows. Macroscopically two main types of kidney were seen. In those who died early the capsule stripped off easily and the cortex had a yellow glistening texture. In those who died later the kidneys were cyanosed, although the cortex was again yellow. The capsule was unwrinkled. The glomeruli were usually normal in appearance although sometimes the vascularity appeared reduced and the capsular space contained granular eosinophilic material. Early degenerative changes were noted in the proximal tubules with a 'catarrh' made up of cell debris or precipitated protein.[9,10] The changes in the proximal tubule were minimal compared to the distal tubule, although amorphous debris was seen in the latter. There was no pigmentation of the contents of the proximal tubules. The descending limb of the loop of Henle looked normal, although some 'mild catarrhal changes' were sometimes present. The major abnormalities were in the thick ascending limb of the loop of Henle and the distal tubules. Focal areas of degeneration, necrosis and regeneration were scattered throughout this part of the nephron. Areas of necrosis were surrounded by an inflammatory infiltrate of lymphocytes and histiocytes. Later fibroblasts appeared and destroyed parenchyma was noted to be replaced by scar tissue. It was noted that degeneration rarely occurred before day 4, but after that necrotic tubule cells disintegrated and disappeared. By day 10 regeneration of necrotic areas had occurred. Sometimes a tubule ruptured with protrusion of a cast into the interstitial tissue. Occasionally tubules ruptured into veins

Hyaline casts were common in the thick ascending limb and pigment casts became more numerous in the distal tubule. According to Bywaters and Dibble they were particularly numerous in the collecting ducts, although Lucke reported that the collecting ducts were

almost normal.[9,10] Darmady suggested that the composition of casts depended on the length of survival.[11] Early casts consisted largely of hyaline material, whereas in those dying later pigment casts were predominant. Sometimes pigment casts formed round a central focus such as a fragment of degenerate epithelium or a sulphanilamide crystal.

The disputes over the pathological lesions occurring in anuria were resolved by the meticulous microdissections of Dr Jean Oliver and co-workers in 1951.[12] Dr Oliver was sent specimens of kidneys by Professor Shaw-Dunn from Glasgow and he also received post mortem material from the United States from patients dying from a variety of nephrotoxins such as mercuric chloride, potassium diclonate and also from patients dying from ARF post operatively. Oliver carefully microdissected hundreds of whole nephrons. Two distinct types of nephron damage were found. The first type showed focal lesions with destruction of the entire tubule wall, including the basement membrane, throughout the entire length of the nephron. He called this tubulorhexis. An example from a victim of the Clydebank Blitz is shown in Figure 1:1. This demonstrates only the distal nephron. Similar changes were found throughout the proximal tubule. The second histological picture was much more localised and related to nephrotoxic damage by specific nephrotoxins (Figure 1:2).

1:1:2 Function

Early micropuncture studies gave rise to three theories as to the aetiology of ARF.

1. Backdiffusion of filtrate through damaged tubule walls causing oliguria was first suggested in 1929.[13] Using kidney micropuncture techniques, Richards noted that in frogs poisoned with mercuric chloride, tubules, which were normally impermeable to dye, allowed leakage of the dye into the interstitium.

2. Tubule obstruction by the casts seen histologically in the dilated tubules. The original theory that ARF was caused by epithelial debris and casts causing intratubular obstruction was put forward by Barratt and Yorke (1909) and supported by Baker and Dodds (1925) and

Morrison (1941).[14-16] Micropuncture studies looking at increasing intratubule hydrostatic pressure were conflicting, depending on the model studied and the stage of evolution of ARF.[14-16] Taken over all, the studies showed that intraluminal hydrostatic pressure was not consistently raised during the early or late stages of experimental ARF. Many believed that in the presence of reduced single nephron glomerular filtration, sludging would occur causing tubule casts to be formed and thus perpetuation of ARF, ie tubule obstruction had a secondary rather than a primary role in the development of experimental ARF.

3. In 1945 Bywaters proposed that tubule ischaemia was critical and that the presence of casts was incidental.[11] Corcoron and Page (1947) divided post traumatic anuria into two groups - those with tubule obstruction by casts and those with tubule necrosis.[17,18] They noted that beef metamyoglobin injected into healthy dogs caused little renal damage but that the addition of renal ischaemia caused the animal to die from renal failure within 24 hours.[17] They also suggested renal ischaemia causing oliguria and associated with acidaemia predisposed to the formation of protein casts in the tubules. Thus renal ischaemia was important for the development of both histological pictures.

Bywaters and Dibble believed that true lower nephron nephrosis was associated with casts in the distal tubule while pointing out that mechanical blockage could not be the sole responsible agent.[8,9] They drew attention to patients who died from anuria complicating crushing injuries but who had very little in the way of casts. They suggested that if tubule obstruction were the only factor involved in anuria, then any urine passed would be of normal composition having been excreted by unblocked, apparently normal nephrons. Of course the urine was not normal and appeared to be poorly concentrated glomerular filtrate, suggesting a disruption in tubule function.

Tomb (1942) first suggested that decreased renal blood flow would cause damage in those areas of the nephron supplied last by the blood ie the loop of Henle, where maximum

damage was found.[19] In 1942 Trueta showed that localised trauma leads to widespread arterial spasm and suggested that this might result in renal anoxia.[20] Cournand (1943) and Lausen (1944) proved that vasoconstriction of renal arteries occurred in shock,[21,22] while others showed that temporary experimental occlusion of renal arteries caused a picture similar to traumatic uraemia.[23] Maegraith (1944) argued that the anuric state was due to a temporary decrease in glomerular filtration resulting from altered vascular flow, rather than primary obstruction of the tubules.[24,25]

1:2 Modification of acute renal failure

'... No measure is known that will restore a physiologically ruined kidney
except time and the organ's intrinsic reconstitutive power'

Homer W Smith (1951)[26]

The earliest attempts to treat patients with acute renal failure did more harm than good. Clinicians assumed that expanding the intravascular volume might initiate a diuresis, but the over administration of fluid merely hastened the demise of the patient by causing fluid overload and pulmonary oedema.[27]

Renal decapsulation, first performed by Edebohl in 1898,[28] was favoured in the early 1940's as a method of relieving the renal oedema seen by some microscopically.[28] Peters proposed that renal oedema caused an increase in intrarenal pressure, effectively preventing the flow of urine.[29] However, by the early 1950's this procedure had been abandoned for several reasons. First of all it was recognised that the renal oedema was probably due to over administration of saline. Secondly, decapsulation was a last resort by which time the patient was usually moribund. Finally the operation itself was potentially harmful in these sick patients. Peters noted that those successes reported in the literature indicated that renal decapsulation was performed between the 8th and 10th day, the time when a spontaneous

diuresis most often occured.[29] Styron and Leadbetter (1945) carried out unilateral decapsulation in a case of ARF and by ureteric catheterisation demonstrated no difference in function in either kidney.[30] Culpepper and Findlay (1947) pointed out that the tubule lesions were most important in maintaining oliguria and said that there was little evidence supporting increased intrarenal pressure as a significant aetiological factor.[31]

In 1944, in a personal communication to Darmady, Trueta suggested that a splanchnic block might prevent renal vasoconstriction.[11] Darmady then successfully tried renal denervation, using procaine infiltration of the splanchnic nerve, on two patients.[11] McGowan and Antry reported the successful treatment of a 19 year old male with a procaine sympathetic block.[32] Others had less favourable results.[33]

Those patients who were not 'drowned' by over zealous administration of fluid died from arhythmias secondary to hyperkalaemia. In 1950 Hicks et al showed that an innovative method of obtaining extra-renal clearance of urea and creatinine was by small intestinal lavage.[34] Peritoneal lavage was also shown to be effective at removing nitrogen from the body.[35,36]

The early treatment of anuria was therefore mainly conservative. Circulatory failure was corrected, salt and water administered as necessary and infection treated. Dietary sodium was restricted in all patients who were not dehydrated, as sodium retention would lead to water retention when water might otherwise have been excreted. Patients were given glucose-fat emulsions to provide calories in a non-protein diet and thus reduce endogenous protein catabolism to 1/3 of its starvation level. It was possible for a patient managed in this way to continue for at least a month before the urea concentration rose above 300mg%.[37]

In 1943 Kolff first used his artificial kidney to treat a patient with ARF. In his book 'The Artificial Kidney' he describes the first 14 patients with ARF treated by this method. [38] The initial experience was not good and 12 out of the first 13 patients died. Kolff admits

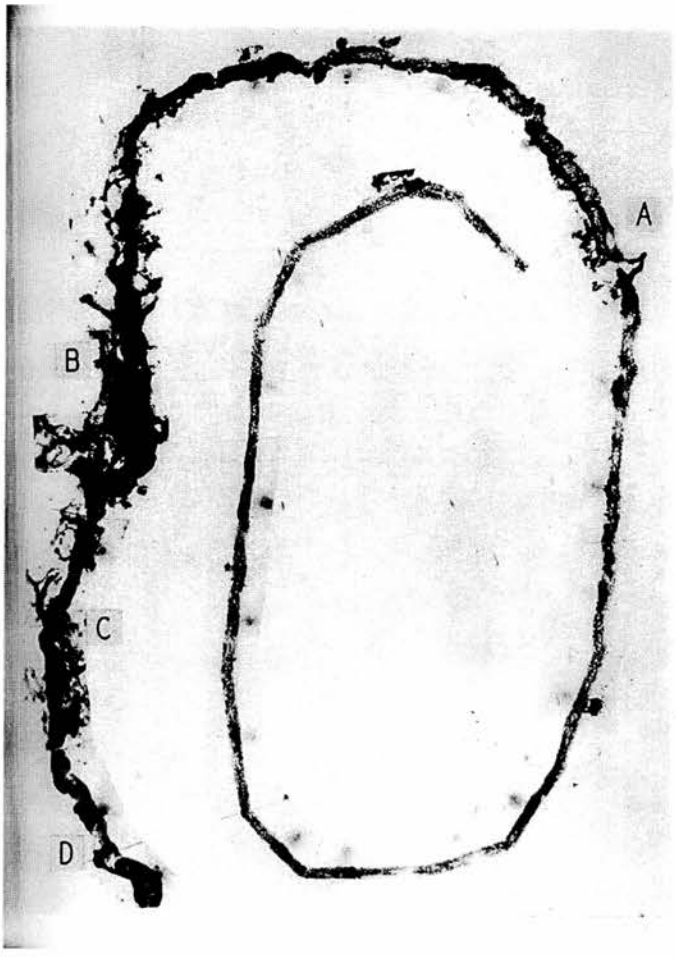
that the patient who survived would probably have done so without dialysis. The high mortality was attributed to the serious clinical condition of the patients and the late referral for dialysis. Despite the depressing statistics, Kolff remained confident that he and his colleagues would one day be able to say 'He is cured, and without the artificial kidney he would have died'.[38]

The 14th patient probably had acute tubular necrosis secondary to acute cholecystitis and proved Kolff to be correct - without dialysis she would have died.

1:3 Summary

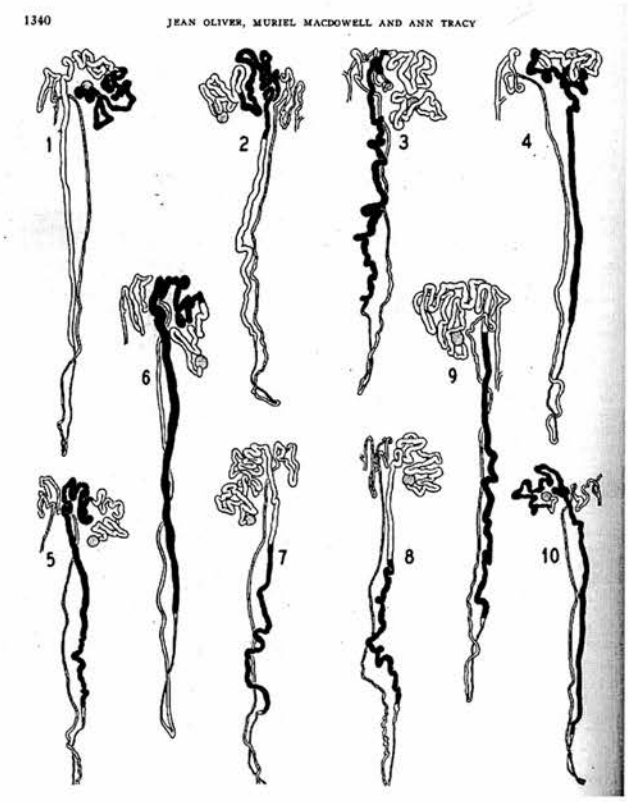
Although peritoneal dialysis and the artificial kidney were in use by the early 1950's, Homer Smith comments that 'they cannot in themselves induce a diuresis and their value in influencing the course of acute anuria, usually a self limited disturbance, defies critical evaluation'.[26] Until renal replacement therapy became standard practice for patients with ARF therapeutic measures were aimed at keeping fluid and electrolyte balance normal, reducing endogenous protein catabolism to minimum and preventing infection and circulatory failure.

Figure 1:1 Crush anuria - young woman pinned under a beam for six hours [12]



A. Rupture of basement membrane and tubular wall
B. Tubule distended with a black pigment cast
C. Fragmentation of the tubule wall
D. Regenerating tubule epithelial cells

Figure 1:2 Localisation of nephrotoxic damage in various forms of poisoning [12]



1. Potassium bichromate; **2.** Uranyl nitrate; **3.** Corrosive sublimate; **4.** Sodium potassium tartrate; **5.** Potassium chlorate; **6.** Di-ethylene glycol; **7.** Carbon tetrachloride; **8.** Mushroom poisoning; **9.** Serine; **10.** Sulfonamides.

2 Current Knowledge - after 1965

2:1 Pathophysiology of acute renal failure

Hypoxic injury to renal tissue results in cell damage due to inadequate energy and substrate provision to meet the tissue's metabolic demand. The pathogenesis of ischaemic acute renal failure due to hypoperfusion of the kidney is complex and there are many interrelated mechanisms which together initiate and promote ischaemic renal damage.

2:1:1 Pathology

After sixty minutes of ischaemia changes are seen in proximal tubule brush borders.[39] Microvilli disintegrate and are shed into the tubule lumen. Tubule cell cytoplasm is extruded into the lumen and forms dense, free floating spherical bodies. There is no plasma membrane and it is unclear how the limiting membrane is formed. These "blebs" of cytoplasm are much more densely packed in the proximal straight tubule than in the proximal convoluted tubule (PCT). Twenty-four hours later these "blebs" have disappeared and have presumably been degraded into amorphous debris. The distal tubules, collecting ducts and thin limbs of the loop of Henle contain casts formed from cellular debris and Tamm Horsfall protein. All the proximal straight tubules and about half of the proximal convoluted tubules have epithelial necrosis. The damage occurring after sixty minutes of ischaemia is much less severe in the proximal convoluted tubules than in the proximal straight tubules.

After 25 minutes of ischaemia, Donohoe showed that damage to the proximal convoluted tubules was much less severe, with interiorisation of microvilli into epithelial cell cytoplasm.[39] These changes are reversible and microvilli are regenerated 30 - 60 minutes after reflow. Irreversible changes with signs of necrosis were present in some of the

proximal straight tubule cells. These cells were shed into the lumen and the epithelium subsequently regenerated. Venkatachalam et al also showed that within 5 minutes of reperfusion after 25 minutes of renal artery occlusion, most of the brush border microvilli in all three segments of the proximal tubule were interiorised into the cytoplasm of the proximal tubule cells.[40] The S_1 and S_2 cells showed complete regeneration of microvilli by four hours and cells appeared morphologically normal. Cells in the S_3 segment however showed signs of irreversible cell injury and were exfoliated into the cell lumen.

Fifteen minutes of ischaemia resulted in mild morphological changes that had completely reverted to normal after 30 minutes of reflow. Thus the severity of morphological damage appears to be related to the duration of ischaemia.

2:1:2 Intratubular obstruction

After sixty minutes of ischaemia a rise in proximal intratubular pressure can be demonstrated which rises further following microinjections of small volumes of fluid.[41,42] Further injections of larger volumes of fluid did not cause a rise in intratubular pressure implying that the initial injection had flushed out the obstruction.[43] Twenty-four hours after reversal of ischaemia proximal tubule pressure was still elevated but only approximately 50% higher than baseline pre-ischaemic pressures.[44,45] Arendshorst et al noted greatly increased proximal and distal tubule pressures of 31 and 16mmHg respectively (controls 11.5 and 5.3mmHg).[41] Twenty four hours later proximal tubule pressure had decreased to 9.2mmHg. However, acute volume expansion at this time caused a rise in intratubular pressure implying that the tubules were still blocked.[41] Morphologically casts are still present a week after the ischaemic event but by two weeks the tubules are patent and fluid injections do not produce a rise in intratubular pressure.[44] Donohoe used intrarenal inulin sequestration as a measure of tubule obstruction.[45] After 15 minutes of ischaemia, and in control animals, recovery of inulin was complete. After 25

minutes of ischaemia 12% of inulin injected into the ischaemic proximal convoluted tubule was not recovered in the urine. This increased to 28% after 60 minutes of ischaemia. About 73% of the missing inulin was found to be sequestered in the ischaemic kidney.

2:1:3 Backleak of filtrate

Using microinjection techniques the tubule epithelium can be shown to be abnormally permeable due to cell necrosis, and the degree of permeability is related to the duration of ischaemia.[45,46] After microinjection of inulin into the PCT of control animals, recovery of inulin from ipsilateral urine was virtually complete. This was also the case after 15 minutes of renal artery occlusion. However, after 25 minutes of ischaemia 10.8% of injected inulin was recovered in the contralateral urine, increasing to 34.9% after sixty minutes of ischaemia.[45] Tanner and colleagues kept the intratubular pressure constant during microinjection of inulin, and showed that backleak is not an artefact due to transient rises in intratubular pressure forcing fluid across the damaged epithelium, but is due to a genuine increase in tubular permeability.[46]

This is in keeping with Richards' original theory of 1929.[26]

2:1:4 Vascular congestion

Following temporary experimental ischaemia there is vascular congestion in the inner stripe of the outer medulla,[47] and the accumulation of erythrocytes is such that normal blood flow is not possible.[48,49] No fibrin deposition or platelet aggregation can be seen histologically,[48] and as prior heparinisation and administration of aspirin had no effect on post ischaemic renal function, the participation of haemostatic mechanisms seems unlikely.[49,50]

The cause of the vascular congestion is not clear but there are several possible contributing factors. The anatomy of the medullary vasculature is thought to predispose to erythrocyte

aggregation: the parallel capillary circuits may slow blood velocity to such an extent that during ischaemic events a further reduction in flow rate promotes stagnation of erythrocytes. It can be postulated that hypoxia and substrate deficiency lead to depletion of intracellular adenosine triphosphate (ATP) and therefore inactivity of the ATP dependent Na/K pump. The resulting impairment in cell volume regulation allows cell swelling due to movement of extracellular fluid into the intracellular space. Karlberg showed that labelled albumin accumulated in the medullary interstitium during recirculation after ischaemia.[51] This leak of plasma would cause a rise in haematocrit. The combination of reduced plasma volume, increased haematocrit, cell swelling, and reduced blood flow rate promotes aggregation of erythrocytes, which restricts reperfusion after the ischaemic event leading to irreversible cell damage and tubular necrosis.

The degree of functional loss has been shown to be related to the severity of vascular congestion, with the most severe degree of hyperaemia associated with the lowest filtration rate, poorest concentrating ability, most depressed reabsorptive capacity, smallest urine volume and lowest blood flow rate.[48] The disappearance of medullary hyperaemia occurred concurrently with restoration of deep glomerular blood flow, and the return of concentrating ability in the recovering kidney.

Tubule cells in the outer medulla normally operate on the brink of hypoxia and are therefore extremely sensitive to episodes of hypoxia.[52] This is particularly so for those tubules situated at a distance from nutrient blood vessels.[53,54] There are three possible mechanisms for preventing tubule cell death by limiting vascular congestion:

1. Increasing perfusion pressure
2. Lowering haematocrit
3. Reducing cell volume

Mason et al have shown that increasing renal perfusion pressure and reducing the number of circulating erythrocytes without any volume expansion, both cause a reduction in the degree of medullary congestion and an improvement in renal function.[55] They showed the filtration rate and tubular reabsorption could be raised from 20% to 60% of normal 3 and 18 hours after ischaemia in rats.

Reducing cell volume can be achieved by administration of hypertonic mannitol. Giving hypertonic mannitol before ischaemia has been shown to confer protection on renal function.[56,57] This is presumably due to the rise in plasma osmolality limiting the movement of extracellular fluid into the intracellular space and so limiting cell swelling and vascular congestion. Mannitol also causes expansion of the plasma volume which will reduce haematocrit.

All three of these manoeuvres had exactly the same effect on renal function, each causing an increase in filtration rate and tubular reabsorption to 60% of control,[58] and this despite completely different modes of action; lowering haematocrit prevents erythrocyte aggregation, increasing perfusion pressure reverses stasis and congestion while mannitol prevents cell swelling. However, the end result common to all three methods was an increase in perfusion to the deep nephrons, delivering oxygen and substrates necessary for recovery.

2:1:5 Renal blood flow

Renal blood flow (RBF) is decreased by up to 50% during the first six hours of reflow following ischaemia.[59] Daugherty et al showed that incomplete renal ischaemia for three hours caused a rise in afferent and efferent arteriolar resistance immediately after relief of partial renal ischaemia.[60] The cause of the increased vascular resistance is not clear, but several hypotheses have been proposed.

1. Endothelial cell swelling leading to a decrease in arteriole diameter and thus increased resistance to blood flow was an early hypothesis.[61] It has since been rejected on the grounds that cell swelling is mainly tubular and not vascular.[40]

2. Ischaemia is known to cause a rise in plasma and intrarenal renin activities which may lead to angiotensin II production, so causing vasoconstriction.[62] In human studies, plasma renin levels have been shown to be elevated during the phase of established ARF.[63,64] During the diuretic phase of ARF plasma renin levels fell to normal. Unfortunately, presumablY due to the difficulty in predicting when ARF is going to develop, renin levels prior to the development of ARF have not been looked at to determine whether an increase in plasma renin is necessary for the development of ischaemic ARF. However, angiotensin converting enzyme (ACE) inhibitors do not appear to modify the course of ARF despite increasing renal blood flow,[65] and chronic suppression of renal renin by salt loading does not prevent the decrease in glomerular filtration rate (GFR) seen after 45 minutes of ischaemia.[66]

3. Increased solute delivery to distal segments causes tubuloglomerular feedback and an increase in vascular resistance.[67]

4. Numerous groups have shown impaired autoregulation of renal blood flow during the maintenance phase of ischaemic ARF.[68-71] Conger et al have shown that in a noradrenalin induced model of ARF (NA-ARF), at one week a reduction in renal perfusion pressure causes a paradoxical increase in renal vascular resistance.[72] Renal nerve stimulation also causes vasoconstriction and prostaglandin I_2 (PGI_2) infusion caused normal vasodilatation. In a renal artery clamp model of ARF (RAC-ARF), there was no change in vascular resistance in response to a reduction in renal perfusion pressure and renal nerve stimulation did not affect renal blood flow.[70] PGI_2 infusion caused marked vasodilatation.

In the first model <10% of resistance vessels showed smooth muscle necrosis, whereas 50% of resistance vessels showed smooth muscle necrosis in RAC-ARF. NA-ARF caused predominantly functional endothelial vascular injury and RAC-ARF caused predominantly smooth muscle injury. This may be because renal blood flow in the noradrenalin model was quantitatively less, although more sustained. The rate of recovery of blood flow may determine the extent of vascular injury, and the slow recovery in NA-ARF may have attenuated the severity of reperfusion oxidant injury.

Angiotensin and thromboxane both increase the vasoconstrictor response to renal nerve stimulation,[72,73] and both hormones are elevated after ischaemic injury.[71,74] Robinette et al have shown that administration of an angiotensin II antagonist or a thromboxane A_2 antagonist partially corrected the vasoconstrictor response to renal nerve stimulation and renal perfusion pressure reduction.[71] A combination of the two eliminated the vasoconstriction due to renal nerve stimulation and also to a reduction in renal perfusion pressure, but in the latter case appropriate vasodilatation was not restored. From this they conclude that there are two components of abnormal renal blood flow autoregulation in NE-ARF.

a. Paradoxical vasoconstriction to renal nerve stimulation or a reduction in renal perfusion pressure, mediated by angiotensin II and thromboxane induced adrenergic hypersensitivity.

b. Inability to vasodilate in response to myogenic or tubuloglomerular feedback stimuli.

In a clinical setting it may be that the maintenance phase of ischaemic ARF is accompanied by additional insults such as repeated ischaemic episodes. Loss of renal blood flow autoregulation would mean the kidney would be unable to protect itself from falls in renal perfusion pressure causing further ischaemic damage. Fresh tubular necrosis would occur and prolong the duration of ARF.

5. Endothelium derived relaxing factor is depressed after ischaemia and it may therefore be involved in the vasodilatation produced after a reduction in renal perfusion pressure.[75] In its absence, appropriate vasodilatation may not occur.

6. Calcium may leak back into the ischaemic smooth muscle cells and so cause vasoconstriction.[76]

2:1:6 The role of prostaglandins

Prostaglandins are involved in autoregulation of RBF and glomerular GFR, renin release, tubule ion transport and water metabolism. However, their role in ARF is unclear. There are several potential prostaglandin sensitive pathophysiological mechanisms in ARF.

a. Autoregulation of renal blood flow. Prostaglandins of the E_2, D_2 and I_2 series dilate the intrarenal vessels.[77] However, there is controversy over whether or not local prostaglandin release is necessary for autoregulation of RBF and GFR.[78-80]

b. Tubuloglomerular feedback, which may be involved in the pathophysiology of ARF, requires an intact prostaglandin system to operate.[81,82]

c. A reduction in the glomerular capillary ultrafiltration coefficient (K_f) may contribute to the decline in GFR. Prostaglandin E_1 has been shown to diminish K_f[83] as has bradykinin - a vasodilator that mediates its effect partly by prostaglandins.[84]

d. Capillary reflow. Endothelial PGI_2 release may prevent vascular aggregation and maintain vascular patency.[85] Indomethacin has been shown to increase the injury in the medullary thick ascending limb of the Loop of Henle in ARF,[86] while an infusion of PGI_2 and PGE_2 has a protective effect on the proximal tubule structure.[87] Also, the vasodilator effect of mannitol in ARF has been shown to be due to PGI_2 release.[88] Thus, impaired prostaglandin synthesis due to endothelial cell injury may contribute to the decrease in RBF in the maintenance phase of ARF.

e. The renin - angiotensin system. Angiotensin II may be involved in tubuloglomerular feedback and also in controlling K_f.[81,89] Prostaglandins modulate renin release and activation of the renin - angiotensin system stimulates local prostaglandin release,[90] therefore it can be seen that the interaction between the two systems may be an important pathophysiological mechanism in ARF.

2:1:7 The role of calcium

The extracellular calcium concentration is 10^4 greater than the intracellular calcium concentration (10^{-3} v 10^{-7}). This difference in concentration is maintained by the relative impermeability of the cell membrane to calcium. There are two main calcium extrusion mechanisms:[91]

1. ATP dependent Ca-ATPase
2. Na/Ca exchange mechanism

Under normal circumstances buffering of cytosolic calcium is regulated by the Ca-ATPase of the plasma membrane and the endoplasmic reticulum. Larger loads of calcium are dealt with by the Na/Ca exchange mechanism and by mitochondrial uptake.[92]

The transport of electrons, derived from Krebs cycle, along the mitochondrial respiratory enzyme chain situated in the inner mitochondrial membrane releases energy. This energy is used to transfer hydrogen ions from the inner surface of the inner membrane to the outer surface of the inner membrane so creating an electrochemical gradient. This electrochemical gradient is the driving force for the formation of ATP from adenosine diphosphate (ADP) and phosphorus (Pi). However, it is also the source of energy for mitochondrial calcium uptake which occurs without being coupled to the movement of another ion.[93] The use of the electrochemical gradient for calcium uptake is at the expense of ATP production. A highly significant correlation between increasing mitochondrial calcium concentration and decreasing mitochondrial respiration has been shown,[94,95]

presumably because mitochondrial calcium accumulation competed with oxidative phosphorylation and ATP synthesis.[96]

During ischaemia, alterations in the cell plasma membrane phospholipid composition allow an influx of calcium into the cell during the period of reflow, when more calcium is delivered to the ischaemic area.[95] Also during ischaemia there is reduced ATP synthesis,[97] which may result in reduced ATP dependent calcium efflux. The rise in free intracellular calcium ($[Ca]_i$) during reflow is therefore buffered by the mitochondria and the result is an uncoupling of oxidative phosphorylation. Arnold showed that mitochondria isolated after ischaemia but prior to reflow were unable to buffer increases in $[Ca]_i$.[97] Buffering capacity returned to normal after 3 - 6 hours of reperfusion but then deteriorated. After 18 - 24 hours of reperfusion mitochondria were completely unable to regulate calcium.

The buffering of calcium by mitochondria causes a rise in mitochondrial calcium concentration which uncouples oxidative phosphorylation. ATP production is reduced and calcium efflux is impaired and there is a net influx of calcium into the cell. Mitochondrial buffering of the cytosol becomes less effective, and as the $[Ca]_i$ rises there is equilibrium between the mitochondria and the cytosol. This appears to be passive transfer of calcium from the cytosol across a mitochondria membrane damaged by ischaemia, as neither an increase in active uptake nor diminished release of calcium by mitochondria can be demonstrated.[97]

The accumulation of intracellular calcium has other detrimental effects: elevated $[Ca]_i$ activates energy consuming reactions such as sarcolemmal Ca ATPase and so further depletes the cell of ATP.[91] Phospholipases are activated causing an alteration in membrane permeability and membrane enzyme activity.[98-101] Degradation of membrane phospholipids leads to the production of lysophospholipids and free fatty acids that may cause further damage. Activation of intracellular proteases in the presence of a raised $[Ca]_i$

will convert xanthine dehydrogenase to xanthine oxidase which in turn leads to the production of hydroxyl radicals.[102,103]

2:1:8 The role of free radicals

Generation

Ischaemia leads to a depletion of intracellular high energy phosphates, and the hydrolysis of ATP generates hypoxanthine.[97] During conditions of ischaemia, the rise in intracellular calcium causes xanthine dehydrogenase to undergo a proteolytic configurational change to xanthine oxidase.[107,108] During reperfusion, and in the presence of molecular oxygen, hypoxanthine acts as an oxidisable purine substrate for xanthine oxidase and is converted to xanthine. In the process superoxide radical is generated (equation 1).[104]

$$\text{Hypoxanthine} + H_2O + 2O_2 \xrightarrow{\text{xanthine oxidase}} \text{Xanthine} + 2O_2^{-}\cdot + 2H^+ \qquad (1)$$

Superoxide dismutases (SOD) are naturally occurring free radical scavengers which rapidly catalyse the conversion of $O_2^{-}\cdot$ to hydrogen peroxide (equation 2), which is itself toxic at high concentrations and also inhibits SOD. The major problem with H_2O_2 accumulation is that it reacts with $O_2^{-}\cdot$ to form molecular O_2, hydroxide and hydroxyl radical (equation 3).

$$O_2^{-}\cdot + O_2^{-}\cdot + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad (2)$$

$$H_2O_2 + O_2^{-}\cdot \xrightarrow{Fe} O_2 + OH^- + OH\cdot \qquad (3)$$

$OH\cdot$ is the most reactive and toxic of all the free radical species. Under normal physiological conditions large concentrations of $OH\cdot$ do not exist as $O_2^{-}\cdot$ is scavenged by SOD, and H_2O_2 by the catalase (equation 4) or glutathione peroxidase systems (equation 5).

$$2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O \qquad (4)$$

$$2GSH + H_2O_2 \xrightarrow{\text{GSSG Peroxidase}} GSSG + 2H_2O \qquad (5)$$

There are no scavenger systems that scavenge excessive concentrations of OH·.

Oxygen free radicals (OFR) are also generated by auto-oxidation of catecholamines, although the contribution of this to ischaemic renal damage is thought to be insignificant.[105] Endothelial cells may contribute to free radical production, either by producing them or acting as a source of chemoattractants for polymorphs (PMNs).[106] However, leukocyte production of free radicals does not play a major role in cell damage until at least two hours after reperfusion,[107] and thus the main source of free radicals during reperfusion after ischaemia is the xanthine oxidase reaction.[104]

Effect of free radicals

In theory, free radicals could damage arterial endothelium, mesangial cells, and tubule epithelial cells. Using a rat model, Galat and colleagues generated free radicals in the absence of ischaemia, and showed a decrease in glomerular filtration out of proportion to the reduction in renal blood flow.[108] Tubule function, however, was not affected in the absence of ischaemia, although this may have been due to reduced delivery of filtrate to the tubules. Had normal or even higher than normal loads of filtrate been delivered to the tubule epithelium, tubule transport might have been impaired. However, the tubules did not have any histological lesions of ischaemia. They propose that during reperfusion following ischaemia the site of OFR generation is the tubules, rather than vascular endothelium. Also neutrophils may be necessary for full expression of post ischaemic reperfusion injury as neutrophil depletion has proved to be as protective as OFR scavengers in some organs.[109,110]

Endothelial cells appear to be more susceptible to oxidant injury than tubule epithelial cells, and tubule epithelial cell lines with proximal tubule characteristics are more sensitive to injury by OFR than distal tubule epithelial cell lines.[111]

It has also been shown that mitochondria in the rat renal cortex exposed to injury by free radicals alone develop only a mild impairment of respiratory function, implying that other factors must be contributing to ischaemic renal damage.[112]

Paller et al demonstrated that inhibition of free radical generation using the xanthine oxidase inhibitor allopurinol, provided protection against renal impairment after ischaemia.[113] Likewise, scavenging of $O_2^{-}\cdot$ with SOD or OH· with dimethylthiourea afforded protection after ischaemia. However, the protection afforded by the scavengers was incomplete, again implying that other factors contribute to ischaemic renal damage.

Others have shown that prevention of free radical formation by a xanthine oxidase inhibitor prevented erythrocyte accumulation in the medulla, which may have contributed to the lower peripheral resistance and higher blood flow observed immediately after reflow.[114] It is postulated that free radicals damage the capillary basement membrane resulting in leakage of plasma, a rise in haematocrit and aggregation of erythrocytes. Prior treatment with allopurinol prevents these effects. Similar results were obtained from rabbit kidneys pre-treated with the free radical scavengers SOD and catalase.[115]

Potentiation of free radical induced injury to mitochondria by prior exposure to calcium has been demonstrated in vitro.[112] Free radical induced injury alone caused a mild increase in mitochondrial permeability and a reduction in ATP synthesis, both of which were more marked after prior mitochondrial loading with calcium. In addition, free radicals had no effect on electron transport, but the combination of free radicals and calcium caused an uncoupling of respiration.

Lipid peroxidation

Free radicals are thought to produce cellular damage through peroxidation of polyunsaturated fatty acids in plasma membranes, resulting in increased membrane permeability.[116,117] Increased permeability of tubule cell membranes may cause impaired

solute transport; increased mitochondrial permeability would allow an influx of calcium; the activity of mitochondrial ATPase and the process of oxidative phosphorylation also depend on the phospholipid content of the mitochondrial membrane;[118,119] increased lysosomal permeability might release hydrolytic enzymes and cause further cell degradation.

Paller showed that ischaemia alone had no effect on lipid peroxidation, but that ischaemia plus reflow caused an increase in the membrane lipid peroxide malondialdehyde.[113] Presumably this is because the production of $O_2^{-}\cdot$ by xanthine oxidase requires the presence of molecular oxygen. SOD reduced lipid peroxidation in renal cortical mitochondria.

2:1:9 The role of endothelin

In 1988 Yanagisawa et al isolated and cloned a potent vasoconstrictor secreted by endothelial cells.[120] This substance, called endothelin (ET), was subsequently shown to be a more potent vasoconstrictor than angiotensin, vasopressin or neuropeptide Y.

Endothelin is generated by proteolytic cleavage of a prohormone by endothelin converting enzyme and exists in three isoforms - ET-1, ET-2, ET-3. [121,122] ET-1 is the most potent and widely distributed of the three isoforms and in the kidney is produced by endothelial cells, mesangial cells and tubule epithelial cells.[123-126]

There are at least two endothelin receptors, ET_A and ET_B, both of which are distributed throughout the body, including the kidney.[127] The ET_A receptor promotes vasoconstriction, cellular proliferation and matrix deposition.[128-130] The ET_B receptor appears to mediate release of vasodilalatory prostaglandins (PG) and nitric oxide (NO).[131,132] However, there is also evidence that ET_B mediates renal vasoconstriction.[133]

Firth and Ratcliffe (1992) showed that renal ischaemia caused an increase in prepro ET-1 expression which was present two hours after ischaemia and still present 7 days later.[34] Ischaemia has been shown to increase the affinity of ET_A and ET_B receptors for ET-1 in the

renal cortex.[135] Various injurious stimuli also appear to upregulate ET receptors - particularly the ET_B receptor.[136] This may be a physiological mechanism designed to protect against the vasoconstricting action of endothelin by increasing production of PGI_2 and NO.

ET has been shown to potentiate cellular proliferation, hypertrophy and collagen deposition.[129,130] Therefore, as well as being a causative factor in the glomerular hypoperfusion that occurs in ARF, ET may also be involved in the long term outcome. The development of renal scaring will of course, prevent full functional recovery from ARF.

2:1:10 The role of intercellular adhesion molecules

Leukocyte adhesion molecules have been shown to regulate the interactions of leukocytes with other leukocytes, endothelial cells and extracellular componants.[137-139] Intercellular adhesion molecule-1 (ICAM-1) expression on endothelial cells is increased by various mediators of inflammation.[139] In the normal kidney ICAM-1 expression is low but it is increased following exposure to cytokines and also in various glomerulopathies.[140,141]

Hypoxia followed by reflow has also been shown to increase ICAM-1 expression in cultured endothelial cells.[142] Thus renal ischaemia, with increased levels of inflammatory mediators, may cause increased expression of ICAM-1. The resulting leukocyte adhesion with activation of leukocytes and release of cytokines will increase vascular permeability. As mentioned in section 2:1:8 activated leukocytes also release free radicals which in turn will cause further tissue damage.

Leukocyte adhesion may also result in capillary obstruction and contribute to vascular stasis.

2:1:11 The role of platelet activating factor

When stimulated, a number of cell types, including glomeruli and renal medullary cells, are capable of producing platelet activating factor (PAF).[143] Intrarenal administration of PAF to anaesthetised dogs caused a dose dependent decrease in RBF, GFR, urine volume and sodium excretion without any significant, systemic, haemodynamic changes.[144]

Several mechanisms are involved in the ARF induced by PAF.

a. Decreased renal blood flow. PAF has a direct contractile effect on vascular smooth muscle.[145]
b. A reduction in K_f. PAF can cause contraction of cultured mesangial cells and has also been shown to reduce K_f in vivo.[146,147]
c. In isolated rat or rabbit kidneys, PAF has been shown to increase the production of thromboxane B_2, a potent vasoconstrictor.[146,148] PAF antagonists can block the PAF induced release of thromboxane B_2 from cultured rat and human mesangial cells.[149]
d. PAF induces neutrophil aggregation and activation.[150] This results in the generation and release of free radicals which can cause tissue damage.

2:2 Modification of acute renal failure

2:2:1 Mannitol

Mannitol is a simple sugar, molecular weight 182 daltons. When given intravenously it is not metabolised, but is freely filtered by the glomeruli into the tubular fluid where it acts as an osmotic diuretic. In healthy individuals intravenous mannitol results in a profound diuresis and natriuresis. There are six theoretical reasons, based on animal studies, why mannitol might ameliorate ARF.

a. Intratubular casts may be flushed out by an increase in tubule flow rate. Morris et al (1972) showed that glomerular filtration and urine flow continued in rats infused with hypertonic mannitol during progressive reduction in renal perfusion pressure produced by

aortic clamping.[151] In hydropenic rats or rats infused with isotonic saline alone, glomerular filtration was abolished. Mannitol infused acutely after the perfusion pressure had been lowered to 40mm Hg in hydropenic rats restored urine flow and glomerular filtration.

b. The same workers also demonstrated an increase in renal blood flow (RBF) after mannitol in rats subjected to partial renal artery occlusion. Isotonic saline was ineffective.[151] The increase in RBF preceded the increase in urine flow rate and was assumed to be due to a decrease in afferent arteriolar resistance.

c. By reducing hypoxic cell swelling, mannitol may limit vascular congestion and prevent the no-reflow phenomenon after hypoxia. Flores et al (1972) in a classic study demonstrated the ability of hypertonic mannitol to reverse the no-reflow phenomenon seen after complete renal artery occlusion in rats.[152] Equivalent expansion of the extracellular fluid volume with isotonic saline or isotonic mannitol had no effect.

d. Mannitol causes plasma volume expansion which reduces the haematocrit. Haemodilution appears to protect against erythrocyte aggregation following an ischaemic insult.[153]

e. Mannitol may protect mitochondrial function after ischaemia. Schrier and colleagues (1984) demonstrated that in dogs with norepinepherine induced renal failure, pre-treatment with mannitol preserved renal cortical mitochondrial function.[154] During reflow following ischaemia the rise in intracellular calcium is buffered by mitochondria at the expense of mitochondrial respiration. Mannitol appears to attenuate this rise in intramitochondrial calcium thereby preventing the decrease in mitochondrial respiration.[154,155]

f. Mannitol has free radical scavenging properties and may help to mop up free radical species generated during reflow after ischaemia.[115]

Can mannitol prevent ARF if given with intravenous fluids to high risk patients?

In a wide variety of animal models of ARF pre-treatment with mannitol does appear to prevent oliguria and attenuate the severity of renal dysfunction. [57,156,157] In clinical studies the results are not so definitive. The use of prophylactic mannitol has been promoted in certain high risk groups - vascular surgery, cardiac surgery, obstructive jaundice and rhabdomyolysis.

a. Vascular Surgery

In 1961 Barry reported a diuresis with mannitol in four patients undergoing aortic cross-clamping for abdominal aortic aneurysm repair.[158] In two patients mannitol was given for post operative oliguric and in two it was given as pre-operative prophylaxis. Although apparently successful, these four patients are simple anecdotes. In a follow-up controlled study the same group showed that patients given mannitol during aneurysmectomy had a higher urine flow rate during the period of aortic cross clamping and post operatively, compared to the controls who did not receive mannitol. [159]

In a prospective study Beall et al (1963) looked at 30 patients undergoing elective repair of an abdominal aortic aneurysm, in whom the aorta was cross clamped below the origin of the renal arteries. [160] Patients were divided into three groups. Group 1 received standard care with no intravenous (IV) fluid preoperatively; group 2 received adequate hydration with 5% dextrose while group 3 received IV dextrose plus pre-operative and post-operative mannitol as required to keep the urine output > 60ml/min. The control group showed only minimal depression of renal function in the post-operative period although urine flow rate fell from 5.8 to 0.8ml/min. There was no change in renal function in either the hydrated group or the group receiving fluids plus mannitol. In both groups urine output fell compared to pre-operative values. The post-operative urine output was not significantly different between the two groups, although intra-operative urine flow rate was better in the mannitol group.

This suggests that the prophylactic use of mannitol had no additional benefit over adequate pre-operative hydration in abdominal aortic aneurysm surgery. Finally, in 1964, Powers et al published observations on 50 high risk surgical patients who appeared to benefit from prophylactic mannitol.[161] In addition 52 post-operative patients with established oliguria also received mannitol. The authors comment that all 104 patients had a diuresis and that no patient developed "acute tubular degeneration".

No further studies have been published recently. However, the use of prophylactic mannitol and IV fluids has become standard practice in many vascular surgical units. Prospective, controlled studies to evaluate a specific role for mannitol as compared to adequate volume expansion alone seem unlikely.

b. Cardiac Surgery

In 1964 Berman et al studied 27 patients undergoing a mixture of aortic and cardiac procedures.[162] Patients receiving mannitol had a higher urine output than controls. However, mannitol did not prevent a drop in GFR which fell to approximately 50% of pre-operative values in both the mannitol and the control groups.

These results were confirmed the following year by Etheredge et al.[163] They studied 18 patients before and after cardiopulmonary bypass surgery. Intravenous mannitol was associated with a diuresis but did not prevent the fall in GFR which occurred during cardiopulmonary bypass. Unfortunately this study was uncontrolled.

In 1972 a retrospective study of 428 patients undergoing open heart surgery showed that the incidence of severe (serum urea > 130mg/dl) or mild (serum urea 80-130mg/dl) renal failure was 4.7% and 26% respectively.[164] Frusemide and mannitol, either singly or in combination, did not appear to prevent this deterioration in renal function. No comment was made about the effect on urine volume. Once again this was an uncontrolled study and was retrospective.

Most recently, in 1984, a prospective controlled study of children undergoing cardiopulmonary bypass has been reported by Rigden et al in which a beneficial effect of mannitol on post-operative renal function was found.[165] They studied 20 patients who received 0.5mg/kg of 20% mannitol and 20 patients who received an equal volume of Hartmann's solution. The serum creatinine rose in both groups post-operatively. Pre-operative values were regained more rapidly by the mannitol group, with a significant difference between the two groups on post-operative days 3 and 5. Interestingly, the group receiving mannitol also had less albuminuria post operatively.

c. Obstructive Jaundice

It is generally believed that obstructive jaundice can predispose to acute post-operative renal failure.[166-168] In 1960 in a study of 350 patients with obstructive jaundice Williams et al described uraemia as the commonest cause of post-operative death.[169] Dawson et al (1965) found a strong correlation between the pre-operative serum bilirubin concentration and the fall in post-operative creatinine clearance.[170] Administration of mannitol pre-operatively reduced this fall to 18%, compared with a fall of 63% in jaundiced patients not given mannitol.

The pre-operative administration

of mannitol and IV fluid has thus become virtually mandatory in surgery on patients with hepatic problems. A different view, however, has been expressed by Gubern and colleagues in 1988.[171] They carried out a prospective, randomly allocated study assessing the effect of mannitol on serum creatinine, creatinine clearance and urinary sodium excretion immediately before and for three days after elective biliary tract surgery. The mannitol group received 50g over 1 hour IV prior to anaesthesia and continued this each day for two days after surgery. An initial loading dose of 1 litre of 5% dextrose was given to all patients prior to surgery. Fluid balance was carefully maintained perioperatively in all patients.

Under these circumstances, there was no significant change in creatinine clearance in the control group (mean pre-operative creatinine clearance 64ml/min; post-operative 54ml/min). Surprisingly there was no beneficial effect of pre-treatment with mannitol on post-operative renal function. Indeed patients whose creatinine clearance exceeded 70mls/min pre-operatively had a statistically significant fall in this post-operatively despite mannitol infusion. The authors conclude that "The routine use of an osmotic diuretic is of no value in preventing renal failure in patients undergoing surgery for the relief of jaundice".

d. Rhabdomyolysis

Animal models of rhabdomyolysis have shown that prophylactic use of mannitol may prevent renal dysfunction by inducing a diuresis and so preventing intratubule obstruction with casts.[172] However, in a clinical setting the prophylactic use of mannitol is rarely possible. Early treatment of rhabdomyolysis with a forced alkaline diuresis and mannitol may prevent or attenuate renal dysfunction. Ron et al (1984) began an alkaline solute diuresis in seven patients with extensive crush injuries immediately after extraction from the debris.[173] None of these patients developed renal failure. This is a case report without any controls, and there are no studies comparing the effect of mannitol versus adequate fluid replacement alone.

Can mannitol convert oliguric ARF to polyuric ARF?

The concept that a mannitol infusion given during the phase of pre-renal failure known to precede acute established organ failure might produce polyuria and prevent progression of renal failure was first made by Barry and colleagues in 1962.[174] Sixteen of 24 patients with prolonged oliguria, low urinary specific gravity and a tentative diagnosis of ARF developed polyuria (urine flow > 60ml/hour) after acute intravenous infusion of mannitol.

Three patients responded to a single dose of 12.5g. The remainder required a mannitol infusion titrated to maintain adequate urine volumes. They felt unable to predict which patients would respond to mannitol and made the plea that it be initiated "the moment oliguria is confirmed, even coincident with resuscitation and in dehydrated patients, since the moderate urinary fluid loss required by improved renal haemodynamics and function can be replaced easily. No patient should be denied a trial".

These observations were strengthened by the studies of Eliahou.[175] Ten patients diagnosed as having "incipient" ARF on the basis of oliguria and "low" urinary osmolality diuresed after 250ml of 10% mannitol intravenously. Renal function returned to normal generally within 2-4 days. Unfortunately, this study, as with all others, was not controlled for volume expansion alone. In an attempt to predict which patients with incipient ARF might respond to mannitol, the Glasgow group reported clinical observations on a total of 72 patients.[176,177] All patients were oliguric (urine flow < 20-30ml/hour) and had a low urine to plasma urea and osmolality ratio. Care was taken to ensure that obvious volume depletion was corrected by the use of intravenous blood, plasma or electrolyte containing fluid. However, central venous pressure was measured in only 4 patients. No information was available on oxygen delivery or blood pressure at the time of mannitol administration. Forty five of the 72 patients experienced a significant increase in urine volume and amelioration of their ARF after mannitol (20-60gm over 6 hours). It was concluded that a response to mannitol should be expected when the urine:plasma osmolality > 1.05, the duration of oliguria < 50 hours and persistent peripheral circulatory failure is absent.

Once again this study was uncontrolled. The effect of continued volume expansion per se in these patients is unknown.

Can mannitol improve GFR and ARF?

A rise in creatinine clearance of, on average 24 ± 3ml/min is shown by Valdes et al in 1979 in 7 ventilator dependant patients after 100ml of 20% mannitol intravenously.[178] However, all patients had good pre mannitol urine volumes (90 ± 22ml/hr); only 2 patients had urine volumes < 50ml/hr, and pre mannitol renal function was only mildly impaired (creatinine clearance 91 ± 27ml/min). Urine osmolality was low in only 1 patient, implying that renal failure was not established and the patients were probably primarily dry.

In summary, while there is much anecdotal evidence in favour of a beneficial role for mannitol, there are no prospective randomly allocated studies in which its effect are compared with those of volume expansion alone. Until such studies are carried out, the case for mannitol must remain, in the words of the Scottish legal verdict, "Not proven".

2:2:2 Loop Diuretics

There are six "loop diuretics" currently available or under investigation in the United Kingdom (Mersalyl, ethacrynic acid, frusemide, bumetanide, piretanide and torasemide). Frusemide and its successors all have a similar principal mode of action. The diuretic effect is due to their ability to bind to and inhibit the Na+/2Cl/K+ transporter in the luminal membrane of the thick ascending limb of Henle - hence the expression "loop diuretic" (Figure 2:1).[179] The blocked uptake of chloride leads to a fall in cytosolic chloride activity and inhibition of short circuit currents. Na/K ATPase activity at the baso-lateral membrane becomes unnecessary as a result of the blocked sodium uptake. The cell enters a "resting state" with no need to expend energy and consume oxygen.

Thus far, frusemide has been the principle loop diuretic studied in ARF. Studies in animal models of ARF have given us several theoretical reasons as to why these diuretics might be of benefit.

a. Loop diuretics produce a "resting state" in cells of the thick ascending limb of Henle as described above. In isolated perfused kidneys and in rats, frusemide has been shown to preserve tissue ATP levels, presumably by suppressing reabsorptive work. As a consequence, cells of the thick ascending limb - those areas farthest from oxygen supply - are protected from hypoxic damage.[53,54]

b. Loop diuretics will increase tubule flow rate. This may flush out intratubular casts and help to prevent intratubular obstruction.

c. Loop diuretics, by inhibiting chloride flux at the macula densa, will inhibit tubuloglomerular feedback. Hence the effects of increased sodium delivery to the macula densa resulting from proximal tubule damage will not in turn cause a fall in nephron filtration rate. This will protect GFR.[180]

d. Loop diuretics reduce renal vascular resistance and increase RBF.[181] The exact mechanism is unknown. Inhibition of prostaglandin dehydrogenase, a recognised effective of loop diuretics may be important.[182] Prostaglandin dehydrogenase degrades PGE_2, a substance known to cause afferent arteriole dilatation. Prostaglandin synthetase inhibitors reduce the vasodilatory effect of loop diuretics.[183]

Do loop diuretics convert oliguric renal failure to polyuric renal failure?

Controversy exists over the answer to this question. In an uncontrolled, retrospective analysis, Minuth and colleagues could not demonstrate a response in 51 of 79 patients (64%) given loop diuretic after the onset of ARF.[184] 14% had a transient diuresis and required repeated doses of frusemide, and 22% developed a sustained diuresis after a single dose of loop diuretic. In agreement with Minuth, Kleinknecht could not show any significant reduction in the duration of oliguria following intravenous loop diuretic.[185] Others have shown that administration of frusemide either as a bolus or as a continuous infusion, can promote polyuria.[186-192]

Do loop diuretics shorten the period of renal dysfunction and reduce the need for dialysis?

In 1971 Cantarovich showed that dialysis dependent patients receiving a progressive dose of frusemide (100 - 3200mg/day in geometric progression) had a smaller duration of creatinaemia than patients receiving a fixed dose of frusemide (600mg/24hours) or those receiving conventional therapy (17.5 days, 25.4 days and 26.6 days respectively).[186] Patients in the fixed dose group were given frusemide 600mg daily for 14 days, while the progressive dose group had 1240mg daily for 7 days. Therefore, the size of the dose appears to be more important than the total dose of drug given.

In a second study, the same group showed that 2000mg frusemide given on alternate days to dialysis dependent patients decreased the number of dialyses required, but not the time taken to recover a normal creatinine.[187] However, the patients in this study were compared with retrospective controls.

Minuth et al also demonstrated that patients who responded to frusemide had a significantly lower need for dialysis than those who failed to respond.[184] This was again a retrospective analysis of case sheets, and the dose of frusemide varied between 40mg and 500mg daily.

Despite an increase in urine output in patients receiving frusemide, Brown et al could not demonstrate a rise in GFR sufficient to decrease the need for dialysis.[190] Kleinknecht et al did a prospective analysis of 66 patients.[185] They could not show any difference in the number of dialyses or the time to reach a spontaneous decrease in the blood urea between treated and control patients. No severity of illness score was performed, but the two groups did not appear to differ.

Do loop diuretics lower mortality?

No! Many studies have addressed this question.[184-186,188,191] Only one, that of Anderson et al, showed any improvement in mortality.[191] In this study, a group of

nonoliguric patients (spontaneously nonoliguric and pharmacologically induced nonoliguric) had a lower mortality than a group of oliguric patients (26% v 50%). However, spontaneously nonoliguric patients had a lower fractional excretion of sodium (FENa) than oliguric patients. Oliguric patients who responded to frusemide had a milder degree of renal impairment as evidenced by lower serum creatinine, lower urine sodium and lower FENa than those who did not respond. Thus the nonoliguric patients who had a better mortality also had less severe renal failure. No severity of illness score was performed to determine if the non- oliguric group was indeed less sick than the oliguric group.

While mortality is an outcome of particular interest it is a difficult question to address in studies examining the effect of various treatment regimens in ARF. Choosing endpoints which allow assessment of renal function such as duration of renal failure, need for dialysis etc are specific. Mortality is not an end point specific to ARF and may be affected by many other factors during the course of the patient's illness. It is well recognised that many patients with ARF die not from ARF, but from the primary illness. Mortality may therefore not be an appropriate endpoint to address when assessing the effect that loop diuretics have on recovery from ARF, although shortening the period of renal dysfunction may have a beneficial effect on prognosis.

However, as a key factor in patient outcome, mortality has been looked at in all studies addressing the role of loop diuretics in ARF. As in the majority of these studies loop diuretics have very little effect other than to increase urine output, it is not surprising that no beneficial effect on mortality has been shown. Anderson's study compares non-oliguric with oliguric patients but as there were patients on frusemide in both groups no comment con be made about the contribution of loop diuretics to the improved mortality in the non-oliguric group.

2:2:3 Dopamine

In normal subjects, low dose dopamine (1-5 μg/kg body wt/min) causes a rise in renal plasma flow, increased inulin clearance and increased FENa.[193] The prophylactic use of low dose dopamine in at risk patients has been shown to reduce the incidence of postoperative ARF.[194,195] Salem et al (1988) showed that aortic cross clamping reduced GFR, which was restored or increased by the addition of dopamine.[195] Schwartz et al (1988) demonstrated a rise in renal blood flow that was not associated with a significant diuresis, in patients undergoing vascular surgery who received dopamine either pre or post operatively.[196]

In oliguric, euvolaemic intensive care patients, low dose dopamine alone caused an increase in urine volume which fell when the dopamine was stopped. The response to dopamine was independent of serum creatinine.[197] Parker et al demonstrated an increase in osmolar clearance, FENa, creatinine clearance and urine volume in 52 patients on low dose dopamine.[198] This study was not controlled and eighteen of the patients with a creatinine clearance of 13+9.9ml/min required a frusemide infusion to increase urine volume. The increase in creatinine clearance was not significant in the patients requiring frusemide.

Three separate groups have shown that patients who remain oliguric despite high dose loop diuretics or hypertonic mannitol, may begin to diurese if dopamine is added.[199-201] Again none of these studies were controlled. Plasma frusemide levels may have been high at the time of dopamine administration and could have contributed to the diuresis observed after the dopamine infusion.

2:2:4 Atrial natriuretic peptide

"...the most promise for an ANP-related drug is in the treatment of acute renal failure."

Pollock and Opgenorth [202]

Animal studies have shown that an intravenous or an intrarenal arterial infusion of atrial natriuretic peptide (ANP) or a residue peptide, atriopeptin III (APIII), can reverse the effects of experimental ischaemic ARF.[202,203] The mechanism by which this occurs is thought to be due to the vasodilatory action of ANP. Schafferhans et al showed that immediate infusion of intrarenal arterial ANP after a 40 minute intrarenal arterial noradrenalin infusion caused an increase in GFR, urine flow and FENa and thus reversed the ischaemic effect of noradrenalin.[204] Shaw et al (1987) induced ischaemia by renal artery occlusion and then infused ANP into the renal artery.[205] Creatinine clearance 4 hours and 24 hours post infusion fell in control rats, but was within the normal range for rats infused with ANP. Rats given ANP also had a greater urine volume than controls at 4 hours and a lower FENa at both 4 and 24 hours. This group also showed that morphological damage was less after a 4 hour infusion of ANP.

Intravenous administration of Atriopeptin III has also been shown to improve GFR, urine flow and tubule function in both in vitro and in vivo studies when given after renal artery occlusion.[206] This study also showed that post ischaemic renal ATP regeneration improved after ANP infusion.

Conger et al (1991) induced renal failure in rats with a norepinephrine infusion.[203] Two days later, when ARF was established, an infusion of APIII plus dopamine (APIII-D) to maintain blood pressure was commenced over a four hour period. At the start of the infusion, serum creatinine had increased in both control and study groups to 1.65+0.6mg/dl and 2.02+0.99mg/dl respectively. At day four, control rats infused with saline had a serum creatinine of 2.78+2.11mg/dl and rats infused with APIII-D had a serum creatinine of

1.21+0.81mg/dl ($p<0.05$). Rats treated with APIII-D also had a lower FENa at day four. In other words, in animal studies APIII-D causes a sustained improvement in GFR and tubule function in established ARF.

Reduced size analogues of ANP have also similar effects in rats with ischaemic ARF. Pollock et al (1992) showed that a short term intravenous infusion of a reduced size analogue of ANP attenuated the histological damage and the rise in creatinine induced by bilateral renal artery occlusion.[202]

Human studies are limited. Gotz et al (1989) studied 13 patients with dopamine and frusemide resistant ARF.[207] Seven oliguric and six nonoliguric patients were given ANP 2.5ug/min for 3 hours. There was no change in urine volume, GFR or FENa in the oliguric patients. In the nonoliguric patients urine flow increased more than 50% and FENa increased by about 40%. There was no change in GFR. It is likely that the nonoliguric group who responded to ANP had less severe renal failure than the oliguric group. The nonoliguric group had a higher pre study GFR compared to the nonresponders (19.8+8.5ml/min v 6.2+1.5ml/min).

Recently, Rahman et (1994) studied 53 patients with established, intrinsic ARF.[208] Patients were allocated to one of 2 groups. Group 1 received intrarenal ANP via catheters in both renal arteries for 8 hours, or intravenous ANP for 24 hours. Some patients also received mannitol or frusemide. Patients in group 2 received mannitol, frusemide or no diuretic and were not given ANP.

Urine flow increased significantly in both groups in the first 8 hours of treatment, while creatinine clearance increased significantly in group 1 only. Significantly fewer patients in the ANP group required dialysis compared to group 2 (23% v 52%, $p<0.05$), But there was no significant difference in survival, although there was a trend towards improved survival in group 1. Interestingly, Rahman points out in his discussion that although '...there was no

convincing evidence that diuretic therapy was of proven benefit in ARF patients, most primary physicians indicated they were reluctant to enter patients if they were randomised to a group that would not at least receive diuretic therapy'.

Two other studies failed to show any consistent benefit from the use of ANP in the post transplant patient.[209,210] However, ANP may offer protection against post transplant ARF if added to the perfusate at the time of harvesting the kidneys.

There are two major problems which limit the clinical use of ANP. Atrial peptides act by peripheral vasodilataion and in large quantities cause hypotension. Endogenous and exogenous ANP are rapidly degraded by neutral endopeptidase. The development of oral enzyme inhibitors of ANP degradation may be one way of ameliorating the development of ARF in the future.

2:2:5 Calcium channel blockers

If the vascular consequences of an ischaemic insult, ie increased renal vascular resistance, loss of autoregulation, hypersensitivity to renal nerve stimulation and decreased glomerular permeability, are due to an increase in intracellular calcium in afferent arteriolar and glomerular mesangial cells, then the administration of a calcium channel blocker might be expected to attenuate or abolish these effects.

Burke and colleagues showed that an intrarenal infusion of verapamil, given for 30 minutes before renal ischaemia was induced by intrarenal noradrenalin (NA), had a protective effect on both function and morphology.[211] Verapamil or nifedipine given intrarenally for 2 hours after an intrarenal NA infusion also protected the kidney.[211] The protection afforded by verapamil in a renal artery clamp model of ARF is not so conclusive.[212] These authors suggest that verapamil may prevent complete cessation of renal blood flow in the NA model.

The concentration of NA, vasopressin and angiotensin are increased in ARF. Verapamil and nifedipine have been shown to block the response of the arterial resistance vessels to these vasoconstrictors.[213] It has been shown that these vasoconstrictors act on vascular smooth muscle and mesangial cells by causing an increase in cytosolic calcium.[213-217]

The effect of calcium channel blockers on renal tubular epithelium is less certain. Calcium channels have not yet been demonstrated in renal tubule cells. However, verapamil has been shown to reduce the uptake of calcium seen in proximal tubules after 30 minutes of anoxia.[218]

Calcium channel blockers seem to be effective when administered prior to or immediately after the ischaemic insult. Their potential may therefore be more fully utilised in renal transplantation. Experimental studies in both animals and humans have shown that this class of drug does indeed have a beneficial effect in renal transplantation. Isolated rat kidneys flushed with verapamil or emopamil had higher inulin clearances and renal cortical ATP levels than controls.[219,220] In clinical studies Duggan showed that verapamil given to donors before harvesting the kidneys improved early graft function.[221] Early rejection was also reduced. Pre treatment of renal grafts and recipients with diltiazem also reduced the incidence of delayed graft function.[222,223] In this study graft survival was not affected.

2:2:6 Other potential therapeutic agents in acute renal failure

Adenosine nucleotides and magnesium chloride infused into rats after an ischaemic injury accelerated recovery of inulin clearance and tubule function, and also preserved cell morphology.[224,225] ATP given alone causes adverse cardiovascular effects by chelating divalent cations. Equimolar amounts of magnesium chloride given with ATP prevent these adverse effects from occuring.[226] As yet there are no human studies looking at the effect of magnesium chloride and ATP in ARF.

Thyroxine stimulates renal tubule transport mechanisms and has been studied in ARF in animals and man with encouraging results.[227,228] Unfortunately the clinical study was uncontrolled.[228] **Glucagon** has beneficial effects if given prior to or immediately after development of ARF, but has no effect on established ARF and is therefore of no therapeutic value, although it may yet have a role in prophylaxis against ARF.[229]

2:3 The future

There have been exciting developments in our understanding of the pathogenesis of acute renal failure which may well contribute to our ability to modify the disease. Evidence for the role of endothelin is accumulating. Tomita et al (1989) have shown that plasma endothelin levels in patients with ARF are raised, while in experimental studies antibodies to endothelin have improved the outcome of ARF.[230,231] Gellai et al (1994) showed that in rats with established ARF, infusion of a selective endothelin receptor antagonist (BQ123) improved Na^+ reabsorption and moderately increased GFR and K^+ excretion.[232] All vehicle treated rats died by day 3, whereas rats given the endothelin$_A$ receptor antagonist were all alive at day 4 and eventually 75% fully recovered. The therapeutic potential for this in established ARF is enormous.

A monoclonal antibody against intercellular adhesion molecule 1 (anti-ICAM-1) improved post ischaemic ARF when given up to 2 hours after the ischaemic insult.[233] Histological evidence of ARF and tissue levels of myeloperoxidase (induced by ischaemia) were both significantly reduced by anti ICAM-1 infusion.

ANP is thought to exert its biological actions by activating guanylate cyclase thus increasing the production of cyclic guanosine monophosphate (cGMP). Therefore, inhibiting the degradation of cGMP would be expected to have a similar effect as administration of ANP. Guan et al induced ARF in rats by clamping the renal arteries for 15 minutes.[234] Twenty-four hours later the rats were given vehicle, ANP or Zaprinest - a cGMP specific

phosphodiesterase inhibitor. Rats given Zaprinest had a significantly greater GFR 24 hours after infusion of the drug. Rats given ANP also had an increased GFR but this did not reach statistical significance.

Urodilatin is another natriuretic peptide whose structure comprises the entire sequence of human ANP plus a four amino-acid N-terminal extension.[235] It is detectable in human urine but not plasma.[236] Unlike ANP, its hypotensive effect is minimal, but it causes a greater increase in GFR and more of a natriuresis than ANP.[237]

In an animal model of ischaemic ARF, Shaw et al demonstrated that urodilatin and dopamine had a greater beneficial effect on GFR than dopamine alone or dopamine plus nitroprusside.[238] In a recent randomised controlled trial in which patients had developed oliguria following cardiac surgery, Wiebe et al showed that an infusion of urodilatin caused a brisk diuresis - 363.6 ± 102ml/hr v 69.4 ± 20ml/hr in the control group.[239] 86% of the control group required dialysis whereas none of the patients who received urodilatin required dialysis.

Several authors have shown that pre treatment with a PAF receptor antagonist improves the recovery of the GFR after acute ischaemic renal injury.[240-242] As yet there are no studies examining the effect of PAF receptor antagonists given after the acute ischaemic insult.

Aggregation of detached tubule epithelial cells causes intratubule obstruction and contributes to the maintenance of ARF. Integrins recognising the argenine-glycine-aspartic acid (RGD) sequence may participate in cell-cell adhesion of exfoliated tubule cells and could thus be targeted to prevent tubule obstruction.[243] In vitro studies have demonstrated that synthetic RGD peptides that mimic the RGD sequence of integrin ligands inhibit cell-cell adhesion of tubule epithelial cells. In rats with experimental ARF, RGD peptides given at the time of reperfusion accelerate recovery from ARF.[244]

The number of potential therapeutic agents in ARF currently under investigation reflects the extremely complex and multifactorial nature of the pathogenesis of ARF (Figure 2:2). The existence of many pathways (some interrelated) all ultimately resulting in tubule cell death means that a single therapeutic agent targeting a particular pathway is probably not the answer. In addition, most of the therapeutic agents show a beneficial effect when given prior to or immediately after reflow. In clinical practice this is rarely possible and therefore attention must be turned to hastening regeneration of tubule cells and thus recovery from ARF.

Growth factors are polypeptides that can stimulate or inhibit cell proliferation. In rats with experimental ARF induced by bilateral renal artery clamping, exogenously administered epidermal growth factor (EGF) attenuated the rise in serum creatinine and increased renal DNA synthesis.[245,246] Binding of EGF in the kidney was increased.

Insulin like growth factor-1 (IGF-1) is mitogenic for renal epithelial cells and in ARF there is an increase in the number of IGF-1 receptors in regenerating tubules.[247] However, IGF-1 appears to be important in differentiation and maturation of regenerating cells as the increase in IGF-1 appears after proliferation has occurred. Recombinant IGF-1 given to rats 5 - 24 hours after induction of ischaemic ARF caused accelerated regeneration and repair of the epithelium and attenuation of ARF.[248,249] When given prior to the ischaemic insult there did not appear to be any additional benefit.[249]

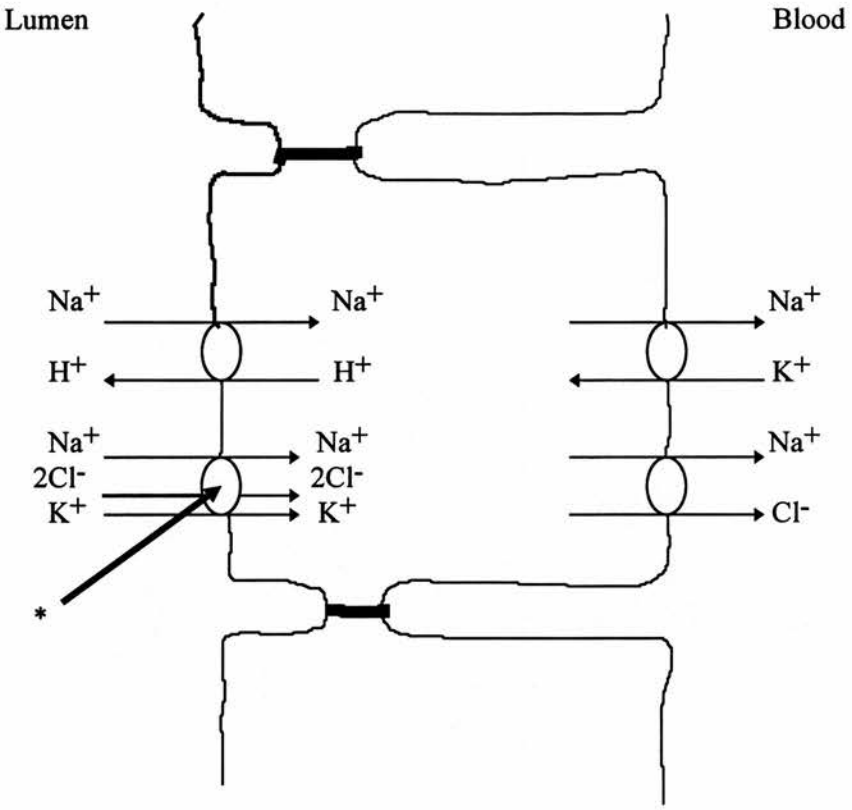
Hepatocyte growth factor (HGF) is also mitogenic to a variety of cultured proximal and distal tubule epithelial cell lines.[250] Human recombinant HGF given intravenously to mice with $HgCl_2$ induced ARF caused a significant increase in DNA synthesis in tubule cells in the outer medulla where an increase in HGF receptor mRNA was also detected.[251] In rats with ischaemic ARF, subcutaneous recombinant human HGF given 30 minutes after reperfusion enhanced recovery and improved survival.[252]

Other growth factors may also have a role in the process of repair following ARF. Transforming growth factor-β (TGF-β) effects a different physiological response in different cell types. It inhibits epithelial cell proliferation; mesenchymal and endothelial cell mitogenesis can be stimulated or inhibited.[253] Therefore TGF-β may regulate the regenerative response. Fibroblast growth factors are potent inducers of blood vessel growth, and induce expression of proteolytic enzymes involved in tissue remodelling.[253] Their role in regeneration after renal cell injury is not clear. Similarly, the role of platelet derived growth factor like protein (PDGF) released by mesangial cells (which also express PDGF receptors) is not clear.[254] Endothelial and mesangial cells have a proliferative response to PDGF, but PDGF does not appear to be mitogenic for renal epithelial cells in culture.[253] Some growth factors seem to have a beneficial effect on each other. IGF-1 and EGF reciprocally enhance the expression of their receptors. Thus a combination of growth factors given post insult may result in a more effective mitogenic response.[255]

2:4 Summary

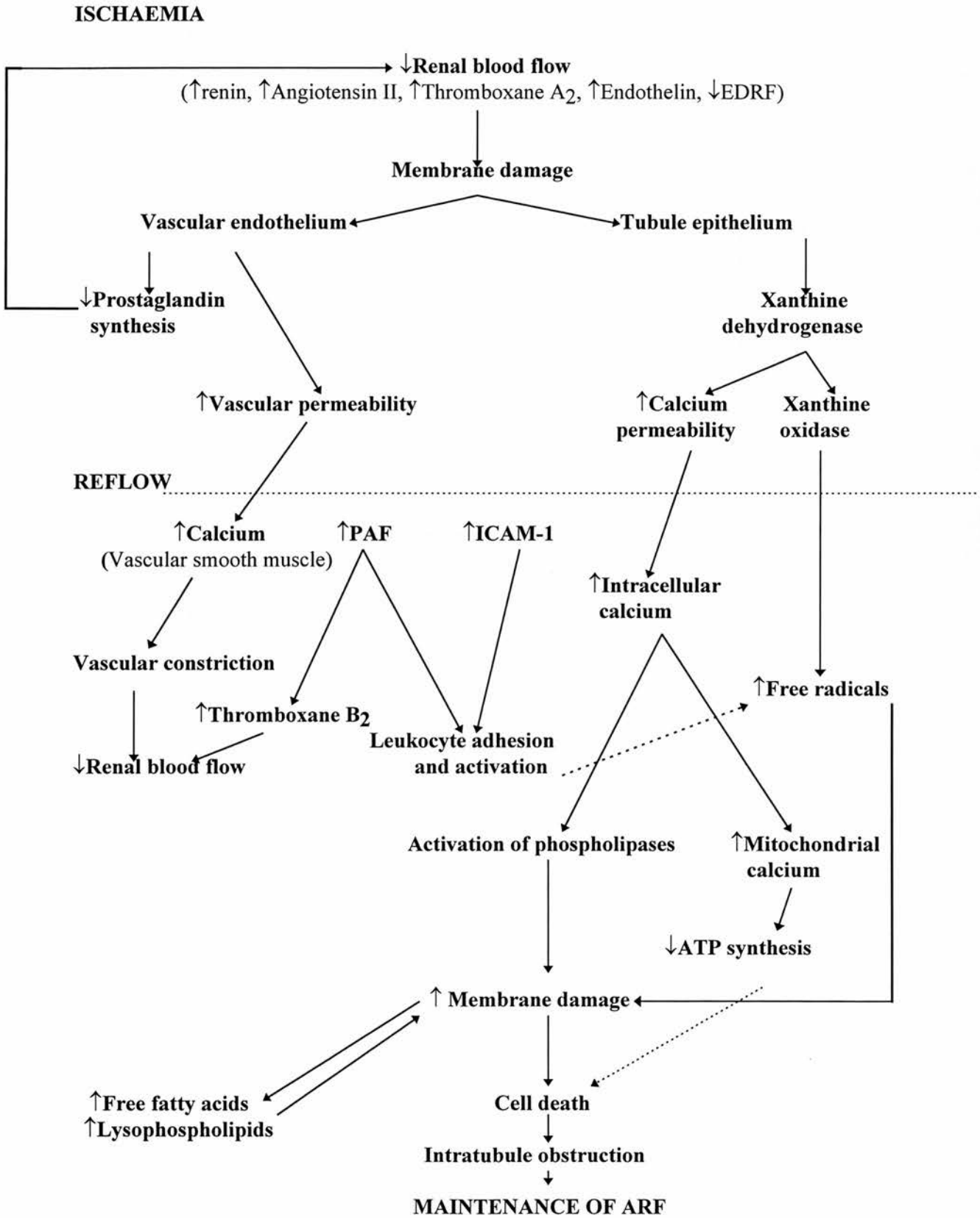
The pathogenesis of ischaemic ARF is extremely complex with a number of factors contributing to the development and progression of ARF (Figure 2:2). There have been many therapeutic attempts to modify the progression of ARF and to date none have proven clinically useful. The role of loop diuretics has been investigated in the past but the studies performed were poorly controlled, retrospective or anecdotal. There is a need therefore for a properly controlled, prospective double blind study of the effect of loop diuretics in ARF.

Figure 2:1 Action of loop diuretics in the thick ascending limb of Henle's loop



* Bold arrow indicates site of action of loop diuretics at the $Na^+K^+Cl^-$ transporter

Figure 2:2 Factors contributing to the development and progression of ARF



3 Energy Expenditure and Outcome in Acute Renal Failure

3:1 Daily energy expenditure

Total daily energy expenditure (EE) is made up from the following components: basal metabolic rate (BMR), resting energy expenditure (REE), dietary thermogenesis, shivering and non shivering thermogenesis, and voluntary physical activity.

The BMR was defined in 1920 by Boothby and Sandiford as "...the minimal heat production of an organism, measured from twelve to eighteen hours after the ingestion of food and with the organism at complete muscular rest".[256] The BMR is a result of the metabolic processes required for cellular integrity, and also the metabolic processes of various organs required to maintain life. The BMR is related to cell mass and can therefore be predicted from variables such as height, weight and body surface area (BSA) corrected for age and sex.[257] The regulation of BMR is largely by changes in peripheral metabolism of thyroid hormone.[258]

3:2 Effect of illness on basal metabolic rate

The BMR is measured in a resting subject in a thermoneutral environment 12 - 18 hours after last eating. Injury, burns and sepsis all cause an increase in core temperature and BMR. The regulation of EE in trauma and sepsis appears to be similar to that of non shivering thermogenesis and is activated by the sympathetic nervous system.[257] As well as increased heat production in brown adipose tissue there is a resetting of the hypothalamic 'thermostat' to maintain a higher core temperature. Thus the patient feels cold resulting in shivering and non shivering thermogenesis with a rise in the core temperature until it meets the new set point. During the process energy expenditure is increased.

Burns cause an obligatory loss of heat from the surface of the burn because blood flow to regenerating granulation tissue is high and not regulated by the autonomic nervous system.[259] Therefore the temperature at the skin surface remains close to the core temperature and heat is lost by radiation, conduction and convection (dry heat loss) and evaporation (wet heat loss). In this instance heat loss and therefore EE can be minimised by keeping the patient in a warm, humid environment.

Trauma and sepsis also cause an imbalance in the glucose-fatty acid cycle with marked hyperglycaemia, even although energy is largely derived from oxidation of fat.[260] This is due to the high concentration of hormones associated with increased sympathetic activity such as glucagon, cortisol and catecholamines. During an infusion of total parenteral nutrition (TPN) there is an increase in noradrenalin excretion with an increase in EE.[257] Thus diet induced thermogenesis from overfeeding results in metabolic stress.

3:3 Resting energy expenditure

REE may be measured at any time of day, as long as the methods outlined in chapter seven are adhered to, and has been shown to be approximately 10% higher than the basal energy expenditure as measured in deep sleep.[261-263] REE accounts for 75-90% of total energy expenditure; diet induced thermogenesis, shivering and non shivering thermogenesis, and exercise make up the rest.[257,264]

A number of factors affect the REE:

1. Size of the individual - REE increases with increased height and weight.[261]
2. Sex - males have a higher REE than females of the same weight. REE correlates closely with lean body mass and males have a higher lean body mass compared to females.[261]
3. Age - older patients have a reduced REE.[261]
4. Diet - intermittent feeding causes an increase in REE of 8-10% whereas continuous enteral or parenteral feeding reduces diet induced thermogenesis by 4-8%.[265]

Thermogenesis peaks within the first hour after a light meal and has been shown to return to baseline within the next hour.[262] Hypercaloric parenteral nutrition increases metabolic rate but the effect is stable when the nutrients are infused at a constant rate.[263]

5. Sleep - REE is reduced during sleep.[266]
6. Work of breathing - impending respiratory failure causes an increase in REE.[264]
7. Disease - release of neurotransmitters, mediators of inflammation, increased catabolism, increased core temperature all increase REE.[265] This effect is not seen until the patient has passed from the ebb phase to the flow phase of injury, usually 8-12 hours after the onset of acute injury.[267]
8. Physical activity causes a rise in REE.[268,269]
9. Body temperature - each degree rise on the Farenheit scale causes a 7% rise in REE (13% rise for each degree on the Celsius scale).[264]
10. Drugs also affect the REE. Pressor agents and catecholamines increase REE while narcotic analgesics, sedatives, general anaesthesia, beta blockers and alpha blockers all cause a reduction in REE.[269-271]

The REE can be predicted using the Harris-Benedict equation (HBREE).[272] This is dealt with in detail in chapter 7.

3:4 Measurement of EE

Indirect calorimetry provides a method of accurately measuring EE at the bedside.[262] A full description of the technique is given in chapter 7. Its main use has been in the design of nutritional regimens suitable for different disease and organ failure states.[264]

The ratio of REE measured using indirect calorimetry to the predicted REE (REE/HBREE) can be used to determine whether patients are hypermetabolic (ratio >110%), normometabolic (ratio >90%, <110%) or hypometabolic (ratio <90%).[264]

3:5 Energy expenditure in acute renal failure

Overall mortality from ARF remains high.[273] However, mortality from various types of ARF is variable ranging from 35% (nephrotoxic ARF) to 75% (ARF associated with sepsis and MOF).[274,275] It has been suggested that the difference in mortality between different types of ARF is due to differences in metabolic status.[276] Hypermetabolism in ARF results in malnutrition, impaired wound healing and impaired immunological function with increased morbidity and mortality.[277]

EE in ARF has been reported to vary between 1.19 - 2.5 times the predicted REE.[278-280] In the study from Bouffard et al the measurements of EE were widely scattered implying that ARF is not associated with a predictable increase in EE.[278] The presence of sepsis did however, significantly increase EE.

The APACHE II score is a marker of physiological stress and, when measured within the first 24 hours of hospital admission, can be used to predict outcome.[281] It is possible, therefore, that the APACHE II score may be related to the REE of the patient.

3:6 Summary

Various disease states cause an increase in REE and in general, the more severe the injury the greater the metabolic response.[269]

Indirect calorimetry is an accurate way of measuring energy expenditure in the critically ill patient, which in turn can be used as a marker of physiological stress. Chapter 7 (Resting Energy Expenditure and the APACHE II Score in Patients with Acute Renal Failure) describes studies which I carried out on patients with ARF. The REE was measured and compared with another objective marker of physiological stress (APACHE II score).[281]

4 Aims and Objectives

The multifactorial pathogenesis of ARF makes it unlikely that a single agent will be able to attenuate the effects of ischaemic ARF. Rather, combination therapy targeting contributory mechanisms may be the answer. To date, the most widely used drugs in the treatment of ARF are loop diuretics, mannitol and dopamine, but a precise role for these drugs in the clinical setting of potential or established ARF has not been established. Available studies are usually retrospective, poorly controlled or merely anecdotal reports.

Intracellular calcium levels have been shown to be raised in experimental ARF and also in white cells and red cells from patients with sepsis.[282-284] It is possible therefore that intracellular calcium levels may be raised in patients with ARF, and that the magnitude of the rise may be related to prognosis.

Resting energy expenditure is increased in various disease states and the more severe the injury the greater the metabolic response.[267] The APACHE II score is a marker of physiological stress and can predict outcome of illness.[281] It may therefore be related to the metabolic response to ARF as reflected in energy expenditure.

The aims of this thesis are to examine the following hypotheses:

1. Patients with ARF who are treated with high dose loop diuretics have a better prognosis in terms of survival and speed of recovery than those who receive placebo.
2. Levels of intracellular calcium may have an effect on outcome.
3. Resting energy expenditure may affect outcome.

The specific objectives of the studies are as follows:

1. Describe the role / usefulness of loop diuretics in managing patients with ARF.
2. Measure free intracellular calcium levels in platelets from patients with ARF.
3. Look at the effect of loop diuretics on free calcium concentrations in platelets from patients with ARF.
4. Examine the relationship between intraplatelet calcium concentration and APACHE II score in patients with ARF
5. Examine the relationship between resting energy expenditure and APACHE II score in patients with ARF

In order to do this I enrolled 96 patients for the study described in chapter 5. Patients for the studies described in chapters 6 and 7 were selected at random from the patients enrolled in the loop diuretic study (chapter 5).

The work was carried out in the Renal and Intensive Care Units of the Royal Infirmary, Glasgow while I held the post of research fellow.

Some of the work I am about to describe (chapter 5) was presented at the American Society of Nephrology meeting in San Diego, November 1995 and has been published in Nephrology, Dialysis, Transplantation.[285] Chapter 6 has been prepared for publication.

5 Study 1 - Loop Diuretics in the Management of Acute Renal Failure

5:1 Introduction

In clinical practice the temptation to use high doses of loop diuretics to increase urine flow rate and thereby perhaps ameliorate the progress of ARF in patients with acute oliguria is strong. The evidence for this practice is poor. Most reported studies have been largely anecdotal, retrospective, non randomised or uncontrolled. [184-192]

Theoretically, administration of loop diuretics should reduce the energy requirements of the cells of the thick limb of the loop of Henle.[286] These drugs act by inhibiting the $Na^+/2Cl^-/K^+$ pump in the luminal cell membrane resulting in a fall in transcellular sodium transport. Basal Na/K ATPase activity becomes unnecessary and the requirement of the cell for oxygen falls. Brezis et al (1984) have shown that reducing active transport with frusemide significantly reduces the damage to the thick ascending limb of Henle's loop in the isolated perfused kidney.[287] It is therefore possible that loop diuretics might 'protect' the cells of the thick ascending limb during the hypoxia which accompanies hypotension and sepsis, frequent predisposing factors in ARF, by reducing the need for energy consumption.

In 1983 a new loop diuretic, torasemide *(Boehringer Mannheim)* became available for clinical trials. This prospective, double blind, randomised, placebo controlled study was designed to compare the effect of frusemide, torasemide and placebo on the outcome of patients with ARF in Glasgow Royal Infirmary. My aim was to answer the following questions:

- Can loop diuretics convert oliguric ARF to non-oliguric ARF and is this associated with an improvement in outcome ?

- Can loop diuretics shorten the period of renal failure and reduce the need for dialysis?
- Can loop diuretics decrease mortality in ARF?

5:2 Subjects and methods

5:2:1 Patient selection

Patients aged 18 years and over referred to the Renal Unit of Glasgow Royal Infirmary with potential ARF were seen by myself over a 3 year period. All had potentially reversible acute intrinsic renal failure as defined by a rise in serum creatinine to over 180umol/l.

When first assessed each patient had bladder catheter drainage established and attempts were made to correct all reversible pre-renal factors. A central venous catheter or pulmonary artery catheter was inserted, arterial blood gases were measured and pulse oximetry established. Obstructive uropathy was excluded by ultrasonography.

Those patients whose ARF did not respond to correction of pre-renal factors and who were not obstructed were then considered for enrolment into the study. Exclusion criteria are listed in Table 5:1. Written consent for the study was obtained either from the patient or, if unconscious, from the next of kin, after explaining the various options and risks. The protocol was approved the Ethics Committee of Glasgow Royal Infirmary. The randomisation code was computer generated by software called Almedica Drug Labelling System.

5:2:2 Methods

Patients were enrolled into the study at time (t) = 0, after a run in period of a minimum of two hours. During this run in period two baseline hourly urine collections were made and blood samples collected for subsequent biochemical tests (Table 5:2). An APACHE II score was calculated for each patient during the run in period.[281]

Serum biochemistry was repeated at t = 24 hours and thereafter on a daily basis at 0800 hours until Day 21 or, if sooner, death. In addition, serum osmolality was measured for the first three days while mannitol was being used. This value was compared with that obtained by calculating the serum osmolality using the formula:

(1.86 x serum sodium) + urea + glucose + 9.[288]

Urine was collected every six hours for the first 48 hours and thereafter every 24 hours. Hourly urine flow rates were calculated. Urine biochemistry tests were carried out on each sample (Table 5:2).

5:2:3 Treatment

All patients were given dopamine (continuous infusion of 2ug/kg estimated body weight/min) and mannitol (infusion of 100ml of a 20% solution for 1 hour every 6 hours for a maximum of 3 days), and were randomised to receive either frusemide, torasemide or placebo as an intravenous infusion over 1 hour every 6 hours for up to 21 days. Previous studies in chronic renal failure suggested that intravenous torasemide is equipotent to frusemide.[289,290] This study has made the assumption that this holds for ARF. The study drug was given in a double blind fashion, initially in a dose of 3mg/kg estimated body weight per dose. If the serum creatinine fell thereafter the dose of study drug was decreased from 3mg/kg to 2mg/kg to 1mg/kg and finally stopped as renal function recovered. Both the study drug and dopamine were continued if the patient became dialysis dependent or failed to recover renal function, for a maximum of 21 days, or until death within 21 days. Although the study ended at day 21 the survivors continued on long term follow up.

Mannitol was discontinued before day three if the patient remained severely oliguric or anuric or became hyperosmolar (measured osmolality - calculated osmolality > 17).

5:2:4 Statistical analysis

A sample size of 90 was determined to be sufficient to detect a clinically significant difference in outcome between the three groups (W Koehler, statistician, Boehringher Mannheim - personal communication). The absolute values of the primary variables (recovery, death or dialysis) were compared using chi-squared analysis. This was the most appropriate test to use as I was predicting that patients given loop diuretic would have different rates of recovery, death and dialysis, without specifying the direction of the association. Other variables were analysed by means of analysis of variance (mean ± S.D.).

5:3 Results

A total of 278 oliguric patients were assessed for entry into the study (Figure 5:1), 25% of whom recovered with adequate rehydration. A further 40% were excluded as they did not fit the entry criteria or refused consent. The remaining patients (n= 96) were enrolled into the study. Of these, 4 patients are excluded from the statistical analysis; 2 died in the run in phase before the study drug was given and a further 2 patients were inappropriately enrolled as they had been in another study within the preceding 30 days. 92 patients are therefore available for analysis on an intention to treat basis. The sample size for each group is as follows: torasemide n=30, frusemide n=32 and placebo n=30.

Table 5:3 shows demographic and clinical features. Patients in each of the groups were well matched for age, sex, severity of illness and degree of renal impairment. The causes of acute renal failure were similar in all three groups and have been amalgamated to show the overall causes of ARF in Table 5:4.

A tabulated list of important screening data for each individual patient is shown in Appendix 1. Appendix 2 is a list of outcome data for each individual patient.

5:3:1 Loop diuretic (torasemide or frusemide) v placebo

Table 5:5 shows the outcome of treatment with loop diuretic or placebo. Patients given torasemide or frusemide had a significant increase in urine output (Figure 5:2) and fractional excretion of sodium (Figure 5:3) in the first 48 hours compared to placebo. These patients did not receive any more mannitol than those in the placebo group. However, there was no significant difference in the final outcome (recovery, dialysis or death) at day 21. Twenty three percent of placebo patients recovered full renal function without requiring haemodialysis compared to 17% and 28% for patients given torasemide and frusemide respectively (p=0.56).

The proportion of patients requiring dialysis were not different in the three groups. The time to dialysis in each group was torasemide 5.0 ± 5.0 days, frusemide 5.4 ± 5.7 days and placebo 2.8 ± 1.2 days (p=0.35). Loop diuretic had no effect on the duration of dialysis (torasemide 5.6 ± 4 days, frusemide 13.4 ± 13.7 days, placebo 13.2 ± 10.7 days, p=0.16).

The number of patients who died by day 21 without requiring dialysis was 47% and 41% in the torasemide and frusemide groups respectively and 37% in the placebo group (p=0.73). The total number of deaths by day 21 (dialysis and non dialysis dependent patients) was 70% in the torasemide group, 66% in the frusemide group and 50% in the placebo group (p=0.24).

Table 5:6 shows the final outcome of all patients who required dialysis. There was no significant difference between the groups. The day the serum creatinine started to fall spontaneously was taken as an indication of renal recovery and there is no significant difference between the groups (torasemide day 7 ± 7, frusemide day 5 ± 5, placebo day 8 ± 7, p=0.46).

Actuarial survival curves for the three groups are shown in Figure 5:4. This is continued up to day 56, since all of the patients who died as a result of their primary illness had done so

by then. At day 56 survival between placebo (43%), frusemide (38%), and torasemide (30%) was not significantly different. Long term survival at 2 years was not significantly different to survival at 56 days. Death after 56 days was due to causes not associated with the primary illness such as trauma, ischaemic heart disease or cancer.

Daily median values for serum creatinine, sodium, potassium, calcium and C reactive protein over the 21 day study period were calculated. There was no significant difference in these between the three groups.

5:3:2 Oliguric v non-oliguric patients

The patients were divided into two groups - oliguric and non-oliguric - based on their urine output during the first 24 hours after starting medication. Those patients whose urine volume in the first 24 hours averaged $\geq$50ml/hour were termed non-oliguric. Conversely those patients with hourly urine volumes <50ml/hour were termed oliguric. One patient has been excluded from statistical analysis because he died prior to the first urine collection after entry into the study. Therefore n = 91.

51 patients remained oliguric during the first 24 hours of the study while 40 were non oliguric either spontaneously or because of diuretic treatment. By day fifty-six, 35 (69%) of the oliguric patients had died compared to 17 (43%) of the non-oliguric patients (p=0.01). The pre-study APACHE II score was better in the group who became non-oliguric (17.2 $\pm$ 5.9 v 20.6 $\pm$5.5, p=0.008), as was the pre-study creatinine clearance (14 $\pm$ 11ml/min v 4 $\pm$ 4ml/min, p<0.0001).

In the group of patients who were non-oliguric, 8 (20%) were on placebo and 32 (80%) were on a diuretic. In the non-oliguric placebo group (n=8) two patients (25%) had died by day 56. In the non-oliguric diuretic group (n=32), 15 (47%) had died by day 56 (p=0.3).

5:4 Side effects

There was a non-significant increase in the incidence of seizures in the patients given loop diuretic - torasemide 6, frusemide 6, placebo 1 (p=0.1). One patient on frusemide became acutely deaf but recovered when the drug was stopped.

Torasemide caused a significant rise in γ glutamyl transferase (γ GT) an effect which is reversible and given as a warning on the international data sheet.

Only 22% of patients continued on mannitol for 3 days. The reasons for stopping mannitol are shown in Table 5:7. The patient who developed pulmonary oedema was not hyperosmolar. Only one of the patients who became hyperosmolar had any symptoms (confusion).

5:5 Discussion

Can loop diuretics convert oliguric ARF to non-oliguric ARF?

The use of high dose loop diuretics in patients with incipient ARF can significantly improve urine output. Thus 57% of patients given torasemide and 48% of patients given frusemide had a significant increase in urine volume in the first 24 hours compared to placebo (23%).

Patients who became non-oliguric had a lower mortality than those patients who remained oliguric (43% v 69% p=0.01). However, patients who became non-oliguric were less ill as evidenced by a significantly lower APACHE II score. They also may have had less severe renal failure as their creatinine clearance was higher. However, this is an imprecise measurement of renal function in these patients. On this evidence I cannot attribute a 'beneficial' effect on mortality solely to the use of loop diuretics.

Of more significance would have been a significant improvement in mortality between those patients who became spontaneously non-oliguric with placebo and those whose diuresis was induced by the use of loop diuretics. There was no difference in mortality at

day 56 between the non-oliguric group who had placebo (spontaneously non-oliguric) and the non-oliguric group given loop diuretic. However, the number of patients in this subgroup analysis is small and larger numbers of patients are required for statistical analysis.

It has been suggested that continuous low dose infusions of frusemide might be preferable to high dose bolus injections.[291,292] It is possible, therefore, that I might have achieved an even greater diuretic effect by the continuous infusion of the loop diuretic. Few controlled clinical trials comparing the diuretic and natriuretic response to infusion versus bolus administration of loop diuretic have been published and most were published after this study began.[188,291-293] They are flawed by the lack of data regarding severity of illness, type of surgical procedure and post operative support required in the two groups of patients. Thus, in view of the lack of definitive evidence in favour of continuous infusion of loop diuretic at the time the study was started, I elected to use bolus therapy as this was standard practice in Glasgow Royal Infirmary at that time.

In 1976 Kleinknecht et al studied 55 patients with established oliguric ARF.[185] Thirty three were given a variable dose of frusemide while 33 were given only intravenous fluids. There was no significant reduction in the duration of oliguria in the group given loop diuretic. Minuth and colleagues found a sustained diuresis in 22% of 104 patients given a variable does (40 - 500 mg) of frusemide.[184] Other groups however, have shown that intravenous frusemide modifies ARF by causing sustained polyuria.[186-192]

Can loop diuretics shorten the period of renal dysfunction and reduce the need for dialysis?

Renal recovery was considered to begin when the serum creatinine started to fall spontaneously, without dialysis. There was no significant difference in the time to renal recovery between the three groups. The need for dialysis was also not different. These findings agree with the studies of Brown and Borirakchanyavat.[190,192]

Minuth and colleagues, however, found a reduction in the need for dialysis in patients given loop diuretic.[184] Cantarovich showed that a progressive does of frusemide (100 - 3200mg/day in geometric progression on continuous days) shortened the period of renal dysfunction, presumably because of the high average daily dose of frusemide (1.24g/day) received by the progressive dose group compared to the fixed dose group (600mg/day).[186] I gave a maximum dose of loop diuretic 1.2g/day.

Can loop diuretics decrease mortality in ARF?.

This prospective randomised double blind study failed to demonstrate an improved mortality in patients treated with loop diuretics, a finding in keeping with most previous less well controlled studies.[184,185,188,190] Only Anderson et al showed a reduction in mortality in the subgroup of patients given frusemide and who were non-oliguric.[191] This subgroup also had a lower fractional excretion of sodium and lower urinary sodium than those who did not respond to the diuretic, implying they had less severe renal failure as renal tubular sodium reabsorption continued. This is in keeping with my observation that the patients who became non-oliguric after loop diuretics had significantly less severe organ failure and better renal function at presentation.

It could be argued that the use of mannitol and dopamine in all the patients may have affected the results. At the time ethical approval for the study was sought the use of these two drugs was 'routine', despite the lack of evidence in their favour. Recently there has been evidence suggesting that both mannitol and dopamine may not be beneficial in ARF and indeed may even be harmful.

In healthy volunteers, dopamine causes a selective increase in renal blood flow resulting in a natriuresis and diuresis.[193] However, Breslow et al have shown that in pigs with endotoxic shock renal vascular autoregulation and vasodilatation may already be maximal and therefore unable to be manipulated further by pharmacological methods.[294] Baldwin et al

studied 37 patients having elective repair of an abdominal aortic aneurysm or aortobifemoral grafting.[295] None of the patients had renal failure, but in this prophylactic study low dose dopamine (n=18) did not show any advantage over adequate peri-operative hydration (n=19). Duke et al showed that low dose dopamine given to critically ill patients with a normal serum creatinine caused a diuresis but did not improve creatinine clearance.[296]

In a porcine model of haemorrhagic shock Segal et al (1992) showed that animals given low-dose dopamine (2ug/kg/min) had an earlier onset of gut ischaemia associated with a decreased ability to extract oxygen than shocked animals not given low dose dopamine.[297] They suggested that low dose dopamine used to treat shocked, oliguric patients may have a detrimental effect on oxygen transport and utilisation in the gut which potentially could cause occult gut ischaemia, translocation of bacterial products and development of multi-organ failure. Scannell et al (1994) have shown that gastric submucosal PO_2 does correlate with gut translocation.[298]

Chertow et al assessed dopamine administration in 497 patients with early progressive renal failure.[299] Dopamine was given 'at the discretion of the attending physician'. 69% of oliguric and 68% of non oliguric patients were therefore given dopamine. The overall mortality at 21 days was 31% with and 21% without dopamine and the tendency to increased mortality in the dopamine group was more obvious in those patients who were oliguric (49% with v 26% without dopamine). Dialysis requirement at day 14 was said to be significantly higher in the dopamine group (47%) compared to the group not given dopamine (36%). Unfortunately no indicators of statistical significance are included in the abstract. Nor is there any mention of severity of illness or renal function in the two groups. It is possible that patients given dopamine were in fact more ill with more severely impaired renal function than the group not given dopamine. The waters are further muddied by the

fact that the study was originally designed to compare the effect of ANP with placebo. The authors do comment that regardless of the original treatment assignment (ANP or placebo) dialysis was still more common in patients given dopamine, although no mention of the effect of the primary treatment assignment and dopamine on mortality is made.

Heymann et al have shown that a mannitol infusion decreased rat outer medullary O_2 tension from 22±4 to 14±3mmHg ($p<0.002$) without changing the cortical oxygen tension.[300] Thus mannitol has the potential to perpetuate the medullary hypoxia seen in ARF. It may do this by increasing the oxygen requirements of the already hypoxic mTAL. By washing out intratubular casts mannitol causes an increase in inulin clearance; it also causes decreased fluid absorption in the proximal tubule and therefore may cause increased delivery of filtered solutes to mTALs in nephrons not blocked by intratubular casts. Both these effects will increase the metabolic demand of the mTAL, but there will be no increase in blood flow to meet the increased metabolic demand as capillaries in the outer medulla are blocked by trapped red blood cells. The result is more hypoxia and further injury.

The prophylactic use of mannitol has been reported to prevent RBC trapping in the rabbit kidney.[115] This may be related to its action as a free radical scavenger as other free radical scavengers have also been shown to prevent RBC trapping.[115] However, Hellberg et al showed that administration of mannitol in a dose dependent manner *after* the ischaemic event was associated with an increase in RBC trapping in the inner stripe of the outer medulla.[301]

Thus the available experimental evidence and the lack of controlled studies suggest that at present mannitol and low-dose dopamine do not have a role to play in the management of ARF. Were the study to be redesigned neither of these two drugs would be included in the protocol. However, as patients in all three treatment groups received mannitol and

dopamine, any differences noted between the groups as a result of treatment would have been due to loop diuretic.

5:6 Conclusion

This prospective, placebo- controlled, double - blind randomised study has shown that loop diuretics have no beneficial effect on the duration of renal dysfunction, the need for dialysis or on mortality in ARF. Patients who become non-oliguric (with or without a loop diuretic) have a better survival but are less ill and have less severe renal failure than patients who remain oliguric.

80% of patients who became non-oliguric were on a loop diuretic, but the difference in mortality between this group and the group receiving placebo who became non-oliguric is not significant. However, the numbers in this subgroup analysis are too small for any firm conclusions to be drawn.

A trial specifically addressing the question of mortality in patients who become non-oliguric either on loop diuretic or placebo would need to enrol 518 patients in each group. This assumes reducing overall mortality from ARF to 40% or less is a significant improvement, a level of statistical significance of 0.05 and a power of 0.9. In my study 44% of patients enrolled became non-oliguric as a result of intervention (placebo or loop diuretic). Therefore a total of 2354 patients would need to be enrolled and given loop diuretic or placebo in order to have the required number of patients in the spontaneously and pharmacologically induced non-oliguric groups. This in turn means that in the order of 6730 patients with ARF would need to be assessed for suitability for entry into such a study. It may therefore never be performed.

In very ill patients many insults contribute to the onset of ARF; most patients suffer from multiple-organ failure. Perhaps it is a naive to suppose that a drug acting primarily on the kidneys would have a beneficial effect on a multisystem disorder and so improve survival.

My studies have shown that loop diuretics have little effect on the natural history of ARF. The conversion of oliguric into non-oliguric ARF however, could be an indication for their use in order to improve fluid balance, despite their lack of evidence on outcome. If we are to significantly improve mortality in these patients attention should be focused on the early detection and management of all aspects of multi organ failure.

Figure 5:1 Patients assessed for entry into the study

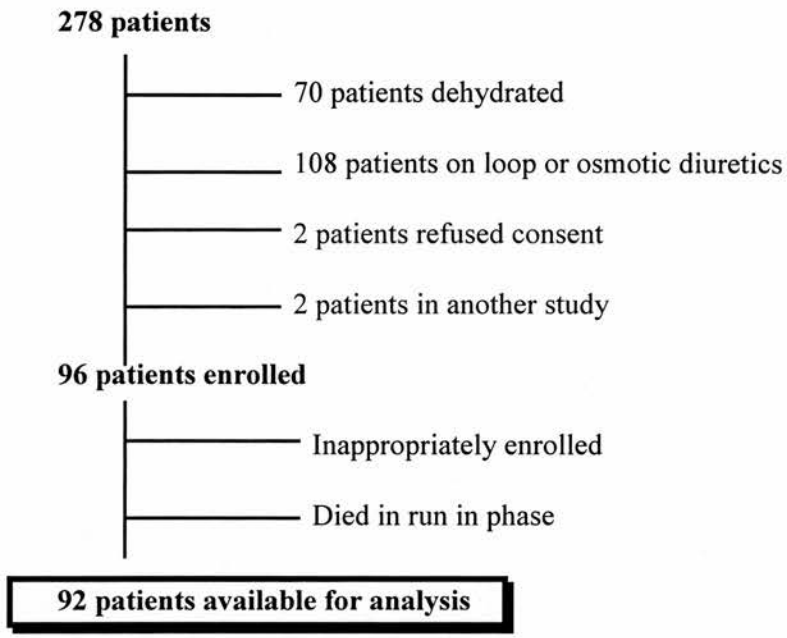
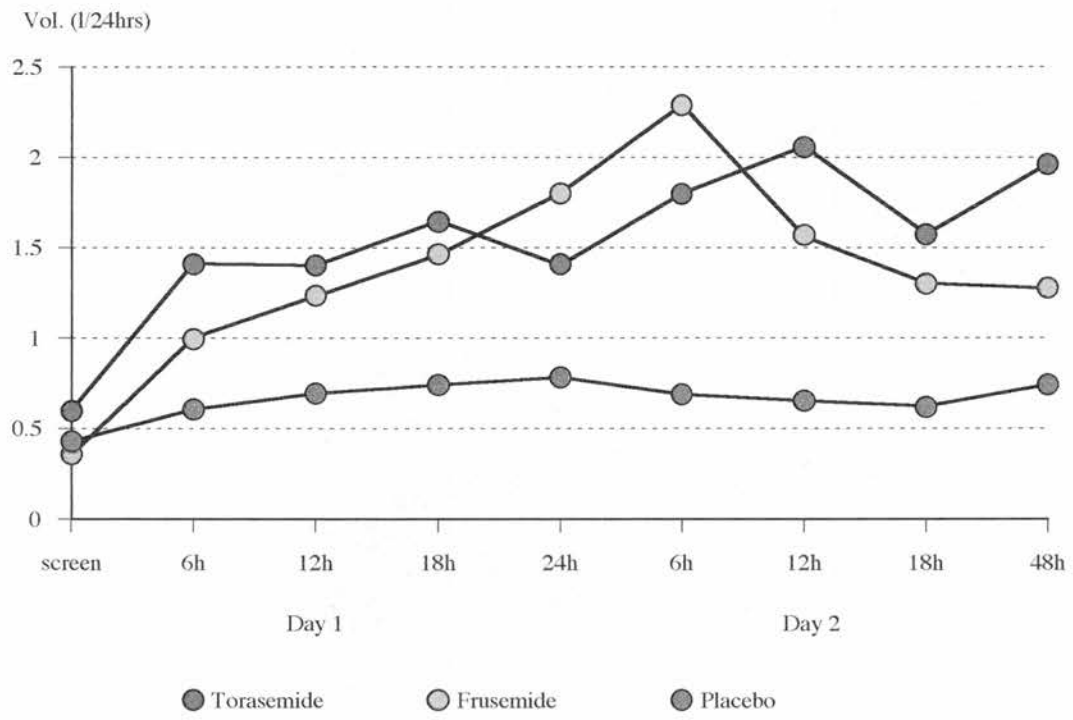


Figure 5:2 Urine output in the first 48 hours (median values)



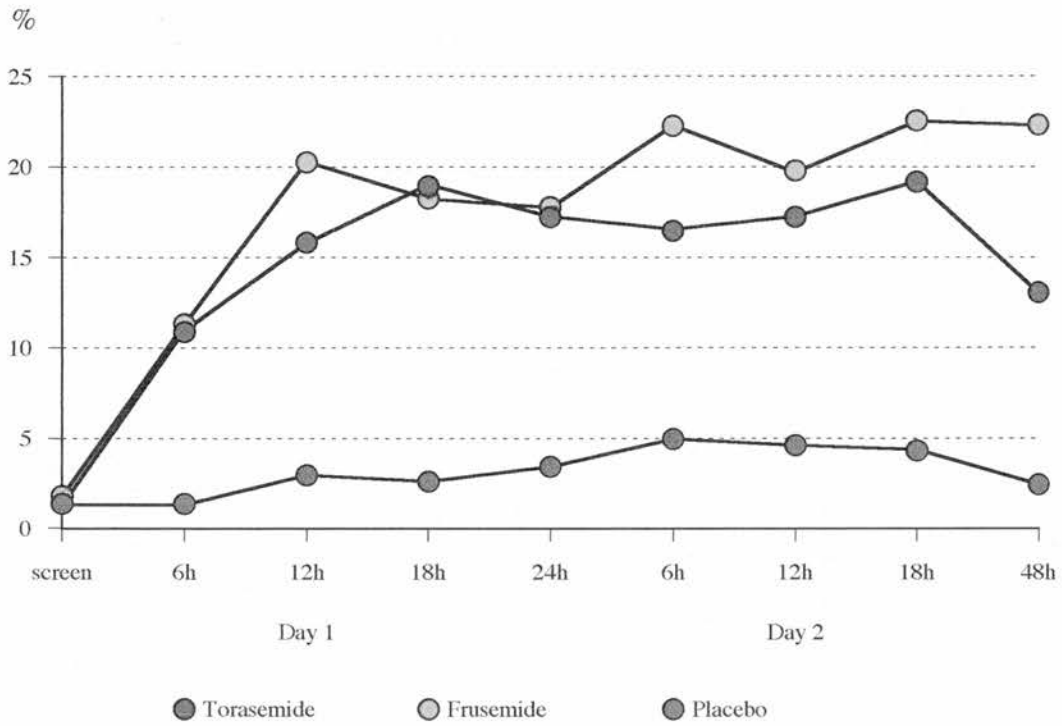
Urine output (l/24 hours)

	Screen			6hr			12hr		
	median	mean	SD	median	mean	SD	median	mean	SD
Tor	0.60	0.63	0.46	1.41	2.69	2.64	1.41	3.18	3.00
Fru	0.40	0.71	0.97	0.99	2.97	4.57	1.24	3.47	4.74
Pla	0.41	0.47	0.38	0.58	0.78	0.70	0.70	0.89	1.10

	18hr			24hr			Day2	6hr	
	median	mean	SD	median	mean	SD	median	mean	SD
Tor	1.64	3.2	3.15	1.41	3.23	3.32	1.86	2.82	2.82
Fru	1.50	3.35	4.03	1.80	3.07	4.05	2.28	2.89	3.80
Pla	0.75	1.11	1.28	0.79	1.4	1.79	0.70	1.29	1.51

	12hr			18hr			48hr		
	median	mean	SD	median	mean	SD	median	mean	SD
Tor	2.06	2.97	3.03	1.60	3.64	3.51	1.90	3.3	3.37
Fru	1.57	2.96	4.08	1.30	2.44	3.30	1.27	2.58	3.31
Pla	0.66	1.22	1.43	0.62	1.21	1.50	0.76	1.08	1.18

Figure 5:3 Fractional excretion of sodium in the first 48 hours (median values)



Fractional excretion of sodium (%)

	Screen			6hr			12hr		
	median	mean	SD	median	mean	SD	median	mean	SD
Tor	1.7	7.8	25.3	11.0	11.6	7.9	15.9	19.8	20.3
Fru	1.7	3.7	5.6	11.3	15.0	12.7	20.2	21.4	16.2
Pla	1.4	5.8	9.3	1.9	4.0	7.0	3.2	4.8	5.8

	18hr			24hr			Day2	6hr	
	median	mean	SD	median	mean	SD	median	mean	SD
Tor	18.7	27.5	28.2	18.0	27.5	37.6	17.7	23.3	25.0
Fru	18.2	22.7	15.7	18.3	22.9	16.3	20.9	21.8	15.2
Pla	3.1	5.3	5.7	4.2	6.2	6.9	5.1	7.2	7.4

	12hr			18hr			48hr		
	median	mean	SD	median	mean	SD	median	mean	SD
Tor	17.3	21.0	17.3	19.3	19.1	13.0	12.9	17.2	12.5
Fru	19.7	18.5	11.0	22.5	23.2	14.0	22.3	23.4	11.7
Pla	4.7	6.8	7.6	4.4	7.2	7.8	2.6	5.9	6.4

Figure 5:4 Actuarial survival for the three groups of study patients up to day 56

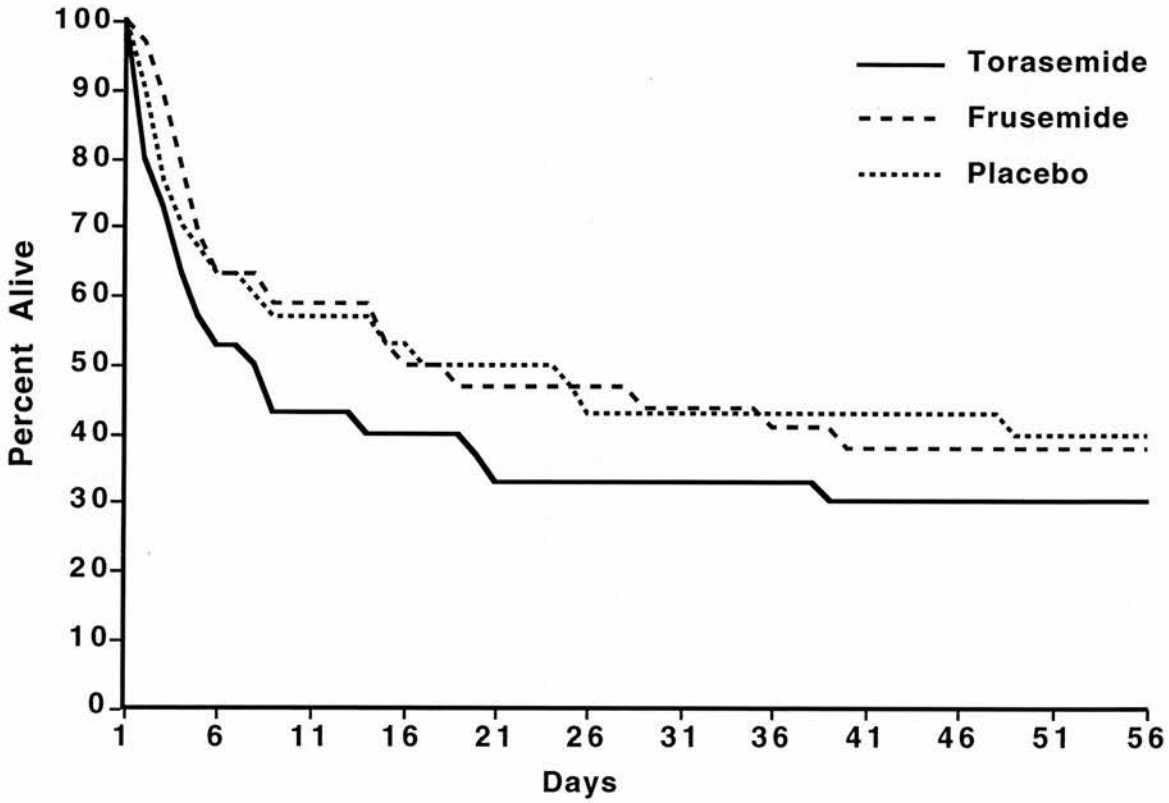


Table 5:1 Exclusion criteria

Exclusion criteria
Patients with either pre or post renal failure.
Patients given a loop or osmotic diuretic within the preceding 12 hours.
Patients given frusemide >100mg (or equivalent loop diuretic) in the preceding 48 hours.
Administration of any investigational substance within 30 days preceding the first dose of the study drug.
Women using inadequate contraception.
Women who were pregnant or lactating.

Table 5:2 Serum and urine measurements

Urine	Serum
Urine flow rate	Creatinine
Creatinine	Sodium
Sodium	Potassium
Creatinine clearance	Calcium
Fractional excretion of sodium	Osmolality
	C reactive protein
	γ glutamyl transferase

Table 5:3 Demographic and clinical features

	Torasemide	Frusemide	Placebo	p
Age (years)	58.7 ± 13.8	59.2 ± 16.5	58.3 ± 14.1	0.97[+]
Sex (%)				
Male	53	50	63	
Female	47	50	37	0.55[*]
Apache II score (Pre-study)	19.6 ± 4.5	19.1 ± 7.2	18.4 ± 5.8	0.77[+]
Creatinine clearance (ml/min)	10 ± 11	8 ± 9	7 ± 8	0.45[+]
Hourly urine volume (ml/hr)	24 ± 18	32 ± 45	20 ± 16	0.32[+]

[*] chi-square test

[+] analysis of variance

Table 5:4 Cause of acute renal failure

Cause of acute renal failure	Number
Septic shock / sepsis	31
Infection	13
Abdominal aortic aneurysm surgery	14
Acute liver failure	5
Rhabdomyolysis / trauma	4
Haemolytic-uraemic syndrome	4
Aminoglycoside toxicity	2
Vasculitis	2
Other	17

Table 5:5 Outcome of acute renal failure at day 21

	Torasemide (%)	Frusemide (%)	Placebo (%)	p
Increase in urine flow	57	48	23	0.02*
Renal recovery	17	28	23	0.56*
Dialysis required	36	31	40	0.87*
Time to dialysis (days)	5.0 ± 5.0	5.4 ± 5.7	2.8 ± 1.2	0.35+
Duration of dialysis (days)	5.6 ± 4	13.4 ± 13.7	13.2 ± 10.7	0.16+
Death by 21 days	47	41	37	0.73*
Alive day 56	30	38	43	0.70*

* chi-square test

+ analysis of variance

Table 5:6 Final outcome of patients requiring dialysis

	Torasemide (%)	Frusemide (%)	Placebo (%)
Dead	64	60	42
Alive	36	40	58

chi-square test, placebo versus frusemide or torasemide, p=0.52

Table 5:7 Reason for stopping mannitol

Reason for stopping mannitol	Number
Severe oliguria / anuria	27
Death before day 3	24
Hyperosmolality	15
Acute pulmonary oedema	1
Renal recovery	2
Withdrawn from study	2

6 Study 2 - Intraplatelet Calcium Levels in Healthy Volunteers and Patients with Acute Renal Failure and Multi-Organ Failure

6:1 Introduction

Elevated free cytosolic calcium($[Ca]_f$), found in cells injured by ischaemia has been proposed as one of the mechanisms important in the pathogenesis of ischaemic cell injury.[302-304] Schanne et al studied the viability of hepatocytes exposed to membrane active toxins. In the presence of extracellular calcium all hepatocytes were killed in 1-6 hours but remained viable in the absence of extracellular calcium.[303] Snowdowne demonstrated a rise in intracellular calcium levels in cultured monkey kidney cells after 1 hour of anoxia and substrate removal.[282] High $[Ca]_f$ causes an uncoupling of mitochondrial oxidative phosphorylation resulting in reduced ATP stores.[94] Wilson et al showed that accumulation of intra-mitochondrial calcium occurs following reperfusion of ischaemic renal tissue, and that there is a highly significant correlation between raised mitochondrial calcium concentration and decreased mitochondrial respiration.95 High $[Ca]_f$ also causes activation of phospholipases resulting in an alteration in membrane permeability and membrane enzyme activity.[98,305,306] Activation of intracellular proteases in ARF in the presence of raised $[Ca]_f$ will convert xanthine dehydrogenase to xanthine oxidase which in turn leads to the production of hydroxyl radicals and tissue injury.[104] In addition calcium has been shown to potentiate the oxygen free radical mediated injury to mitochondria.[112]

Two clinical studies have shown that sepsis increases erythrocyte free cytosolic calcium and lymphocyte free cytosolic calcium.[283,284]

Loop diuretics have been shown to cause a significant decrease in $[Ca]_f$ in platelets from patients with essential hypertension and in red blood cells from normal volunteers.[307,308] The authors do not speculate on the possible mechanism for this effect. In an experimental model of ischaemic ARF loop diuretics prevented the rise in mitochondrial calcium normally seen in oliguric ARF.[309] If loop diuretics could be shown to reduce cytosolic calcium in the cells of patients with ARF then the use of loop diuretics should theoretically attenuate renal tubule cell injury and perhaps improve the clinical outcome.

Studying renal tubule cells in a clinical setting is not possible and a suitable alternative is needed. White blood cells, red blood cells and platelets are easily accessible. I elected not to use white blood cells as the cell population is not homogenous. Haemoglobin in red blood cells interferes with the fluorescence of quin2, the fluorescent probe used to measure intracellular calcium levels in my studies. Although non of the three cell lines considered bear any resemblance to tubule epithelial cells, platelets do have some features in common with vascular smooth muscle cells, namely a calcium dependent contraction-coupling mechanism and α_2-adrenoceptor operated calcium channels.[310,311] As with vascular smooth muscle angiotensin II and vasopressin may also promote calcium influx.[312] Thus platelets are a good model to study in clinical ARF to assess the possible role of intracellular calcium in the initiation (vascular) phase of ARF. It is possible that similar changes may occur in tubule epithelial cells, contributing to cell death and the maintenance of ARF.

I therefore measured free intracellular calcium levels in platelets ($[Ca]_i$) in patients with ARF, patients with multi-organ failure (MOF) but normal renal function, and in normal subjects. Patients with ARF and normal subjects also had $[Ca]_i$ levels measured after administration of loop diuretic or placebo.

6:2 Subjects and methods

6:2:1 Normal volunteers

16 healthy adult volunteers were entered into the study (10 males and 6 females). All subjects had normal renal function, no history of acute or chronic illness and were not taking any medication. The mean age of controls was 30 years (range 25 - 40). Informed written consent was obtained.

On the first study day (control) at 1300 hours a 16G indwelling plastic cannula was inserted into the forearm, the tourniquet removed, and 45 ml blood taken into sodium citrate for measurement of $[Ca]_i$ in platelets. The following day (day 2) at 1000 hours an intravenous injection of either frusemide 20mg/2ml, torasemide 10mg/2ml or placebo 2ml was given. The study was double blind and neither the investigator nor the subject were aware of what was being given. Three hours later a further 45 ml blood was taken into sodium citrate as before for measurement of $[Ca]_i$. During the second study day patients were encouraged to drink freely to prevent dehydration. Estimation of $[Ca]_i$ took place immediately after venepuncture.

A one week wash out period was allowed and days 1 and 2 repeated one week and two weeks later. Each subject therefore received each of the loop diuretics and placebo in a random order.

6:2:2 Acute renal failure patients

Seven patients with ARF participated in this study - 2 females and 5 males; mean age 60 years (range 46 - 69). They were patients who were in the study described in chapter 5. 45ml of blood was taken for intraplatelet calcium estimation as for the normal volunteers and patients were started on frusemide, torasemide or placebo 3mg/kg over 1 hour every 6 hours, a continuous IV infusion of low dose dopamine and 20g mannitol IV over 1 hour every six hours. Five of the patients received loop diuretic. 24 hours later a further 45ml of blood was taken for estimation of intraplatelet calcium.

6:2:3 Multi-organ failure patients

Five patients with multi-organ failure but normal renal function were entered into this part of the study. The mean age was 50 years (range 32 - 64). A single sample of 45ml of blood was taken for intraplatelet calcium estimation as above.

MOF patients were not given diuretic.

6:3 Laboratory methods

6:3:1 Measurement of intraplatelet calcium

$[Ca]_i$ was measured using a fluorescent probe - quin2 acetoxymethylester.[313] Platelet rich plasma (PRP) was prepared by collecting whole blood into 3.9% sodium citrate (9ml whole blood/1ml sodium citrate), and centrifuging at 250 ***g*** for 5 min at room temperature. PRP was incubated at 37°C for 30 min with quin2 (2ul quin2 were added to each ml PRP). Ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA; 5mmol/l; Fluka) was added to bind to any extracellular calcium, and PRP then centrifuged at 250 ***g*** for 10 min at room temperature. The supernatant was removed and the pellet resuspended in platelet buffer titrated to pH 7.3 at 37°C (recipe for platelet buffer shown in Table 6:1). The buffer was calcium free to prevent platelet aggregation during centrifugation. The cells in the suspension were counted in a Coulter Counter (Technicon H-1TH system). The concentration of cells was adjusted to 1.5-2 x 108/ml and the suspension was dispensed into 9 cuvettes and placed in a water bath at 37°C.

The first cuvette was placed into the spectrofluorometer and the background fluorescence was measured with a Perkin-Elmer LS-3B spectrofluorometer at 339nm excitation and 492nm emission.

20ul of 0.1M $CaCl_2$ was then added to the cuvette (Figure 6:1:a). In health there should be no change in the fluorescence signal since the added calcium should not leak across the plasma membrane into the platelets (Figure 6:2).

The cells were disrupted with 20ul of digitonin thereby releasing the intracellular quin2 which then binds to the added calcium giving rise to the maximum fluorescence signal, F_{max} (Figure 6:1:b). The quin2 is then fully saturated. 20ul of $MnCl_2$ was added; manganese displaces calcium from the quin2 and therefore gives rise to a very low signal, F_{min} (Figure 6:1:c).

The intraplatelet calcium concentration can then be calculated using the equation:

$$[Ca]_i = 115(F-F_{min.}/F_{max.}-F)\ 314$$

where 115 is the equilibrium dissociation constant in nmol/l, F the fluorescence of the intact cell suspension, $F_{max.}$ the maximum fluorescence after the cells have been disrupted with digitonin (20ul) and $F_{min.}$ the minimum fluorescence obtained after addition of 20ul 2mmol/l $MnCl_2$.

The procedure was repeated for cuvettes 2 and 3.

6:3:2 Effect of arginine vasopressin

Arginine vasopressin (AVP) stimulates influx of calcium from the suspension into the platelet (mechanism 1, Figure 6:3), as well as a release of intracellular stores of calcium into the cytosol (mechanism 2, Figure 6:3). As quin2 is only found inside the platelet, the fluorescence signal obtained after addition of AVP gives an indication of total intracellular calcium (Figure 6:3).

In cuvettes 4, 5 and 6 the response of platelet $[Ca]_i$ to AVP (1umol/l) was measured in the presence of 1mmol/l calcium (20ul of 0.1M $CaCl_2$ added to the suspension). The maximum fluorescence obtained was therefore due to influx of calcium from the suspension and release of intracellular stores.

6:3:3 Effect of AVP and EGTA

The extracellular calcium concentration was then reduced to negligible concentrations in cuvettes 7, 8 and 9 by adding 50ul of 200mM EGTA. In the presence of EGTA, there is no calcium influx from the suspension into the cell and the fluorescence signal obtained when AVP is added comes solely from release of intracellular stores into the cytosol (Figure 6:4).

6:4 Statistical analysis

Statistical analysis of intracellular calcium levels was performed using the Mann-Whitney U-tests. Results are expressed as the median and 95% confidence interval for the absolute difference between A minus B. Apache II scores were compared using students' t-test. Measures of relationship were calculated by Pearson's correlation test. P values <0.05 were taken to indicate statistical significance.

6:5 Results

6:5:1 Normal volunteers

Tables 6:2, 6:3 and 6:4 show the $[Ca]_i$ (measured as the level of fluorescence) obtained at the start of the experiment (Table 6:2) and after the addition of AVP (Table 6:3) and then AVP/EGTA (Table 6:4), pre and post loop diuretic or placebo. There was no significant difference in the basal $[Ca]_i$ before or after loop diuretic or placebo (Table 6:2). The presence of a negative value in the confidence interval means that although Basal A is greater than Basal B, it is possible within the 95% confidence interval range that the absolute value of Basal B may be greater than Basal A.

The addition of AVP in the presence of 1mmol/l extracellular calcium caused a rise in the fluorescence signal as expected (Table 6:3). In the absence of extracellular calcium the rise in fluorescence was much less - the signal coming solely from the intraplatelet stores of calcium (Table 6:4).

There was no significant difference in the rise in $[Ca]_i$ after either AVP or AVP/EGTA in normal subjects before or after loop diuretic.

6:5:2 ARF patients

The baseline results before administration of loop diuretic are shown in Table 6:5.

Basal levels of $[Ca]_i$ were significantly higher in ARF subjects than normal volunteers (109nm v 92.4nm, p=0.04). The rise in $[Ca]_i$ after the addition of AVP tended to be higher in control subjects although it did not reach significance (584.8nm v 346nm, p=0.4); after the addition of AVP/EGTA there was a smaller rise in the fluorescence signal obtained from the ARF group compared to controls which was not statistically significant (116nm v 165.8nm, p=0.14).

Post diuretic there were no significant changes in basal levels of $[Ca]_i$ nor in the response to AVP (Table 6:6).

The higher basal levels of [Ca]i in the ARF patients were compared with the APACHE II scores but there was no correlation between the two.

6:5:3 MOF patients

To see whether the changes in the ARF patients were due to ARF or the presence of severe illness a group of patients with multi-organ failure but normal renal function were compared with the normal volunteers (Table 6:7). The APACHE 11 score in the MOF patients was similar to the APACHE 11 score in the ARF patients (13.8 ± 6.9 v 17.7 ± 6.2 respectively, p=0.36) indicating that the groups were comparable in terms of severity of illness. Basal $[Ca]_i$ was higher in the MOF group than the normal volunteers, but not significantly so (105.9nm v 92.4nm, p=0.1). As for the ARF group the rise after AVP was less than that in normal volunteers, significantly so this time (298.7nm v 584.8nm, p=0.04). The smaller rise after AVP/EGTA compared to normal volunteers was not significant (128.8nm v 165.8nm, p=0.2), (Table 6:7).

6:5:4 Summary of findings

1. Basal $[Ca]_i$ was significantly higher in ARF patients than in normal volunteers.
2. Basal $[Ca]_i$ tended to be higher in patients with MOF than in normal volunteers, but the difference was not statistically significant.
3. After AVP the rise in $[Ca]_i$ was higher in normal volunteers compared to ARF patients, but not significantly so.
4. After AVP the rise in $[Ca]_i$ was significantly higher in normal volunteers compared to MOF patients.
5. After the addition of AVP/EGTA there was a smaller rise in $[Ca]_i$ in ARF patients compared to the normal volunteers that did not reach statistical significance.
6. Diuretics had no effect on the basal level of $[Ca]_i$ nor the response to AVP.
7. There is no correlation between $[Ca]_i$ and the APACHE II score in ARF.

6:6 Discussion

Normal volunteers

Platelets from normal volunteers behaved as expected with a rise in $[Ca]_i$ after AVP due to an influx of calcium across the plasma membrane and release of intracellular stores of calcium into the cytosol. The calcium then bound to free quin2 in the cytosol and caused a rise in the fluorescence signal. In the presence of EGTA, and therefore in the absence of extracellular calcium, the increase in the fluorescence signal was less, coming solely from released intraplatelet stores of calcium. The magnitude of the difference suggests that in the presence of AVP and extracellular calcium, most of the rise in intracellular calcium comes from the influx of calcium across the plasma membrane rather than from the intracellular stores.

Volunteers in the control group were significantly younger than patients in the two study populations. However, basal levels of $[Ca]_i$ in normal volunteers in this study are not different from those found in normal subjects in a previous study looking at platelet cytosolic free

calcium in essential hypertension where the age range of subject was 15 - 71 years.[307] Age itself probably has no effect on intraplatelet levels of calcium, although hypertension does. None of my normal volunteers had hypertension. One patient in the ARF group and none of the patients in the MOF group were hypertensive.

ARF and MOF patients

Basal levels of $[Ca]_i$ in the ARF patients were significantly higher than in controls. It is possible that the small sample size could give rise to a Type I error. Although basal [Ca]i levels were also higher in the MOF group this was not statistically significant. The rise after AVP tended to be less in ARF and MOF compared to controls - perhaps because a greater percentage of the quin2 in the cytosol was already bound to calcium. The smaller rise in $[Ca]_i$ after EGTA/AVP would imply that the stores of calcium were depleted. It is unlikely that there was not enough quin2 to bind to the calcium released from the stores after AVP/EGTA. This is because in the presence of extracellular calcium and AVP without EGTA, there was enough quin2 to bind to calcium released from stores and calcium from the extracellular fluid.

Depletion of intracellular stores of calcium may reflect alteration of membrane phospholipids in very sick patients. The platelet cell membrane was clearly abnormally permeable to calcium as the fluorescence signal rose after the addition of extracellular calcium (Figure 6:5) implying transfer of calcium across the plasma membrane from the extracellular fluid to the cytosol. Compare this with the unchanged signal obtained from platelets from a normal volunteer (Figure 6:2). It is known that ischaemia alters the composition of plasma membrane phosopholipid.[98,305] Presumably in these sick patients, despite optimisation of oxygen delivery, oxygen uptake and utilisation is impaired and cellular hypoxia results leading to alteration of membrane phospholipids. This would allow efflux of intracellular stores of calcium into the cytosol as well as influx of extracellular calcium. A reduction in Ca ATPase activity due to reduced ATP levels would reduce calcium efflux from the cell.[91]

Gram negative sepsis often results in MOF with ARF. Lipid A from the gram negative bacterial cell wall is believed to be responsible for the cytotoxicity.[315] Mayeux and Shah showed that the cytotoxicity of lipid A in the renal tubular epithelial cell line LLC-PK_1 was mediated by a rise in intracellular calcium that preceded cell death and that buffering of intracellular calcium with quin2 was protective.[316] Interestingly they showed that extracellular calcium was not necessary for the rise in $[Ca]_i$ and that TMB-8 which inhibits release of intracellular stores of calcium protected the LLC-PK_1 cells from lipid A cytotoxicity. Their results suggest that in endotoxaemia the release of intracellular calcium is important for the cytotoxic effect of lipid A. Todd and co-workers demonstrated a significant rise in erythrocyte free cytosolic calcium following endotoxin (84.523nm ± 5.019 v 40.451nn ± 2.923, $p<0.001$), and a similar rise occurred in septic patients (septic 96.261nm ± 7.511 v non-septic 45.376nm ± 2.925, $p<0.001$).284 However, others did not find an increase in lymphocyte free intracellular calcium levels following endotoxin and tumour necrosis factor (TNF), although lymphocyte free intracellular calcium levels were significantly higher ($p<0.05$) in septic patients (176nm ± 12) compared to non-septic head injured (110nm ± 11), cardiac surgical (112nm ± 9) or healthy controls (112nm ± 5).283 This group did show that lysophosphatidylcholine (an endogenous membrane lipid released during ischaemia) did significantly increase lymphocyte free intracellular calcium in a dose dependent manner.

Intramitochondrial calcium levels have been shown to be raised after renal ischaemia.[97,317] The rise in calcium may have been secondary to reduced ATP dependent efflux of calcium and also increased permeability of the cell membrane to calcium.

In a clinical setting renal tubule cells cannot be studied directly and a suitable, easily accessible, alternative model is needed. I therefore elected to study the effect that ARF has on the concentration of intraplatelet calcium. As the renal medulla is susceptible to hypoxia[53] it is

unlikely that platelets would be hypoxic without the tubules also being affected, although the reverse is not necessarily true.

Levels of intracellular calcium in patients with ARF have not been measured before, although sepsis is associated with increased free erythrocyte calcium and lymphocyte free cytosolic calcium.[283,284] Presumably the effect of sepsis on intracellular calcium is also due to hypoxia. It is possible that many cells, including renal tubule cells, are similarly affected by hypoxia. Studies on isolated tubule suspensions show an early rise in permeability to calcium after anoxia.[317]

I have shown that in ARF the basal levels of $[Ca_i]$ are significantly higher than in normal volunteers and that in MOF the basal $[Ca_i]$ levels tend to be higher although the difference is not statistically significant. I have also shown that the intracellular stores of calcium are significantly reduced in MOF and tend to be low in ARF. The fluorescence signal obtained after addition of calcium would suggest that the permeability of the platelet membrane is abnormal and appears to allow leakage of added extracellular calcium into the cytosol. During the post ischaemic reflow period calcium could leak back through the abnormal membrane into the cytosol. In addition, abnormally permeable internal membranes would allow leakage of stored calcium into the cytosol.

I hypothesised that this rise in intracellular calcium might be related to the severity of illness and thus correlate with the APACHE II score, an objective scoring system used to stratify patients according to prognosis. However, I could not show any correlation between the APACHE II score and the intraplatelet calcium level in patients with ARF.

Loop diuretics had no effect on intraplatelet calcium levels in platelets from patients with ARF. It is possible that the alteration in membrane structure due to phospholipase activity affects the binding sites of drugs resulting in reduced drug action. Another possibility is that any lowering

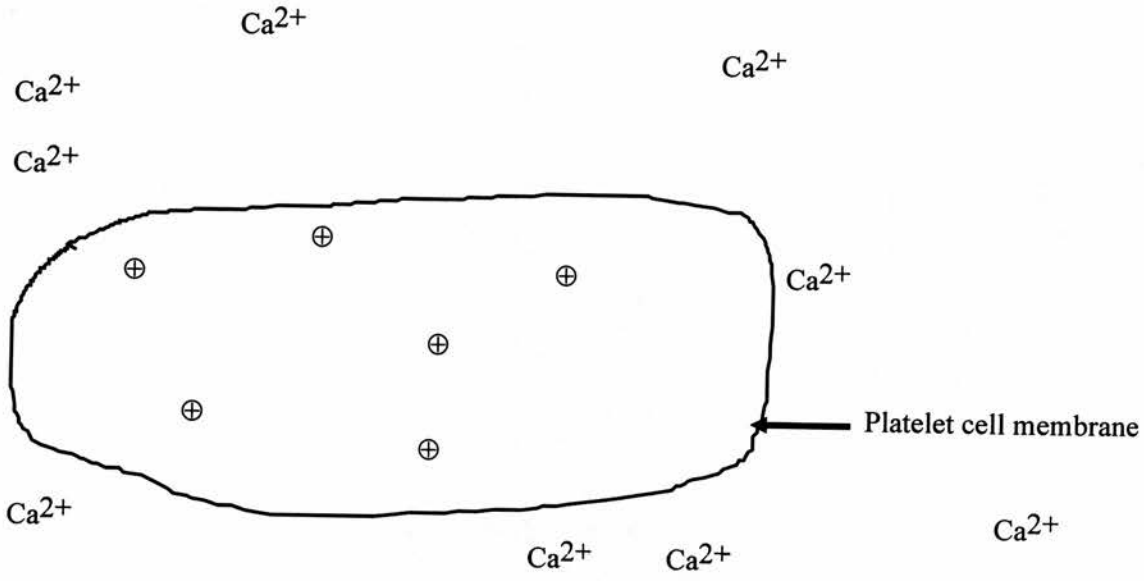
effect of loop diuretics on $[Ca]_i$ may be overwhelmed by the leakage of calcium into the cytosol from the extracellular fluid and internal calcium stores resulting in a net rise in $[Ca]_i$.

The loop diuretics were not given until after the hypoxic or nephrotoxic insult. Had they been given prior to the renal insult one could speculate that the results may have been quite different. However, in a clinical setting this is usually not possible.

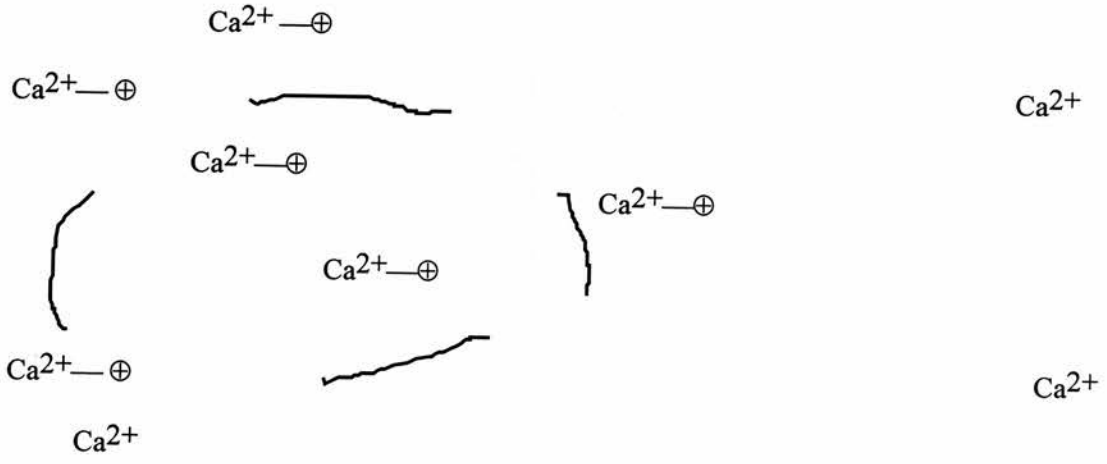
6:7 Conclusion

In summary, abnormally permeable cell membranes may allow an influx of calcium during the post ischaemic reflow period in ARF. Mitochondria buffer the calcium at the expense of oxidative phosphorylation and ATP production, thus reducing the efficiency of the Ca ATPase extrusion mechanism. I have shown that $[Ca]_i$ is high in platelets from patients with ARF, possibly due to an influx of calcium from the extracellular space across the damaged cell membrane, and also release of intracellular stores of calcium. The administration of loop diuretics have been shown to prevent the rise in mitochondrial calcium concentration in animal models of ARF.[309] I could not detect this response to loop diuretics in patients with ARF.

Figure 6:1 Quin2 / calcium interaction

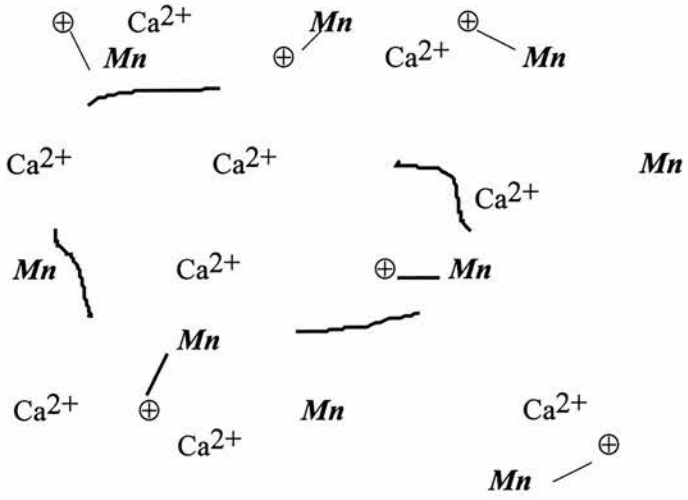


a. Platelet containing quin2 (⊕). 0.1M $CaCl_2$ added to the suspension.



b. Cell disrupted with digitonin. Quin2 binds to Ca resulting in *F max.*

Figure 6:1 (continued) Quin2 / calcium interaction



c. After addition of manganese. Calcium is displaced from quin2 resulting in *F min.*

Figure 6:2 Fluorescence signal from platelets from a healthy volunteer

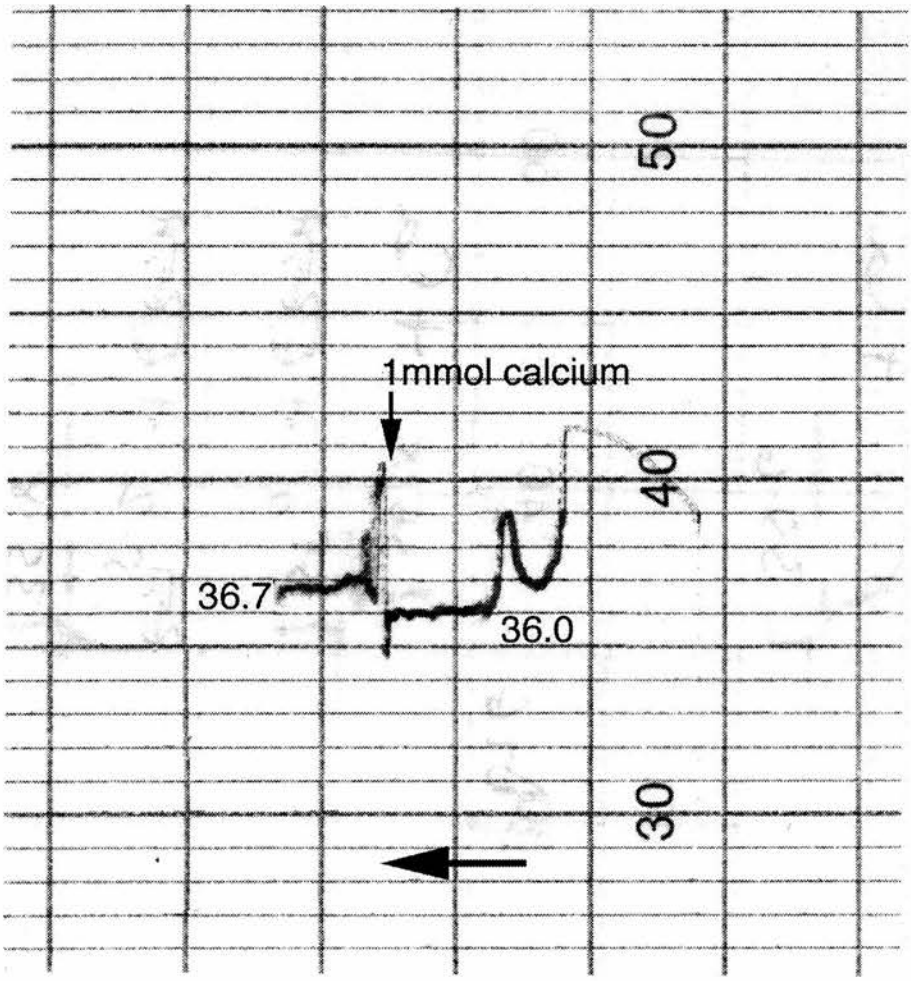
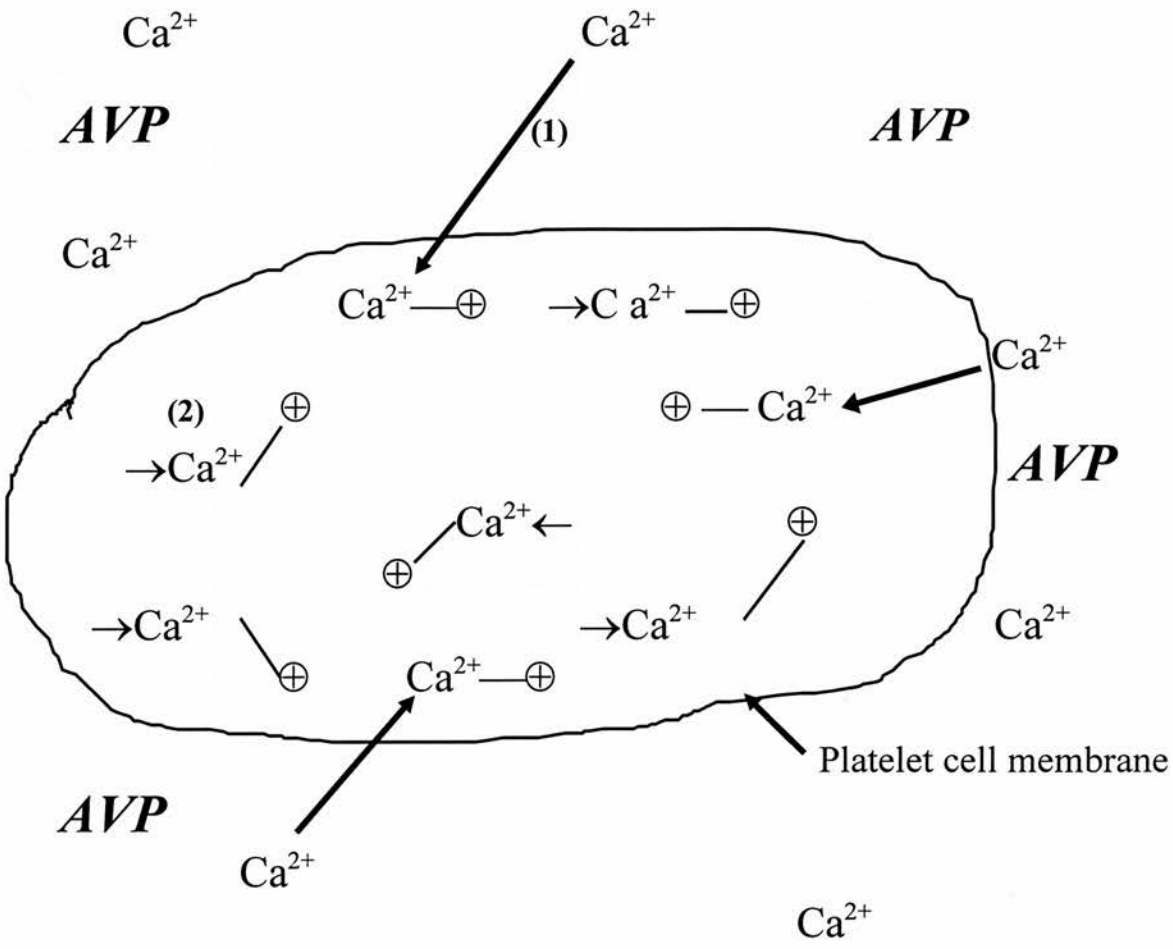


Figure 6:3 Effect of AVP

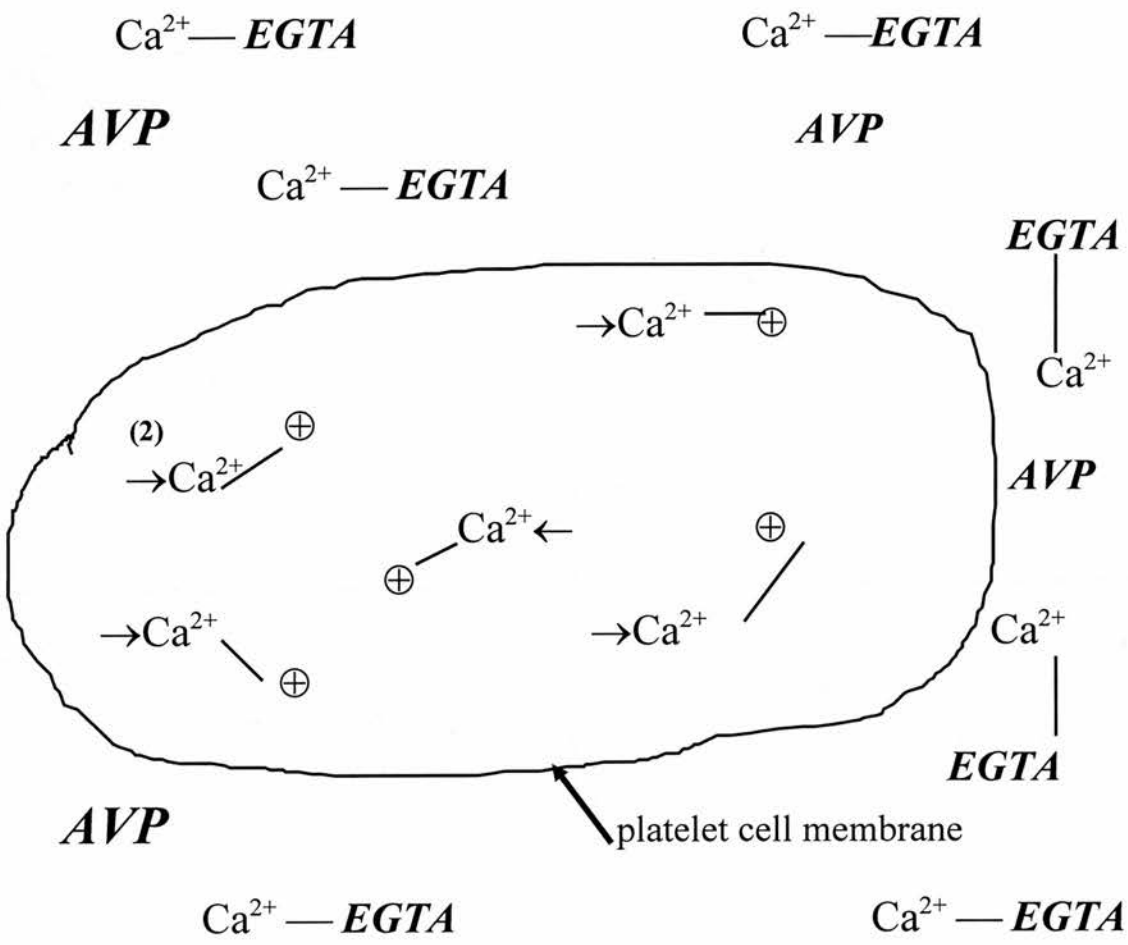


⊕ Quin2

Intracellular stores of calcium

F max due to calcium influx from the suspension, mechanism (1) and release of intracellular stores, mechanism (2)

Figure 6:4 Effect of AVP and EGTA



⊕ Quin2

Intracellular stores of calcium

EGTA binds to calcium in the suspension. Intracellular stores of calcium released after addition of AVP, mechanism (2)

F max due to release of intracellular stores

Figure 6:5 Fluorescence signal from platelets from a patient with ARF

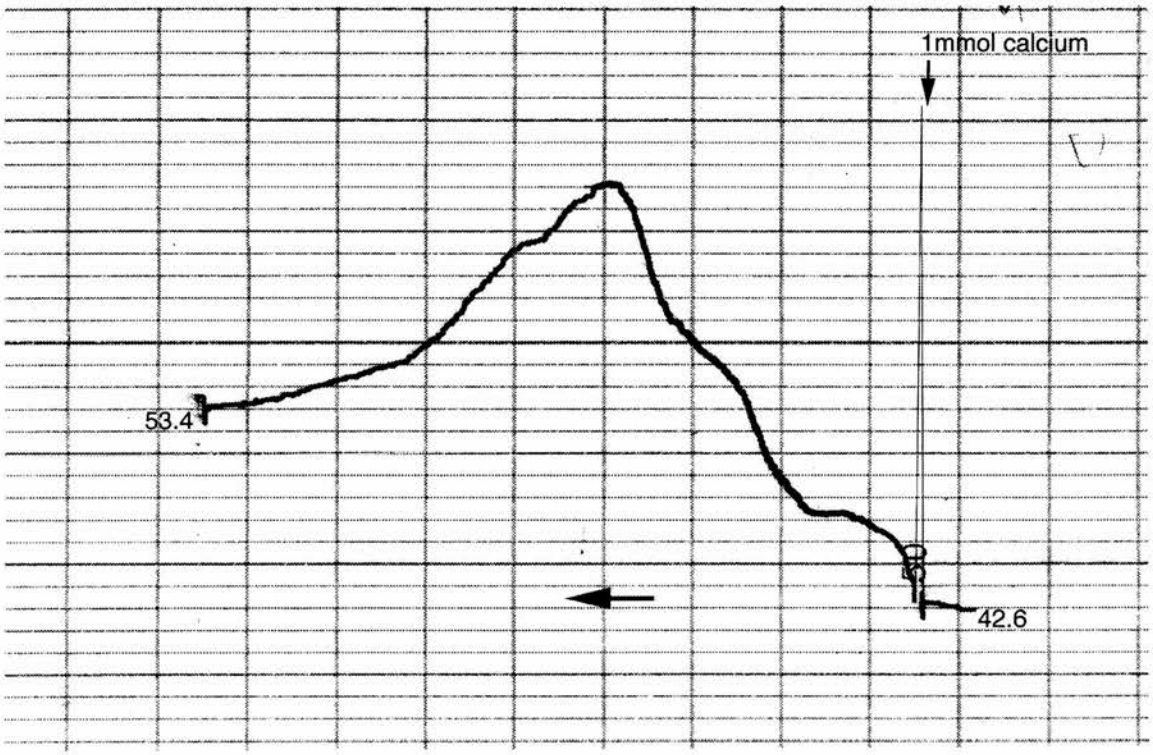


Table 6:1 Recipe for platelet buffer

	per 500ml distilled water
NaCl 140 mM	4.092g
KCl 1mM	0.0374g
$MgCl_2$ 1mM	0.5ml
Glucose 10mM	0.902g
HEPES 20mM	2.384g

Titrated to pH 7.3 with KOH 1M

Table 6:2 Basal levels of $[Ca]_i$ (control group)

	Basal A	Basal B	95% confidence interval	p
Placebo	84.4	75.8	-11.4, 21.6	0.4
Torasemide	103.3	82.4	-1.3, 33.6	0.07
Frusemide	84.4	92	-24.1, 15.6	0.6

A - pre diuretic / placebo
B - post diuretic / placebo

All units are nm.

Table 6:3 Response to AVP (control group)

	AVP A	AVP B	95% confidence interval	p
Placebo	428.5	625.7	-450.5, 118.1	0.5
Torasemide	666.8	520.0	-38.9, 69.3	0.5
Frusemide	613.2	630.0	-337.2, 230.8	0.7

A - pre diuretic / placebo
B - post diuretic / placebo

All units are nm.

Table 6:4 Response to AVP/EGTA (control group)

	EGTA A	EGTA B	95% confidence interval	p
Placebo	172.0	174.1	-47.4, 41.1	1.0
Torasemide	176.0	172.0	-57.3, 38.4	0.9
Frusemide	160.8	176.8	-57.8, 32.9	0.7

A - pre diuretic / placebo
B - post diuretic / placebo

All units are nm.

Table 6:5 ARF patients v controls (before loop diuretic)

	ARF	Control	95% confidence interval	p
Basal	109.0	92.4	-62.8, 1.0	0.04
AVP	346.0	584.8	-358, 510	0.4
EGTA	116.0	165.8	-34.9, 111.3	0.1

All units are nm.

Table 6:6 ARF patients - pre and post diuretic

	A	B	95% confidence interval	p
Basal	109.0	166.0	-167.0, 47.0	0.4
AVP	346.0	289.0	-157.9, 747.0	0.8

A - pre diuretic
B - post diuretic

All units are nm.

Table 6:7 MOF patients

	MOF	Control	95% confidence interval	p
Basal	105.7	92.4	-55.5, 6.2	0.1
AVP	298.7	584.8	9.4, 639.4	0.04
EGTA	128.8	165.8	-155.2, 89.0	0.2

All units are nm.

7 Study 3 - Resting Energy Expenditure and the APACHE II Score in Patients with Acute Renal Failure

7:1 Introduction

7:1:1 APACHE II score

The APACHE II score is an objective scoring system used to stratify patients according to prognosis.[248] The score is compiled from various physiological measurements including vital signs, laboratory investigations, Glasgow Coma Scale score and in addition points are given for significant chronic health problems and age. The higher the final score the greater the risk of hospital death.

By providing a measure of disease severity it is useful in clinical trials to determine whether control and treatment groups are similar. It also acts as a marker of physiological stress.

7:1:2 Resting energy expenditure and the respiratory quotient

REE is the energy expended by an awake, alert subject at rest in the post absorptive state.[249] This value has been shown to be approximately 10% higher than the true basal energy expenditure (BEE) as measured in deep sleep.[248,249] Several factors - BSA, sex, age, diet, sleep, breathing, exercise and disease have been shown to influence the REE.[248,249,251,253-255]

Resting energy expenditure accounts for 75-90% of total energy expenditure; diet induced thermogenesis, shivering and non-shivering thermogenesis and exercise make up the rest.[244,251]

Resting energy expenditure (REE) may be measured at any time of the day as long as the measurement conditions outlined in the methods section are adhered to.

The Harris-Benedict equation was reported to predict BEE, but the conditions of the original test imply that the results are more akin to the REE.[259,294-296] Therefore, REE can be predicted using the Harris-Benedict equation (HBREE).[259] The ratio of REE measured using indirect calorimetry to the predicted REE (REE/HBREE) can be used to determine whether patients are hypermetabolic (ratio >110%), normometabolic (ratio >90%, <110%) or hypometabolic (ratio <90%).[251] It is not known if the hypometabolic response is an energy efficient response carrying a better prognosis or if it carries a worse prognosis as a 'harbinger of adverse events to come'.[251] It has been suggested that the difference in mortality between different types of ARF is due to differences in metabolic status.[277]

The respiratory quotient (RQ) reflects net substrate oxidation at the time of measurement and is derived from measurement of VO_2 and VCO_2. It is the ratio of CO_2 produced to O_2 consumed. The normal value is 0.7 - 1.0; values >1.0 are due to non steady state hyperventilation (which should settle) or a continuous infusion of parenteral nutrition in excess of energy requirements resulting in net lipogenesis. A RQ <0.7 occurs during starvation and ketosis.

The APACHE II score is a valid marker of physiological stress. REE has been shown to correlate with severity of illness, but its measurement is time consuming.[264] If the APACHE II score and REE both reflect physiological stress, it may be simpler to use the APACHE II score as part of a predictive equation to estimate REE. I examined the relationship between REE and the APACHE II score to determine whether or not the APACHE II score could be used in this way.

7:2 Subjects and methods

7:2:1 Subjects

Twenty patients were enrolled into the study (Appendix 3). There were 7 males and 13 females, average age 60.7 years (range 24 - 80). All had acute renal failure with a serum

creatinine >180umol/l. Five were ventilated. All ventilated patients had an inspired oxygen concentration (FiO2) ≤0.6; patients requiring an FiO_2 >0.6 were not included due to inaccurate measurement of VO_2 at higher FiO_2 levels. All non ventilated patients had a good oxygen saturation (>95%) breathing air.

7:2:2 Measurement of REE and RQ

All measurements were performed at the patients' bedside using an open system indirect calorimeter, DELTATRAC^{TM} MBM-100. Measurements were performed at the time of referral to the renal unit or ICU (measurement 1) and again after at least 48hours (measurement 2).

The calorimeter was calibrated before each period of metabolic monitoring. Pressure calibration was done using actual barometric pressure at the time of the test. Following this gas calibration was performed using separate bottles of O_2 and CO_2, as opposed to a mixture of both gases. All equipment coming into contact with patients' skin, expired air and secretions was either disposed of or sterilised after use.

For patients not on a ventilator measurements of REE were made using a canopy. All canopy measurements were performed on patients able to breath room air. A hose (A) connected the canopy to the flow generator inlet (B) on the DELTRATRAC^{TM} (Figure 7:1). The outlet of the canopy (C) was placed close to the mouth of the patient. Sampling of inspired air was done through the inspiratory sample line (D) placed at the air inlet (E) of the canopy where pure ambient air was sampled. The plastic sheet (F) surrounding the canopy was folded under the pillow so that the air inlet was the only entrance for air. Patients were not allowed to fall asleep as this would result in a falsely low measurement. Talking was not allowed as this would cause a rise in the CO_2 concentration in the canopy and prevent equilibration. Figure 7:2 is a photograph of the indirect calorimeter (canopy mode) in use on a patient.

For patients who were ventilator dependent a disposable 22mm tube (A) connected the expiratory outlet of the ventilator (B) to the mixing chamber inlet (C) of the calorimeter (Figure 7:3). A 22mm stainless steel adapter (D) attached to the inspiratory sample line (E) was inserted in the inspiratory tubing of the ventilator at the outlet of the humidifier. The sampling port for inspired gas was at least 50cm from the patient's Y piece to avoid contamination with expired air.

For both ventilator and canopy measurements results were displayed at one minute intervals. The length of the test varied and was determined by how long it took to achieve a steady state and obtain five consecutive artefact free readings.

7:2:3 Measurement conditions [262]

1. Patients must have rested in the supine position for at least thirty minutes prior to measurement of REE.
2. To avoid the transient rise in REE in the post absorptive state, measurements were made at least four hours after any oral intake or on patients receiving a constant rate infusion of parenteral nutrition.
3. Measurements were made in a thermoneutral environment.
4. Patients were asked not to perform any voluntary movements during the measurements.
5. Any sources of supplemental oxygen were turned off during routine room air measurements.
6. The sampling system was checked for leaks.
7. The data used to calculate REE and RQ were taken from a period of equilibration ie a period of five or more consecutive one minute data points with a standard deviation for both VO_2 and VCO_2 of $\leq 5\%$ of the respective mean.

7:2:4 Calculations

The REE is calculated using the Weir formula[318]

$$REE = [3.941(VO_2) + 1.106(VCO_2)]1.44 - 2.17(UN)$$

UN = 24 hour urine nitrogen excretion.

However, the difference between this and the abbreviated Weir formula[318]

$$REE = [3.9(VO_2) + 1.1(VCO_2)]1.44$$

is less than 2% and therefore the abbreviated Weir formula was used to calculate REE.

The RQ was calculated as follows

$$RQ = VCO_2 / VO_2$$

7:2:5 Statistical analysis

Measures of relationship were calculated by Pearson's correlation test. Mortality as a variable was compared using chi-squared analysis. APACHE II scores were compared using students' t-test. P values of <0.05 were taken to indicate statistical significance.

7:3 Results

The mean APACHE II score of the group was 16 ± 5.8. The causes of ARF are outlined in Table 7:1. Patients had been in hospital for a mean of 3.75 days (range 2 hours - 24 days) before referral. However, some patients had been convalescing after elective surgery before deteriorating. All patients were referred within three days of becoming acutely unwell and developing acute renal failure.

7:3:1 Measurement 1

Three patients (15%) had a RQ <0.7 implying starvation and/or ketosis. Five patients (25%) had a measured REE that was $\geq$ 10% below the predicted REE and were therefore hypometabolic. 60% were initially hypermetabolic and 15% normometabolic.

There was no correlation between the initial measurement of REE and APACHE II score (Table 7:2). I adjusted the measured REE for size by dividing REE by BSA but still could not show any correlation between the adjusted REE and the APACHE II score. Nor could I show any correlation between measured REE and a number of other variables (Table 7:2). Table 7:3 shows the relationship between independent variables and the percentage difference in measured REE from predicted, ie 100% - (REE/HBREE)%. The measured REE ranged from 35% below to 64% above the predicted value.

7:3:2 Measurement 2

Repeat testing after 2 days showed 54% to be hypometabolic, 23% normometabolic and 23% were hypermetabolic. Once again there was no correlation between REE (measurement 2) and the initial APACHE II score. Nor was there any correlation between the APACHE II score and the percentage difference in measured REE from predicted. This time the measured REE ranged from 57% below to 21% above the predicted value The percentage difference in measured REE from predicted was significantly lower after 2 days in the renal unit or ICU (10.8% v -8.2%, p=0.03, admission v after at least 2 days respectively), and as noted above 54% of patients were now hypometabolic.

7:3:3 Hypometabolic v hypermetabolic

Patients were divided into two groups based on their initial REE (hypometabolic and hypermetabolic) and the drugs being prescribed at the time of testing were compared. The hypometabolic group were not taking more sedating medication than the hypermetabolic group, although those patients who were most hypermetabolic were receiving pressor agents. The APACHE II scores in the hypermetabolic and hypometabolic groups were 16.6 ± 6 and 14.4 ± 6.4 respectively (p=0.5).

Mortality between the three groups (hypometabolic, normometabolic and hypermetabolic) was compared and no difference was found (p=0.96).

7:4 Discussion

7:4:1 Results of study

I compared the APACHE II score at the time of referral to the renal unit or ICU with the REE measured at the time of referral and again after a minimum of two days and could not show any correlation between the two. In 1987 Swinamer et al measured REE in ten mechanically ventilated ICU patients and showed that the degree to which REE exceeded predicted EE correlated significantly with the admission APACHE II score (r=0.64, p<0.02).[268] The metabolic studies were performed 7±4 days after admission to ICU. I could not confirm this finding in our group of patients either on admission or several days after admission. When I excluded any patient who did not have metabolic measurements and an APACHE II score calculated within 24 hours of admission I still could not show any correlation between the two variables.

Swinamer et al published a second study examining the relationship between the APACHE II score and REE in 112 mechanically ventilated, critically ill patients and could not show any correlation between the APACHE II score and the REE measured within 48 hours.[321] This time they did not compare the APACHE II score with the percentage difference between measured EE and predicted EE.

After I had started this work a second group showed a statistically significant correlation (r=0.2, p<0.001) between the APACHE II score and the measured EE in seventy critically ill patients.[322] They adjusted the measured EE to obtain the REE by eliminating the effects of fever and diet induced thermogenesis and the REE was still significantly related to the APACHE II score (r=0.18, p≤0.001). Further adjustment of true REE to take account of size by dividing true REE by BSA resulted in an even greater correlation with the APACHE

II score (r^2=0.23, p≤0.001). I also adjusted the measured REE for size by dividing REE by BSA but could not show any correlation between the adjusted REE and the APACHE II score (p=0.6).

In this study the difference in measured REE from predicted on admission was significantly different to the percentage difference after at least 2 days in the renal unit or ICU (10.8% v -8.2% respectively, p=0.03) and more patients were hypometabolic (25% v 54%, initial measurement v after at least 2 days respectively). After the initial measurement of REE several of the patients were being sedated which very likely contributed to the hypometabolic response. There was no difference in severity of illness at presentation between the hypometabolic and hypermetabolic groups and, in this study, there was no difference in mortality between the two groups.

As mentioned before most disease states increase REE and in general the more severe the injury the greater the metabolic response.[267] There appears to be a physiological plateau of energy expenditure (EE) reached in response to injury that is approximately twice the REE. Further insults do not cause a rise in REE because respiratory and circulatory responses are already maximal.[270,323] However, not all patients develop the hypermetabolic response.[264,319,324] Some are normometabolic and up to 15 - 20% are hypometabolic. Some authors have attributed hypometabolism to the oxygen debt of anaerobic metabolism.[325] Others suggest that a decrease in VO_2 and EE may be the earliest event preceding cardiac instability.[326]

7:4:2 Potential sources of error

There are many potential sources of error when measuring REE by indirect calorimetry. First, any change in the concentration of inspired oxygen will affect the REE.[264] Particular attention therefore, must be paid to ensuring that there are no air leaks around the canopy. Ventilators cannot be relied upon to deliver a stable FiO2, particularly at high O_2

concentrations. Therefore, indirect calorimetry cannot be used on any ventilated patient requiring an FiO_2 >60%.

Second, there was a huge variability in the percentage difference in measured REE from predicted. This highlights the heterogeneous nature of a group of critically ill patients. Swinamer also showed a marked variability in the percentage difference between measured EE and predicted EE (measured 34 ± 19% above predicted, range -11% to 82%).[321] In their study patients with low values tended to sleep most of the time, while those with higher values were undergoing stressful procedures. Thus, the physical state of the patient has a significant impact on the REE, emphasising that the measurements must be performed on an awake patient in the resting state.

Third, Weissman et al monitored critically ill patients in ITU for a twenty-four hour period and showed that EE ranged from -10% to 23% around a "steady state REE".[324] REE measured on different days showed a variability of 15%, and the more critically ill the patient the greater the variability - REE 46% (range 37% - 56%) in the more severely ill patients compared to 12% (range 4% - 18%) in the less ill patients in ICU. My two measurements were carried out at the same time of day and under the same measurement conditions in order to minimise variability.

7:5 Conclusion

More severely ill patients have been shown to have a higher APACHE II score and some have a rise in REE. However, some patients may be normometabolic and 15 - 20% may even be hypometabolic.[270]

I could not show any correlation between the APACHE II score calculated at the time of referral and the REE measured at the time of referral and again after at least 2 days. This may have been because, despite being severely ill, a large number of the patients (25%) were hypometabolic. There was no significant difference in severity of illness between the

hypometabolic and hypermetabolic groups nor was there any difference in mortality. Whether or not a hypometabolic responds heralds a better prognosis is not clear, although these results would suggest that this is not so. The second time REE was measured 54% of patients were hypometabolic, mainly due to the effect of drugs. The APACHE II score was not repeated at this time.

Indirect calorimetry is an accurate way of measuring energy expenditure in the critically ill patient, and as such is a marker of physiological stress. The APACHE II scoring system accurately determines severity of disease. The lack of correlation with the REE means that the APACHE II score cannot be used as part of a formula to predict energy expenditure. Serial measurements of REE using indirect calorimetry are required.

Figure 7:1 Canopy measurements of REE

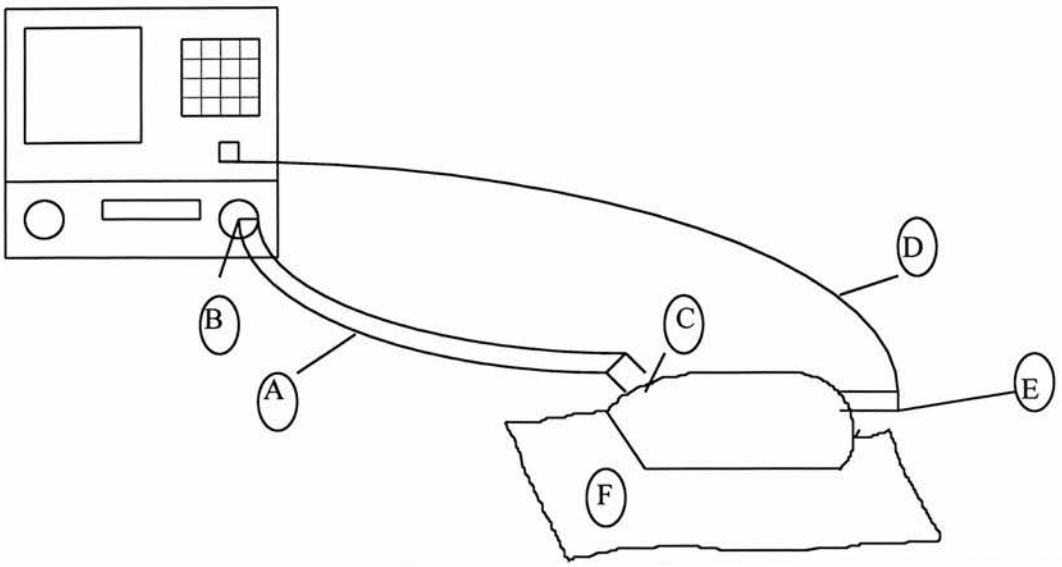


Figure 7:2 Patient demonstrating use of the indirect calorimeter (canopy mode)

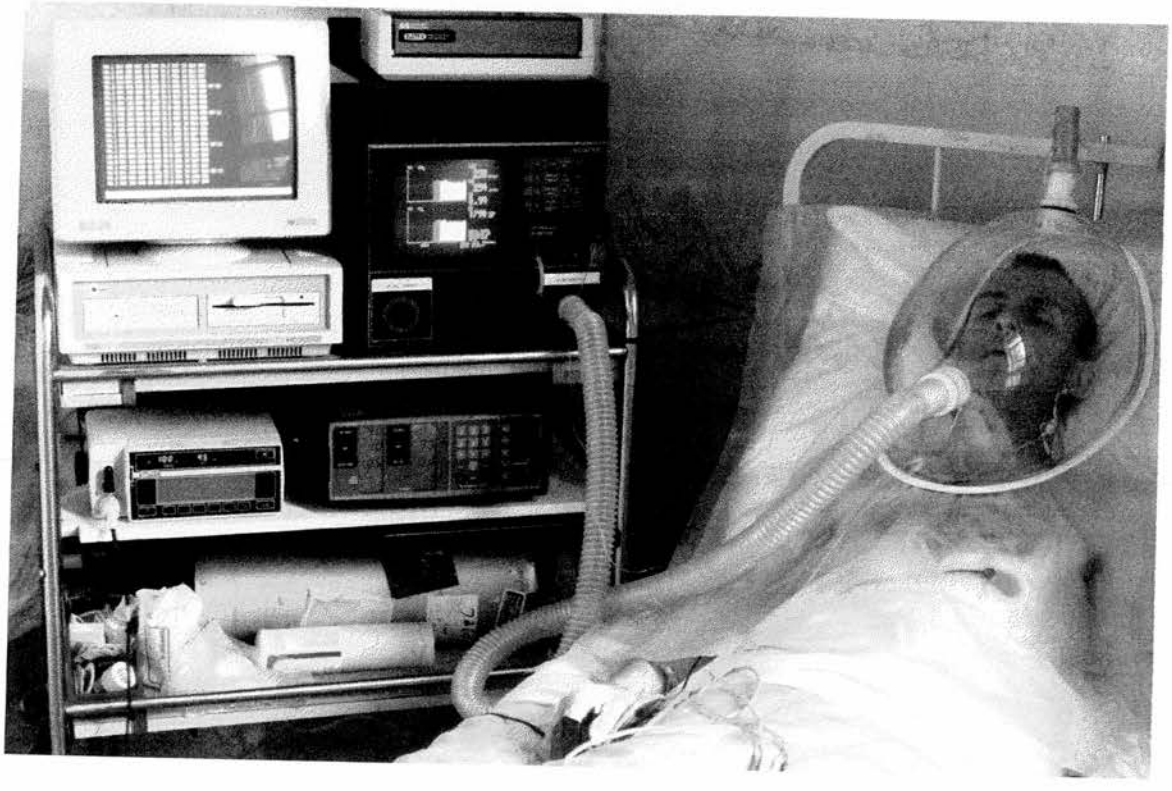


Figure 7:3 Ventilator dependent measurements of REE

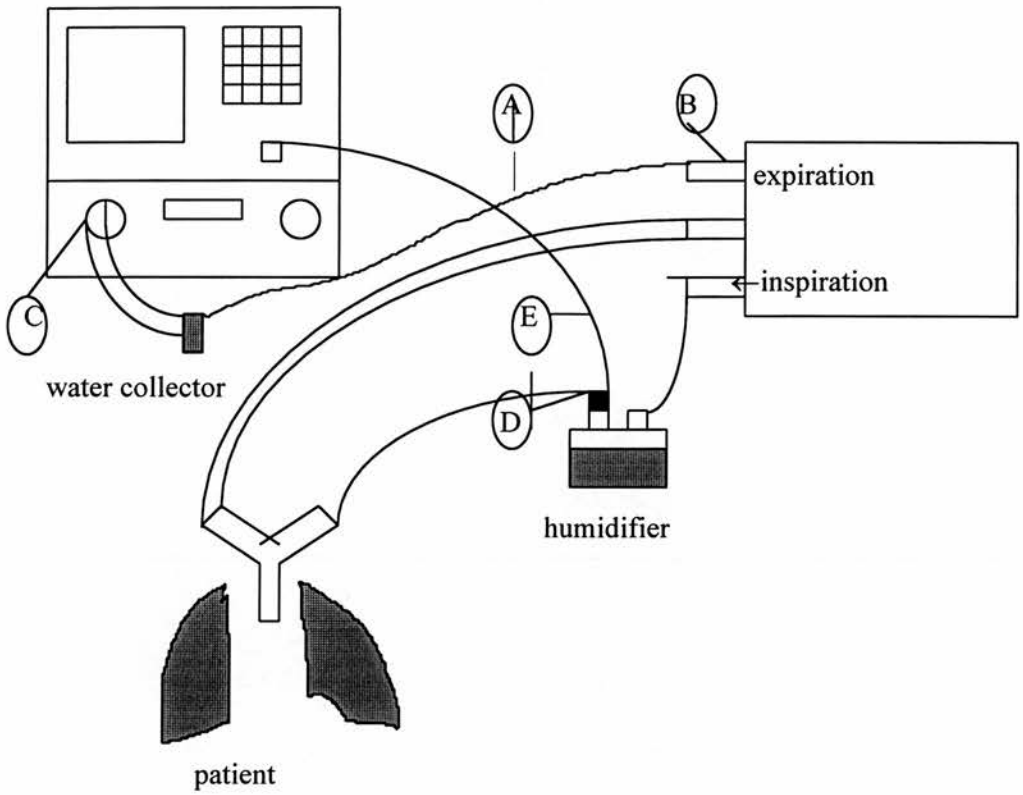


Table 7:1 Cause of acute renal failure

Cause of acute renal failure	Number
Infection / septic shock	9
Post abdominal aortic aneurysm surgery	3
Nephrotoxins	2
HUS / TTP	2
Rhabdomyolysis	1
Scleroderma crisis	1
Goodpasture's syndrome	1
Acute liver failure (alcohol)	1

Table 7:2 Relationship between measured REE and independent variables

Variable	r Value	p Value
APACHE II score	0.09	0.7
Cardiac output	0.4	0.08
Temperature	0.4	0.07
Respiratory rate	-0.103	0.7
MAP	0.03	0.9

Table 7:3 Relationship between the 'percentage the measured REE differed from predicted' and independent variables

Variable	r Value	p Value
APACHE II score	0.2	0.4
Cardiac output	0.14	0.6
Temperature	0.46	0.04
Respiratory rate	-0.02	0.9
MAP	-0.09	0.7

8 Summary and Conclusions

> 'Acute renal failure is the result not of a specific sort of altered kidney that can be given a name but of the formation of various structural functional lesions in several million nephrons'.
>
> Jean Oliver (1951)[12]

8:1 Pathogenesis of ischaemic acute renal failure

The pathogenesis of ischaemic acute renal failure is extremely complex. A number of factors contribute to the development and progression of ARF namely decreased renal blood flow,[41] intratubular obstruction,[41,42] backleak of filtrate,[41] and vascular congestion.[48,49] Less clear are the roles played by prostaglandins,[78-80] free radicals[108] and calcium.[95]

To perform their function of absorption and secretion the renal tubules require an adequate supply of oxygen. The anatomy of the nephron predisposes the medullary thick ascending limb (mTAL) to hypoxia. The vessels and tubules of the medulla are arranged in a hairpin loop manner which allows the exchange of solutes between the descending and ascending limbs leading to efficient concentration of urine. Active transport of sodium in the mTAL creates an osmotic gradient and is a high energy requiring process. Oxygen also diffuses from the arterial to the venous branches of the vasa recta and therefore the oxygen supply to the deeper parts of the medulla is reduced. In addition there is limited blood flow through the medullary vessels to prevent washout of the solute gradients. Thus a limited blood supply and a high oxygen requirement make the mTAL particularly susceptible to hypoxia and '...medullary hypoxia is the unavoidable price we pay for the ability to concentrate our urine'.[53]

Several groups have shown that the medullary pO_2 is low and is in fact close to the pO_2 below which mitochondrial respiration is threatened. [52,327,328] One way of protecting the kidney would be to reduce the energy required by the mTAL by modifying active transport.[286,287]

8:2 Physiology of loop diuretic action

Loop diuretics are powerful diuretics which act mainly on the loop of Henle to prevent sodium and water reabsorption. Some also prevent sodium reabsorption in the proximal tubule. The distal tubules have a limited ability to reabsorb sodium and water and therefore are not able to counteract the diuresis and natriuresis caused by the loop diuretic. Their diuretic effect is due to their ability to bind to and inhibit the $Na^+K^+2Cl^-$ transporter in the thick ascending limb (Figure 2:1) resulting in a fall in transcellular Na transport.[179] Brezis et al (1984) have shown that modifying transport activity with frusemide attenuates damage to the mTAL.[287] 44 ± 2% of mTALs in kidneys perfused with glucose were severely damaged compared to 7 ± 2% in kidneys also perfused with frusemide ($p<0.001$). Acetazolamide, which exerts its diuretic action on the proximal tubule, had no protective effect on the mTAL.

More recently the same group have measured the medullary PO_2 in rat kidneys perfused with frusemide and shown that inhibition of active transport with frusemide increased medullary PO_2 from 16±4mmHg to 35±4mmHg ($p<0.0005$) without affecting cortical PO_2.[286] This effect was thought to be due to decreased tubule oxygen consumption as medullary blood flow (measured by laser-Doppler probe) was significantly reduced by frusemide (-28 ± 6% from baseline, $p<0.0001$). These effects were reproduced by ethacrynic acid and bumetamide. Acetazolamide on the other hand, had no effect on medullary PO_2 but increased cortical PO_2 by a selective reduction in proximal tubule metabolism. Cortical blood flow was not affected by acetazolamide. Loop diuretics,

therefore, increase medullary oxygen availability. Epstein et al showed an increase in the oxidative state of cytochrome aa_3 after the addition of loop diuretic to perfusate.[329]

The beneficial effect of loop diuretics on medullary PO_2 led me to wonder if loop diuretics might attenuate ARF by reducing tubular reabsorptive work and thus, in theory, make more ATP available for regeneration.

8:3 Study 1 - effect of loop diuretics in ARF

Clinical studies of loop diuretics in ARF have been limited by bad study design and poor control groups. The outcome of these studies is shown in Table 8:1. The results are inconclusive, although all authors agree that a large enough dose of diuretic will increase urine output to some extent in the majority of patients.

I carried out a double blind, randomly allocated, placebo controlled, prospective study to examine the effect of loop diuretics in ARF. This study did not show any beneficial effect on mortality, need for dialysis or duration of dialysis. Patients given a loop diuretic had a significant diuresis and natriuresis compared to patients given placebo (Figures 5:1 and 5:2). Those patients who became non-oliguric in the first 24 hours, either spontaneously (placebo group) or pharmacologically induced (diuretic group), had a significantly lower mortality compared to those who remained oliguric (43% v 69% respectively, $p=0.001$). However, they were less ill (APACHE II score 17.2 ± 5.9 v 20.6 ± 5.5, $p=0.008$) and had less severe renal failure at entry into the study (creatinine clearance 14 ± 11ml v 4 ± 4ml, $p<0.0001$).

I conclude that loop diuretics can indeed cause a diuresis in ARF which may help with fluid balance. However, they do not affect the natural history of the disease and are not indicated as definitive treatment.

8:4 Study 2 - intraplatelet calcium in ARF

Elevated free cytosolic calcium has been proposed as the final common pathway of ischaemic cell death.[303] It has been shown that intra-mitochondrial calcium rises following reperfusion after experimental renal ischaemia, and that there is a significant correlation between raised mitochondrial calcium concentration and decreased mitochondrial respiration.[94] Uncoupling of mitochondrial respiration results in reduced ATP stores.[94] Kramer et al showed that in an experimental model of ischaemic ARF, loop diuretics prevented the rise in mitochondrial calcium normally seen in oliguric ARF.[309] Loop diuretics have been shown to decrease red cell $[Ca]_i$ in normal volunteers and patients with essential hypertension.[283,307] I hypothesised that a similar effect might occur in platelets from patients with ARF who had been given loop diuretic, with the potential therefore for less severe cell injury and a more favourable outcome.

Basal levels of $[Ca]_i$ were significantly higher in ARF patients than control subjects (109nm v 92.4nm, p=0.04). In the presence of 1mmol/l calcium and AVP, which stimulates influx of calcium from the suspension and release of intracellular stores of calcium into the cytosol, the rise in the fluorescence signal tended to be higher in the control group, although it did not reach statistical significance (584.8nm v 346nm, p=0.4). In the presence of EGTA, which binds to any extracellular calcium, AVP caused a smaller rise in the fluorescence signal in ARF patients compared to controls which again did not reach statistical significance (116nm v 166nm, p=0.14). In this experiment the increased signal is due solely to the release of intraplatelet stores of calcium and combined with the results from the studies with AVP and 1mmol/l calcium suggests that intraplatelet stores of calcium are low in patients with ARF. This would not be surprising as ischaemia is known to alter the composition of plasma membrane phospholipid.[98,305] In critically ill patients impaired uptake and utilisation of oxygen would cause cellular hypoxia resulting in altered membrane

composition which in turn would allow influx of extracellular calcium and efflux of intracellular stores of calcium into the cell cytosol, resulting in high $[Ca]_i$ and low intracellular stores. The study results tend to suggest that this is occurring. In addition the fluorescence signal obtained from the ARF patients showed that the platelet membranes were abnormally permeable to calcium (Figure 6:5).

The addition of loop diuretics had no effect on $[Ca]_i$ in patients with ARF. One possible explanation for this is that alteration of cell membrane composition due to ischaemia may alter drug binding sites so rendering them ineffective, unless given in a prophylactic manner before the renal insult.

Since a rise in intracellular calcium is associated with illness,[283,284] I wondered if there might be a correlation between the severity of illness reflected in the APACHE II score and the intraplatelet calcium level. However, I could not show any significant correlation between the $[Ca]_i$ and the APACHE II score in ARF patients

8:5 Study 3 - resting energy expenditure and APACHE II score

Resting energy expenditure is affected by a number of factors as outlined in chapter 7. In particular most disease states increase REE and in general the more severe the injury the greater the metabolic response.[266] However, some patients are normometabolic and 15 - 20% are hypometabolic.[264] It has been suggested that the hypometabolic response may be an energy efficient response carrying a better prognosis.[264]

The APACHE II score is a scoring system which can be used to stratify acutely ill patients according to severity of illness, and the more ill the patient the higher the APACHE II score.[281] As the REE has also been shown to correlate with severity of illness I compared the APACHE II score with the REE measured by indirect calorimetry in 20 patients with ARF and a mean APACHE II score of 16 ± 5.8. I could not show any correlation between the REE and APACHE II score measured on admission, even when the measured REE was

adjusted for size by dividing REE by BSA. This may have been because 25% of the patients were hypometabolic at the time of the first measurement of REE. There was no significant difference in severity of illness between the hypometabolic and hypermetabolic group (APACHE II score 14.4 ± 6.4 and 16.6 ± 6 respectively, p=0.5), and the hypometabolic response did not seem to portend a better prognosis as mortality was not improved (40% v 50%, p=0.8, hypometabolic v hypermetabolic).

The REE measurement was repeated after at least 2 days (mean 5 days, range 2 - 11days) and 54% of patients were noted to be hypometabolic. This was thought mainly to be due to the administration of sedative drugs which are known to reduce REE.

Indirect calorimetry is an accurate way of measuring energy expenditure in critically ill patients and is thus is a marker of physiological stress. The lack of any correlation with the APACHE II score means that the APACHE II score should not be used as part of a formula to predict energy expenditure. Serial measurements of REE using indirect calorimetry are required.

8:6 Conclusion

The nature of the pathogenesis of ARF provides many points for targeting therapeutic intervention; manipulation of renal haemodynamics, prevention of intratubular obstruction, prevention of tubule epithelial cell injury and restoration of tubule epithelial integrity.[330]

Loop diuretics have the potential to preserve tissue ATP by suppressing resorptive work, preventing and reversing intratubule obstruction, increasing renal blood flow and, according to some, reducing intracellular calcium levels. Their role in the management of ARF has been unclear mainly due to the poor design of previous studies. My work provides conclusive evidence that loop diuretics have no beneficial effect on the natural history of ARF. This is confirmed at a cellular level where I was unable to detect any change in cytosolic calcium concentration. Loop diuretics are thus are unable to reverse one of the

effects of hypoxia that ultimately leads to cell death. Despite this they may still have a role to play in ARF as they cause a significant increase in urine flow rate in some patients, which in turn may make management of fluid balance easier.

As a result of these findings the routine use of loop diuretics in ARF is no longer standard practice in the Royal Infirmary, Glasgow and Stobhill Hospital, Glasgow.

A rise in intracellular calcium is associated with illness and therefore might be expected to correlate with the severity of illness and outcome as measured by the APACHE II score.[283,284] I could not demonstrate any correlation between the APACHE II score and $[Ca]_i$ in patients with ARF.

Similarly REE is affected by illness[266] and it is possible that those patients with a higher REE have a worse prognosis. Once again I could not demonstrate any correlation between the APACHE II score and REE in patients with ARF.

Table 8:1 Outcome of clinical diuretic studies in acute renal failure

Author	Year	Less dialysis	Shorter period of renal insufficiency	Lower mortality	Increased urine output
Cantarovich et al[187]	1973	Yes	No	No	Yes
Minuth et al[184]	1976	-	No	No	Yes
Brown et al [190]	1980	No	No	No	Yes
Kleinknecht et al 185	1975	No	No	No	Yes
Cantarovich et al[186]	1971	Yes	Yes	No	Yes
Borirakchanyavat et al[192]	1978	No	No	-	Yes

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Appendix 1- Screening Data

Torasemide group

Patient	Urine vol (ml/hr)	Creatinine clearance (ml/min)	APACHE II score
1	65	18.7	11
3	43	9.8	14
8	35	13.6	16
12	26	4.7	25
13	0	0.0	22
15	36	7.5	14
20	53	50.6	18
21	19	13.2	32
28	7	16.0	16
29	7	17.0	17
36	6	23.0	23
38	15	2.4	18
42	28	11.5	20
48	0	0.0	19
49	42.5	1.7	18
51	31	5.1	24
55	10.5	3.0	25
57	5	3.4	23
61	36	12.5	18
63	37.5	23.8	15
68	45	14.7	19
70	50	10.7	15
73	55	10.1	20
77	8	12.4	23
80	15	4.8	27
83	0	0.0	24
86	37.5	28.4	20
87	42.5	24.1	18
91	0	0.0	18
95	32.5	4.1	14

Appendix 1 (continued)

Frusemide group

Patient	Urine vol (ml/hr)	Creatinine clearance (ml/min)	APACHE II score
2	12	3.4	16
6	21	2.3	14
7	75	10.6	12
11	8	4.2	26
16	45	28.5	8
18	43.5	16.5	19
19	17	4.65	19
22	7	1.1	7
25	82	5.4	18
30	17.5	4.6	13
34	115	27.9	38
35	4	0.7	13
37	17	3.9	37
39	192	10.6	11
46	13.5	15.6	18
47	5	0.8	23
50	70	9.7	12
52	5	2.8	23
59	21.5	21.3	17
60	10	3.7	18
62	3	0.2	-
64	28	5.0	13
67	12.5	2.3	13
76	0	0.0	19
78	39	37.6	22
79	0	0.0	27
82	5	1.3	25
89	25	12.9	21
90	32.5	2.7	20
92	0	0.0	27
93	0	0.0	25
97	17.5	10.2	18

Appendix 1 (continued)

Placebo group

Patient	Urine vol (ml/hr)	Creatinine clearance (ml/min)	APACHE II score
4	13.5	1.0	14
5	15	0.6	14
9	0	0.0	25
10	10	3.0	32
14	22.5	9.5	14
17	13	2.4	23
23	17.5	18.2	21
24	7.5	3.2	-
26	32.5	3.3	14
27	26.5	1.3	23
31	52.5	3.0	-
32	22.5	24.3	-
40	22	7.2	11
41	52.5	1.6	15
43	7.5	3.0	19
44	25	25.1	15
53	5	1.0	23
54	15.5	-	-
56	19	-	9
58	0	0.0	17
66	21	11.3	11
69	30	7.6	20
72	0	0.0	15
75	70	29.0	18
81	24.5	13.1	24
84	10.5	10.4	10
88	19	14.2	19
94	7	3.4	27
96	6	3.0	24
98	14.5	4.9	21

Appendix 2 - Outcome at day 21

Torasemide group

Patient	Outcome	Time to dialysis (days)	Duration of dialysis (days)	Time to renal recovery (days)	Urine output at 24 hours (ml/hr)
1	Alive	4	2	11	104
3	Alive	-	-	2	285
8	Alive	-	-	1	353
12	Dead	-	-	-	-
13	Dead	-	-	-	0
15	Dead	16	4	-	287
20	Alive	-	-	1	300
21	Dead	-	-	-	-
28	Dead	-	-	-	56
29	Dead	-	-	-	-
36	Alive	10	15	18	3
38	Dead	1	4	-	59
42	Dead	2	8	-	96
48	Dead	1	4	-	0
49	Dead	4	3	-	41
51	Dead	-	-	-	45
55	Dead	-	-	-	-
57	Dead	-	-	-	-
61	Dead	3	2	7	212
63	Alive	-	-	2	395
68	Dead	-	-	-	427
70	Alive	11	3	-	197
73	Dead	-	-	-	267
77	Dead	-	-	-	32
80	Dead	2	5	-	33
83	Dead	-	-	-	0
86	Dead	-	-	-	76
87	Dead	-	-	-	48
91	Alive	1	10	17	5
95	Alive	-	-	3	47

Appendix 2 (continued)

Frusemide group

Patient	Outcome	Time to dialysis (days)	Duration of dialysis (days)	Time to renal recovery (days)	Urine output at 24 hours (ml/hr)
2	Dead	3	DD	-	67
6	Alive-DD	3	DD	-	133
7	Alive	-	-	1	450
11	Dead	-	-	-	777
16	Alive	-	-	1	-
18	Alive	14	DD	-	231
19	Alive	-	-	4	75
22	Alive	16	DD	-	7
25	Alive	-	-	2	260
30	Alive	-	-	1	207
34	Dead	-	-	-	202
35	Alive	-	-	7	5
37	Dead	-	-	-	8
39	Alive	-	-	2	170
46	Dead	-	-	-	25
47	Dead	1	3	-	12
50	Alive	-	-	-	131
52	Dead	-	-	-	3
59	Dead	-	-	2	175
60	Dead	-	-	-	-
62	Dead	-	-	-	-
64	Alive	-	-	-	130
67	Alive	6	11	18	23
76	Dead	18	DD	-	0
78	Dead	-	-	-	156
79	Dead	-	-	-	12
82	Dead	-	-	-	0
89	Alive	2	5	10	0
90	Dead	-	-	4	300
92	Dead	1	3	-	0
93	Dead	1	DD	-	0
97	Dead	-	-	2	148

DD - dialysis dependent

Appendix 2 (continued)

Placebo group

Patient	Outcome	Time to dialysis (days)	Duration of dialysis (days)	Time to renal recovery (days)	Urine output at 24 hours (ml/hr)
4	Dead	-	-	2	322
5	Alive	-	-	6	54.8
9	Dead	-	-	-	-
10	Dead	-	-	-	-
14	Alive	-	-	2	207
17	Dead	-	-	-	1
23	Dead	-	-	-	160
24	Dead	-	-	-	-
26	Alive	2	DD	-	33
27	Alive	-	-	5	35
31	Alive-DD	1	DD	-	9
32	Alive	2	2	4	52
40	Alive-DD	5	DD	-	54
41	Alive	-	-	5	79
43	Dead	4	12	-	7
44	Alive	-	-	2	105
53	Dead-DD	2	DD	-	50
54	Dead	-	-	-	-
56	Alive	3	3	7	43
58	Dead	-	-	-	0
66	Alive	3	12	17	18
69	Alive	3	12	19	23
72	Alive	4	DD	-	0
75	Alive	-	-	3	107
81	Dead	-	-	-	25
84	Alive	-	-	-	33
88	Alive	2	15	19	25
94	Dead	-	-	-	-
96	Dead	-	-	-	0
98	Dead	2	2	-	19

DD - dialysis dependent

Appendix 3

Patients enrolled in study 3 (chapter 7)

Patient	Cause of ARF	APACHE II score	Ventilated
1	Scleroderma crisis	11	No
2	Bronchopneumonia, respiratory failure	16	No
3	Aminoglycoside nephrotoxicity	18	No
4	Perforated oesophagus	14	No
5	Infection	14	No
6	Aminoglycoside nephrotoxicity	12	No
7	Septic shock	32	Yes
8	Acute liver failure (alcohol)	25	No
9	Bacterial pneumonia	14	No
10	Fungal infection of Hickmann line	8	No
11	Post aortic-bifemoral graft (elective)	23	Yes
12	Thrombotic thrombocytopenic purpura	19	No
13	Post aortic-bifemoral graft (emergency)	19	Yes
14	Septic shock	14	Yes
15	Gallstone pancreatitis	17	No
16	Shigella infection	13	No
27	Post aortic-bifemoral graft (elective)	13	Yes
18	Haemolytic uraemic syndrome	11	No
19	Goodpastures syndrome	11	No
20	Rhabdomyolysis	15	No