USE OF AN ISOLATED PERFUSION SYSTEM IN THE STUDY OF NORADRENALINE-INDUCED ACUTE RENAL FAILURE IN THE DOG

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Abstract

In this study an isolated system for normothermic perfusion of dog kidneys has been developed for use in the study of noradrenaline-induced acute renal failure (ARF). Stable hemodynamics and tubular function were obtained with a bicarbonate buffer containing 3.75% albumin. Other perfusates were less satisfactory.

Infusion of noradrenaline into one renal artery resulted in the hemodynamic and functional changes characteristic of human and experimental acute renal failure. When the ARF kidney was perfused its RVR was not different from the RVR of its contralateral control. The GFR of the ARF kidney was significantly less ($p<0.05$) than its contralateral control. This persistance of low GFR in the absence of high RVR suggests that a defect at the level of the glomerulus is responsible for maintenance of ARF. In addition, the ARF kidney did not respond as well as the control to noradrenaline, angiotensin or angiotensin potentiation of noradrenaline. This suggests that, in addition to the glomerular defect, a defect in vascular smooth muscle is present, and may reflect events involved in the pathogenesis of noradrenaline-induced acute renal failure.

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List of Abbreviations

ACh	- acetylcholine
AORF	- acute oliguric renal failure
ARF	- acute renal failure
ATN	- acute tubular necrosis
A II	- angiotensin II
BUN	- blood urea nitrogen
C_{Cr}	- creatinine clearance
DA	- dopamine
D6%S	- 6% dextran in saline
DOCA	- desoxycorticosterone acetate
EGTA	-[ethylene bis(oxyethylenenitrilo)] tetraacetic acid
EKG	- electrocardiogram
GFR	- glomerular filtration rate
K_f	- ultrafiltration coefficient
K.H.B.	- Krebs-Henseleit bicarbonate buffer
^{85}Kr	- radioactive krypton
MAP	- mean arterial pressure
NORA	- noradrenaline
% $Reab_{Na}$	- percent sodium reabsorption
π_{GC}	- oncotic pressure in the glomerular capillary
P_{GC}	- glomerular capillary hydrostatic pressure
P_T	- proximal tubule hydrostatic pressure
P113	- 1-sarcosine-8-alanine angiotensin II
PAH	- para-aminohippurate
PGA	- prostaglandins of the A series

PGE	- prostaglandins of the E series
PITP	- proximal intratubular hydrostatic pressure
POB	- phenoxybenzamine
PRA	- peripheral renin activity
PRU	- peripheral resistance unit; ratio of pressure to flow
PTH	- parathyroid hormone
RBF	- renal blood flow
RVR	- renal vascular resistance to flow
SNGFR	- single nephron glomerular filtration rate
SQ 20881	- converting enzyme inhibitor
$U_K V$	- potassium excretion rate
$U_{Na} V$	- sodium excretion rate
U/P Osm	- ratio of urine to plasma osmolality
$\dot{V}$	- urine flow rate
^{133}Xe	- radioactive xenon

INTRODUCTION

I. Historical Review

A. Acute oliguric renal failure in man.

The term acute renal failure is applied to a clinical syndrome, of diverse antecedents, which is characterized by abrupt and sustained loss of renal function which, if the individual survives, is spontaneously reversible. Precipitating events can be broadly divided into those preceded by a circulatory event and those following exposure to nephrotoxic agents. Acute loss of intravascular volume is common at onset and acute renal failure has been described as a complication of trauma and hemorrhage, intravascular hemolysis, rhabdomyolysis and septicemia (Smith, 1951; Thomson, 1952; Swann & Merrill, 1953; Merrill, 1960; Kjellstrand, 1973; Dunnill, 1974; Knochel & Carter, 1976; Epstein, 1976; Stein, et al., 1978). In addition, a variety of nephrotoxic agents such as methyl alcohol, ethylene glycol, carbon tetrachloride, lithium, aminoglycosides and cephaloridine have also been found associated with acute renal failure (Redish, 1947; Sirota, 1949; Oliver, et al., 1951; Bailey, et al., 1973; Lavender, et al., 1973; Hagnevik, et al., 1974).

Individuals who develop acute renal failure have glomerular filtration rates usually less than 5% of normal. Urine produced resembles an ultrafiltrate of plasma and the volume

is frequently less than 500 ml/day. In the absence of effective renal function uremic acidosis supervenes (Eliahou, et al., 1973; Epstein, 1976). Histologic examination of the kidneys by light microscopy shows normal glomeruli and variable evidence of tubular necrosis (Oliver, et al., 1951, 1953; Sevitt, 1959). Oliver, et al. (1951) used microdissection of nephrons after shock, burns or a nephrotoxic insult in man, dog or rabbit in order to determine the nature of distribution of the tubular lesion. They described two different tubular lesions. One, termed tubulorrhexis, consisted of destruction both of the tubular cells and underlying basement membrane at random sites in the proximal and distal convoluted tubule. There was no correlation of the lesion with intraluminal debris. A second type of lesion, which they termed nephrotoxic, was characterized by necrosis limited to the proximal convoluted tubular cells with preservation of underlying basement membrane.

Because of the variety of precipitating factors and the lack of positive correlation between the structural and functional lesion, the terminology has often been confusing. Strauss (1948) used the term lower nephron nephrosis or hemoglobinuric nephrosis to define the critical condition. Swann and Merrill (1953) listed twelve names that were synonymous with this condition of functional failure. By 1960 the term acute renal failure (ARF) was used to define the general condition in which the kidneys were unable to

excrete the normal metabolic load present in the plasma (Merrill, 1960). At that time the precipitating events were further classified in respect to whether the primary insult was pre-renal, renal or post-renal. Acute tubular necrosis (ATN) was another alternative which was used because of the relatively common occurrence of some renal tubular degeneration (Epstein, 1976). Another alternative was vasomotor nephropathy (Oken, 1971; Merrill, 1973). Hollenberg, et al. (1968) and Eliahou, et al. (1973b) suggested that if ATN was present with oliguria and renal failure, the term acute oliguric renal failure (AORF) be used. This term served to differentiate this condition from non-oliguric renal failure in which similar changes in function occur although urine flow is normal or increased (Vertel & Knochel, 1967; Epstein, 1976). However, there is still no universal agreement of terminology as indicated by Olsen (1976) who divided acute renal failure into two groups, those with or those without glomerular or vascular lesions.

The renal vasculature was first definitely implicated in ARF by Homer Smith, who, according to Oliver, et al. (1951), said that functional disturbances in ARF were concomitant with changes in renal blood flow. Determinations of renal blood flow prior to 1955 were based on the direct Fick principle using the clearance of radioactive iodopyracet (Diodrast) or para-aminohippurate (PAH) (Bobey, 1943; Redish, et al., 1947; Sirota, 1949; Bull, et al., 1950). These

determinations were subject to error when the patient was oliguric (Walker, et al., 1963; Reubi, et al., 1966). Brun (1955) used ^{85}Kr in an inert gas washout technique to measure renal blood flow in normal and anuric patients. This procedure obviates the necessity for a high urine flow. Other techniques have since been developed to determine blood flow in clinical acute oliguric renal failure, but regardless of technique, or the precipitating factor(s), there usually is both a decrease in total flow to approximately one-third of normal, and renal cortical ischemia in established renal failure (Finckh, et al., 1962; Hollenberg, 1970; Hollenberg, et al., 1968; 1970; Ladefoged & Winkler, 1970; Hollenberg & Adams, 1973).

The clinical course of AORF is varied, depending upon antecedent events (Bull, et al., 1950; Oliver, 1953; Strauss, 1948; Epstein, 1976). In general there is an oliguric phase (urine volume < 500 ml/day) of variable duration in which effective renal function is absent. The inability of the kidney to remove catabolic waste products is associated with acidosis. In the absence of renal function, fluid overload and hyperkalemia are common causes of morbidity. The oliguric phase is followed by a polyuric phase in which blood flow and glomerular filtration increase but tubular function is still impaired. Failure of tubular salt reabsorption during this phase may result in sodium depletion and hypovolemia. There is a gradual recovery of function which may

take weeks to months to reach normal values. Urine volumes gradually decrease as the tubular function returns.

In the absence of a clear knowledge of the pathophysiology of ARF a variety of empiric measures have been tried in an attempt to prevent or reverse renal failure. Early efforts included x-radiation and renal decapsulation (Abeshouse, 1945; Peters, 1945; Strauss, 1948). More recently the use of osmotic loading and diuretics such as furosemide or ethacrynic acid to increase renal blood flow have been proposed (Silverberg, et al., 1970; Kountz, et al., 1973; Cantarovich, et al., 1971; Kjellstrand, 1972; Linton, et al., 1973; Fillastre, et al., 1973; Anderson, et al., 1977). To date no therapeutic intervention has been shown to measurably improve renal function or shorten duration of oliguria (Munck, 1975; Baek, et al., 1973b; Epstein, et al., 1975).

Current therapy is directed to prevention or correction of fluid overload, hyperkalemia and acidosis by means of conservative dietary regimes, use of Na/K exchange resins and dialysis (Merrill, 1973; Abel, et al., 1973; 1974).

In 1948 Strauss reported that the mortality from clinical ARF was fifty percent. Deaths resulted from fluid overload and pulmonary edema, potassium induced arrhythmias or complicating sepsis. In the intervening 3 decades, with the development and widespread use of dialysis and availability

of effective antibacterials, these complications can be avoided or successfully managed. Nonetheless, overall mortality has remained essentially unchanged (Epstein, 1976). In part this may be related to changes in the spectrum of antecedent events. Intravascular hemolysis and accidents of pregnancy, which have a good prognosis, accounted for about 1/3 of cases in early series. These are seldom current causes of ARF, their place being taken by a higher incidence of extensive trauma, often complicated by sepsis. These cases, many of which would formerly have succumbed to shock are now surviving to develop ARF. In general they have a higher ultimate mortality.

Several theories have been proposed to account for filtration failure in ARF. The earliest theory, based on clinical evidence, was related to tubular obstruction (Ponfic, 1875; as cited by Finckh, 1962). Because intraluminal casts and debris were found in biopsy and autopsy material, it was suggested that filtration continued normally until the intratubular hydrostatic pressure rose to equal glomerular capillary pressure, and then filtration ceased (Peters, 1945; Meroney & Rubini, 1959; Merrill, 1960; Finckh, 1962; Shaldon, et al., 1963). An alternate theory proposed that the normally produced filtrate passively diffused into the interstitial space through damaged tubules and basement membranes (Redish, et al., 1947; Sirota, 1949; Oliver, et al., 1951; Oliver, 1953; Merrill, 1960; Flores, et al., 1972).

These two theories, based on morphological, not functional events, were open to speculation. Renal decapsulation, which was believed might alleviate intrarenal edema, did not improve the course of renal failure (Abeshouse, 1945; Merrill, 1960). There was a poor correlation between the degree of tubular injury and duration or severity of functional failure (Sevitt, 1951; Finckh, et al., 1962; Bohle, et al., 1976). This observation was expanded by Sevitt who, in 1959, reported that tubular damage could not be the basis of filtration failure because: the functional disturbances of oliguria followed by diuresis were not paralleled by the tubular damage; filtration failure was not always accompanied by necrosis; some proximal and distal tubular function was maintained; tubular necrosis could occur in situations when oliguria with potassium and other electrolyte imbalances were not seen.

Because of the reduction in blood flow seen clinically, another hypothesis, based on a hemodynamic abnormality was proposed. Goormaghtigh (1945) postulated that the release of a vasopressor substance possibly from juxtaglomerular cells, which showed increased granularity, was responsible for contraction of the vasculature at the glomerulus. In a follow-up study, Goormaghtigh (1947) proposed that the postglomerular arterioles constricted first, and then the glomerular tuft. The vasoconstriction was believed to be followed by a paralytic dilation at which time the glomerulus

became nonfiltering.

Renal vasoconstriction could produce a reduction in renal blood flow and consequent tubular necrosis (Sevitt, 1959). Tissue hypoxia and resulting cellular swelling could lead passively to tubular obstruction (Flores, et al., 1972; 1973). Vasoconstriction of the afferent arteriole and/or vasodilation of the efferent arteriole could reduce glomerular capillary pressure to a level which would stop filtration. The restoration of filtration during subsequent diuresis might be due to recovery of the vascular disturbance (Finckh, et al., 1962).

Intravascular coagulation (Hjort & Rapaport, 1965; Clarkson, et al., 1970; Wardle, 1975) and fibrinogen deposits (Koffler, et al., 1966) have been implicated in hemodynamic changes leading to failure, but they are not consistently found in all patients with AORF (Epstein, 1976), and, if seen, do not appear to modify the course or duration of the failure (Conte, et al., 1974).

Hollenberg, et al. (1968) reported that the blood flow to the cortex, measured by ^{133}Xe washout, decreased in patients with AORF of diverse etiology. This reduction in cortical perfusion could account for oliguria. They suggested that the decrease in filtration could be a conservation measure, designed to protect against extreme sodium and water

loss. When a decrease in glomerular filtration was occasionally seen without a concurrent decrease in renal blood flow, they suggested that there had been a selective efferent arteriolar dilation.

Hollenberg, et al. (1968) discussed the possible mechanisms of the persistent ischemia and suggested that it could be either an active or passive event. Continued activation of the renin-angiotensin system, the thesis suggested by the data presented by Goormaghtigh in 1945, was one possibility. The other was that renal edema produced vascular compression. The passive vascular compression theory could not be further tested clinically, and further elucidation necessitated use of infrahuman models. The theory of activation of the renin-angiotensin system has been indirectly studied clinically by measuring peripheral renin activity (PRA) in patients with established renal failure. Tu (1965) and others (Kokot & Kuska, 1969; Ochoa, et al., 1970; Kokot & Kuska, 1976) reported elevated levels for PRA in patients in the oliguric phase, but not the diuretic phase of renal failure. Brown, et al. (1970) found peripheral plasma renin levels to be elevated at least once in twenty-two out of twenty-five patients with ARF. Del Greco and Krumlovsky (1970) and Paton, et al. (1975) corroborated the work of Brown, et al. (1970). They suggested that this was sufficient evidence to link angiotensin to both initiation and maintenance of renal failure. However, it will have to be shown that the

renal bed does not exhibit tachyphylaxis to angiotensin before it can be seriously suggested to be involved in the maintenance of ARF (Hollenberg, et al., 1973). Also, very high levels of renin and angiotensin occur in clinical situations, for example accelerated hypertension, where AORF is uncommon. The role of other vasoactive substances, such as the prostaglandins, bradykinin, histamine and serotonin has not been evaluated in a systematic fashion in ARF.

Clinical studies to date are not united in support of a vascular mechanism for AORF pathogenesis. Ladefoged and Winkler (1970) said that because they could record a temporary increase in renal blood flow from five patients in ARF who were given dihydralazine, a vasodilator, this was indicative of a primary vascular lesion not being the cause of the renal failure. In a later study the same research group reported their results from a study on kidneys in ARF from persons in shock (Pedersen & Ladefoged, 1973). Their ^{133}Xe washout studies indicated that cortical flow was reduced, but their histological examination suggested that the vasculature was normal. This indicated to them that there was not a primary vascular injury, but did not discount the possibility of a defect in the regulatory mechanism.

B. Experimental models of acute oliguric renal failure.

Studies of human ARF have so far failed to provide a clear insight into the pathophysiology of the filtration failure. Antecedent events are diverse and of variable duration. In addition, non-invasive techniques permitting the study of intrarenal events are not available or necessarily applicable to human studies. Consequently a variety of animal models have been developed in an attempt to duplicate the essential features of human ARF.

Studies using animal models have generally lent support to one or other of two major hypotheses: a) filtration failure due to either tubular obstruction followed by loss of effective transcapillary hydrostatic gradient or back diffusion of filtrate, b) filtration failure due to changes in vascular resistance leading to a reduction in blood flow and a loss of effective hydrostatic pressure gradient. The loss of hydrostatic pressure gradient may be secondary to changes in afferent and/or efferent arteriolar resistance to blood flow, or to changes in glomerular capillary surface area or permeability (Table 1).

1. Tubular Defect

Experiments done by a variety of researchers have led to the suggestion that the primary renal lesion leading to

Table 1: Pathophysiology of ARF. Proposed sites of primary defect.

A. Tubular Defects

1. Obstruction of lumina with rise in intratubular pressure and secondary cessation of filtration.
   a) Casts
   b) Edema

2. Damage to the tubular epithelium.
   a) Structural - passive back diffusion
   b) Biochemical

B. Hemodynamic Defects: high resistance to flow.

1. At the level of the arteriole
   a) Nervous
   b) Hormonal - the renin-angiotensin system
      - an angiotensin-prostaglandin imbalance
      - other
   c) Intrinsic smooth muscle defect

2. At the glomerulus --- glomerular ultrafiltration
   a) Surface area
   b) Permeability

failure is within the tubule. Using a variety of animal species and models, numerous authors have reached the conclusion that the defect is the result of blockage of the lumen by casts or edema. Others feel that the results are best explained as the result of damaged epithelium, either grossly, which allows passive back diffusion, or biochemically, which is manifested by alterations in enzymes for electrolyte transport. Histologic sections in which intratubular debris was seen were the basis for several reports (Flink, 1947; Mason, et al., 1963b; Menefee, et al., 1964; Jaenike, 1967; Gras, et al., 1975; Schubert, 1976; Frega, et al., 1976). These observations, coupled with micropuncture data which indicated a measureable increase in proximal tubular hydrostatic pressure, seemed to be further evidence that the stoppage of flow through the tubule occurred because of cast deposits (Henry, et al., 1968; Biber, et al., 1968; Jaenike, 1969; Tanner, et al., 1973; Arendshort, et al., 1974; Finn, et al., 1975; Tanner & Steinhausen, 1976; Mason, 1976). Finn, et al. (1975) used an electronic servo-nulling pressure apparatus and directly measured glomerular capillary hydrostatic pressure in a strain of rats with surface glomeruli. By continuously monitoring both glomerular and tubular pressures in rats in which renal failure had been produced by ischemia, they reported that the intratubular hydrostatic pressure was elevated before there was a decrease in either glomerular capillary hydrostatic pressure or preglomerular vasoconstriction.

However, many other micropuncture experiments indicate that the debris deposit is a secondary event. The first micropuncture study on rats in mercury-induced renal failure indicated an early low hydrostatic pressure with subsequent intratubular obstruction due to the reduced flow rate (Flanigan & Oken, 1965). The single nephron glomerular filtration rate (SNGFR) was depressed prior to any visible tubular injury. Oken, et al. (1966) found that it was possible to wash the casts down the tubule at pressures equivalent to normal proximal intratubular pressures. Other studies supported the concept of a reduced intratubular hydrostatic pressure in renal failure in rats (Oken, et al., 1966; Wilson, et al., 1967; Biber, et al., 1968; Wilson, et al., 1969; Thiel, et al., 1970; Oken, et al., 1970; Flamenbaum, et al., 1971; 1974). It has been difficult to puncture tubules because they have been either collapsed or devoid of fluid (Flanigan & Oken, 1965; Oken, et al., 1966; Wilson, et al., 1967; Ruiz-Guinazu, et al., 1967; Biber, et al., 1968; Henry, et al., 1968; Wilson, et al., 1969; Jaenike, 1969; Oken, et al., 1970; Flamenbaum, et al., 1971). Flamenbaum, et al. (1971) reported a dissociation between tubular damage and functional abnormalities. He studied rats in which renin production was diminished or enhanced by alteration of sodium balance, and found that there was equal tubular damage when mercury was used to induce renal failure. However, on the basis of micropuncture data, functional parameters of renal failure were much more pronounced in those rats on a low

salt diet (i.e. elevated renin).

Other support for a tubular defect was on the basis of indirect evidence. A decreased glomerular filtration rate (GFR) without visible alteration in blood flow suggested that a hemodynamic parameter could not be involved (Conn, et al., 1954; Goldberg, 1962; Braun & Lilienfield, 1963; Jaenike, 1967; Gottschalk, et al., 1975; Dach & Kurtzman, 1976). It was generally assumed that the glomerular filtration coefficient (K_f) was normal. This assumption is currently under investigation and will be discussed later (Sec. I.B.3).

The data which supports passive back diffusion with or without resulting edema is indirect, and as Oken (1975b) suggested, equally explained by a theory of filtration failure with depressed ionic transport. These studies were based on disappearance of markers such as inulin, creatinine, lissamine green dye and others, from the tubular lumen. The first definitive study was that done by Bank, et al. (1967) who reported the visual disappearance of lissamine green dye as the dye travelled down the tubule after being injected intravenously to rats in renal failure. They reported that the tubules were also slightly dilated (29.5 μ compared to 23.5 μ in controls) and that obstruction occurred in distal segments after flow to those regions had stopped. They believed that inulin, their reference for transtubular water movement, leaked

into the peritubular space as well. Their conclusion was that there was a complete absorption of the glomerular filtrate through the tubular epithelium by an unknown mechanism. Steinhausen, et al. (1969) used a comparable protocol, but reported that the lissamine green dye was being discolored as it came in contact with the mercury poisoned epithelium. They also noted a decrease in inulin, and the appearance of this molecule in urine from the contralateral unexposed kidney. Blantz (1975) reported significant back diffusion of inulin and mannitol. He suggested that this was the result of transfer through damaged epithelium because radiolabelled inulin was not completely excreted from the experimental kidney in AORF after being microinjected into the proximal tubule whereas it was in the control, hydropenic rat kidney.

However, this explanation of the data is not generally accepted. Daugharty, et al., (1974) found no back diffusion or "leakiness" in tubules after incomplete renal ischemia was produced in rats. They suggested that the tubular lesion was the result of the reduction in filtration, and not the reverse. Flamenbaum, et al. (1974) reported that single nephron glomerular filtration rate (SNGFR) in rats with failure from uranyl nitrate was reduced, but stable along the length of the nephron. Oken (1975b), in his review of micropuncture evidence in renal failure, stated that inulin loss could have been produced through the damaged, then

punctured, tubular cells.

A biochemical defect has been suggested by those investigators who found early alterations in sodium or organic acid handling (Braun & Lilienfield, 1963; Mason, 1976; Flamenbaum, et al., 1976). Two recent papers suggest that enzymes in the brush border of the proximal tubule (alkaline phosphatase, 5'nucleotidase, acid phosphatase) are subnormal for fifteen minutes to three hours after a mercury insult to rats (McDowell, et al., 1976; Zalme, et al., 1976). This would inhibit sodium transport. During the first six hours they saw enzymatic and electron microscopic evidence which indicated damage to mitochondrial membranes. They suggested that this led to the tubular necrosis subsequently observed. The hypothesis awaits further testing before it can be considered applicable in forms of failure other than those in which known nephrotoxins directly alter tubular membranes.

2. Hemodynamic Defect

The theories implicating the tubule as the primary site of renal damage have not, as yet, provided an adequate explanation of the decreased renal blood flow or the depressed glomerular filtration in ARF. Many reports support a vascular mechanism for the failure on the basis of direct measurements of decreased renal blood flow (Selkurt, 1946a; 1946b; Ayer, et al., 1971; Chedru, et al., 1972; Sherwood, et al., 1974; Daugharty, et al., 1974) or on the basis of the recorded low proximal intratubular hydrostatic pressure (PITP) where obstruction is seen as a consequence of the low driving pressure for fluid movement (Flanigan & Oken, 1965; Oken, et al., 1966; Ruiz-Guinazu, et al., 1967; Thiel, et al., 1967; Jaenike, 1969; Thiel, et al., 1970; Oken, et al., 1970; Chedru, et al., 1972; Eisenbach & Steinhausen, 1973; Wilson, et al., 1969; Flamenbaum, et al., 1974; Arendshort, et al., 1974; Flamenbaum, et al., 1976b). A vascular mechanism has also been suggested as the result of histological evidence which does not illustrate a gross morphological tubular defect (Eggleton, et al., 1944; Suzuki & Mostofi, 1970b; DiBona, et al., 1971; Reimer, et al., 1972; Brown, et al., 1972; Helmchen, et al., 1972; Ryan, et al., 1973; Loew & Meng, 1976). With the use of micropuncture on rats in various forms of renal failure, it has been shown that SNGFR was markedly depressed (Flanigan & Oken, 1965; Oken, et al., 1966; Wilson, et al., 1967; Bank, et al., 1967; Biber, et al.,

1968; Barenberg, et al., 1968; Henry, et al., 1968; Jaenike, 1969; Wilson, et al., 1969; Thiel, et al., 1970; Oken, et al., 1970; Flamenbaum, et al., 1971; 1976b). An increase in preglomerular resistance in conjunction with unaltered or decreased postglomerular resistance could theoretically decrease glomerular capillary pressure to a point which would stop filtration (Flanigan & Oken, 1965; Oken, et al., 1966; Ruiz-Guinazu, et al., 1967; Thiel, et al., 1967; Wilson, et al., 1967; Biber, et al., 1968; Barenberg, et al., 1968; Henry, et al., 1968; Wilson, et al., 1969; Jaenike, 1969; McDonald, et al., 1969; Oken, et al., 1970; Flamenbaum, et al., 1971; DiBona, et al., 1971; Ayer, et al., 1971; Oken, 1973; Daugharty, et al., 1974; Venkatachalam, et al, 1976). Cessation of filtration in conjunction with a decreased blood flow could render the renal parenchyma hypoxic, and this could lead to cellular necrosis and death as a secondary event (Flores, et al., 1972; 1973; Leaf, 1973; Reimer, et al., 1972; Eliahou, et al., 1973). The collecting duct, which gets its energy by anaerobic metabolism, is not damaged in AORF (Guder & Schmidt, 1976). The glomerular injury may heal faster than the tubular injury, as reflected experimentally by the reduced proximal tubular sodium transport and low fractional water reabsorption (Oken, et al., 1970).

Ayer, et al. (1971) discussed the possible mechanisms which could mediate afferent arteriolar constriction. These could be stimulation of the renal nerves, or high levels of

circulating catecholamines, activation of the renin-angiotensin system or release of other, perhaps unknown vasoactive substances. Although a role for enhanced sympathetic activity has been suggested in the initiation of renal failure (Fung, 1972), the fact that denervated and recently transplanted kidneys are capable of developing acute renal failure (Kjellstrand, et al., 1973) indicates that the neural influence need not be a primary determinant in the initiation or maintenance of AORF.

Study of the renin-angiotensin system was stimulated as the result of the histological data reported by Goormaghtigh (1945; 1947) who first implicated an increase in renin release from the juxtaglomerular apparatus in kidneys in failure. Brown, J.J., et al. (1973) discussed the theories of the possible role of renin in acute renal failure. Renin converts renin substrate, a plasma alpha-2-globulin, to angiotensin I in the plasma. This decapeptide is not a vasoconstrictor. The subsequent conversion to the active octapeptide, angiotensin II by the converting enzyme, could occur in peripheral blood, the renal circulation or in the renal extravascular space. DiSalvo, et al. (1971) have shown that angiotensin I to angiotensin II conversion can occur intrarenally, therefore the converting enzyme must be present in the kidney. Semple, et al. (1976) suggested that angiotensin may be produced in the intravascular space or in the wall of the afferent arteriole, two sites which could be resistant

to routine attempts to deplete renin or inactivate the converting enzyme. Active angiotensin could vasoconstrict the afferent arteriole by a direct action on the smooth muscle (Elkin, et al., 1966) and produce the reduced RBF and GFR seen in AORF.

The stimulus for an increase in renin release has not yet been delineated. Flamenbaum, et al. (1970; 1971; 1972a; 1973; 1976a) have proposed that an initial defect in sodium reabsorption in the proximal convoluted tubule increases the sodium delivery to the macula densa. This stimulates the release of renin and ultimately the production of angiotensin which vasoconstricts the afferent arteriole and reduces SNGFR, producing oliguria and azotemia. They suggest that the decreased blood flow is a further stimulus for renin release, and the cycle is perpetuated. Alternatively, an unknown hormone may directly stimulate the juxtaglomerular apparatus to release renin (Brubacher & Vander, 1968).

Dehydration, which increased renin levels, enhanced the failure induced by glycerol in rats (Oken, et al., 1966; Thiel, et al., 1967). Wilson, et al. (1969) suggested that this was due to the increase in urine osmolality which resulted in a decreased flow through the distal tubule. To test this they used the Battleboro strain of rats with hereditary Diabetes insipidus to study the effect of dehydration on glycerol-induced ARF. They believed that if the

primary effect of dehydration on the ARF kidney was mediated through the urine concentrating mechanism or the release of antidiuretic hormone, then rats under control conditions which excrete a maximally dilute urine in volumes equal to 80% of their body water every day, should be protected. They found that the rats were not protected, and the functional and structural lesions seen were typical of those found in normal rats with glycerol-induced renal failure. Therefore, contrary to the opinion of Oken (1973b), the status of water balance cannot be involved.

Renin depletion by chronic sodium loading offers some protection against production of renal failure (Henry, et al., 1968; McDonald, et al., 1969; Thiel, et al., 1970; DiBona, et al., 1971; Chedru, et al., 1972; Ryan, et al., 1973). Flamenbaum, et al. (1971) found that salt loading protected the rat from acute renal failure but not acute tubular necrosis. DiBona, et al. (1971) agreed with this but never measured renin activity, so it is difficult to determine how effective their regime was in depleting renin. In all the above studies, the GFR remained below normal despite alterations in the renin level. If peripheral renin stores are depleted as a result of acute volume expansion, kidneys are not protected from renal failure according to Thiel, et al. (1970). Pretreatment with desoxycorticosterone acetate (DOCA), which depletes plasma renin and perhaps intrarenal renin (Bailey, et al., 1973) was

effective in suppressing PRA but did not protect rats from renal failure induced with mercury (Flamenbaum, et al., 1973). It was suggested that the pretreatment had not effectively depleted intrarenal renin stores, but this was not supported experimentally.

Intrarenal arterial infusion of another salt, potassium chloride, in normal dogs inhibited plasma renin activity and renal renin concentration, but had no effect on blood pressure or glomerular filtration rates (Vander,1970; Flamenbaum, et al., 1975). Unlike most vascular beds, the renal bed does not dilate in response to potassium chloride (Flamenbaum, et al., 1975). Flamenbaum, et al. (1973) found that potassium loading affords comparable protection to sodium loading presumably by suppression of renal renin content, although they could not exclude the possibility that plasma volume expansion during salt loading contributed to the amelioration of the failure. It has since been reported that plasma volume expansion has no effect on the level of AORF produced, or on the renin levels (Thiel, et al., 1976).

A direct action of exogenous angiotensin to produce renal failure has been attempted, but with variable results. Angiotensin, when given as an infusion (Carriere & Friborg, 1969), or in an intravenous bolus of 0.5 to 4.5 ug/kg to dogs, had a direct action on the vascular smooth muscle to reduce RBF, GFR, urine flow rate and increase the filtration fraction

(Elkin, et al., 1966). However, intrarenal arterial infusion of 7.5 µg/kg b.wt./min to dogs produced tachyphylaxis in the renal vascular bed after 15 minutes of infusion (Fung, 1972). Hollenberg, et al. (1975) reported that the renal interlobar and arcuate arteries in dog and man were relatively insensitive to angiotensin. In rabbits, Gavras, et al. (1971) reported that infusions of angiotensin (0.9 to 1.8 µg/kg b.wt./min) for 72 hours did not produce tachyphylaxis, but produced ARF without ATN. In another study with rabbits, angiotensin infusion for 24 hours (0.5 to 0.9 µg/kg/min) produced acute renal failure lesions comparable to those seen after glycerol. Dach and Kurtzman (1976) infused angiotensin to rats concurrently receiving glycerol. They found that angiotensin aggravated the renal failure, and suggested that this was evidence for the hypothesis that angiotensin plays a role in the pathogenesis of ARF.

If angiotensin is the agonist responsible for the reduced renal blood flow and depressed glomerular filtration, then competitive antagonism of angiotensin II should block or reverse the failure. 1-sarcosine-8-alanine angiotensin II (P113) blocks the pressor action of exogenous angiotensin by competitive antagonism with no agonistic action in rats (Brunner, 1971). Use of this antagonist has had no effect on the severity of renal failure produced in rats (Powell-Jackson, et al., 1973; Shapira, et al., 1976). Ishikawa and Hollenberg (1976) reported that P113 was effective in blocking the renal

vascular response in renal failure, but had no effect on the depressed renal function. Use of the converting enzyme inhibitor, SQ 20881, was also ineffective in improving renal function in kidneys in AORF although it was effective in blocking the pressor response to exogenous angiotensin (Powell-Jackson, et al., 1973; Ishikawa & Hollenberg, 1976).

Immunization against A II was first reported to protect rats from renal failure (Powell-Jackson, et al., 1972). Rats were immunized against A II by using an A II antiserum, and then the protective effect of the lack of circulating angiotensin was determined in glycerol-induced renal failure. It was found that those rats protected with the antiserum did not get renal failure, that is there was no elevation of the BUN, although they were in ATN, as were those rats which had not received the immunization. Subsequent reports indicated that neither active nor passive immunization to renin or to angiotensin protected rats from AORF (Flamenbaum, et al., 1972b; Powell-Jackson, et al., 1973; Matthews, et al., 1974; Oken, et al., 1975a).

It has been suggested that the renal vasculature is hypersensitive to angiotensin in renal failure; local angiotensin conversion can be enhanced without a corresponding increase in measureable renin activity; renin is not important in the maintenance of AORF (Oken, 1973a). PRA did not increase in rats in which failure was induced by folate (Helmchen, et

al., 1972). Propranolol, which decreases PRA in control and thrombin-treated rats did not affect the functional or morphological aspects of failure produced (Rammer & Stahl, 1976).

Oken, et al. (1975) studied rats in which AORF was induced by either an initial challenge with glycerol or mercury. Five days later the rats were rechallenged with the agent different from that used to induce the initial failure. They found that the rechallenge did not produce a subsequent increase in BUN comparable to that seen initially. The renal renin titer did not change significantly throughout, therefore suggesting that renal renin depletion was not involved in protection of the kidney from the second challenge. They suggest that their data could be explained by a non-homogeneity of renin production which could result in the visible lack of change in the renin titer, but with differences in renin produced by filtering or non-filtering nephrons. Alternatively, there could be an impaired responsiveness of the renal vasculature after the initial failure.

Prostaglandins in the kidney are produced in the medulla and metabolized by 15-hydroxydehydrogenase in the cortex (Oken, 1975b; Anderson, et al., 1976). One hypothesis for the renal physiological role of prostaglandins has been discussed by Oken (1975b). Under normal conditions, perhaps as the result of a vasoconstrictor stimulus, prostaglandins are

released from the medullary parenchyma, enter the Loop of Henle and travel through the distal tubule to the macula densa. There they may act to diminish the vasoconstriction, and therefore help maintain normal glomerular filtration. If the prostaglandin delivery to the macula densa is reduced, either as the result of decreased production, or a decreased stimulus for release, then the control mechanism is removed, and vasoconstriction can be prolonged. Reduced GFR and oliguria would result, and could persist in spite of reversal of the precipitating factor(s) (Fine, 1970; Held, 1976; Romero, et al., 1977).

This hypothesis has some experimental support. Prostaglandins of the E series were released when noradrenaline or angiotensin II was infused into the dog renal artery (Lonigro, et al., 1973). Indomethacin, an inhibitor of prostaglandin synthesis, decreased blood flow in addition to decreasing the concentration of prostaglandins in the venous effluent. It also has been suggested that prostaglandin release may be responsible for tachyphylaxis to angiotensin (Gryglewski & Ocetkiewicz, 1974).

It has been suggested that the maintained decrease in RBF and GFR in AORF is the result of either a decrease in prostaglandin levels or a renin-prostaglandin imbalance (Oken, 1975b). Gerhard and Mulrow (1974) found that the level of PGA was reduced in rats in AORF. However, because

indomethacin did not alter the severity of failure although it further decreased PGA levels, they suggested that prostaglandins were not involved in the maintenance of the failure. Torres, et al. (1974b) found that renal prostaglandin release increased the severity of failure in rabbits, but not rats (Torres, et al., 1974a; Solez, et al., 1976). The effect of indomethacin involvement in renal failure in a dog model of renal failure has not been reported, although Moskowitz, et al. (1975) reported that an infusion of PGE into the renal artery of dogs in noradrenaline-induced renal failure increased RBF but not GFR.

Although little is known about the factors which normally determine the intrinsic contractility of the renal vasculature, a lesion in the vascular smooth muscle has been suggested to be significant in AORF states. It is possible to dilate the vascular bed of the kidney in failure with acetylcholine (Hollenberg, et al., 1968; Fung, 1972; Malindzak, et al., 1972; Thomson & Fung, 1973; Newhouse & Hollenberg, 1974) and hydralazine (Ladefoged & Winkler, 1970). The abnormality in vessels in animal models which is seen arteriographically, disappears at autopsy. Furosemide dilates both the afferent and efferent arteriole and therefore increased RBF but decreased GFR (Stein, et al., 1973). Thomson & Fung (1973) reported that the maximum response to cholinergic stimulation was reduced in dogs in renal failure, and that this implied a change in intrinsic vascular tone rather than the presence

of an abnormal mediator. This was supported by Newhouse & Hollenberg (1974) who reported that a dose-related increase in renal blood flow occurred in dogs in failure. They did not believe that angiotensin or noradrenaline could be the mediators of the sustained vasoconstriction seen in AORF.

3. Defect at the Level of the Glomerulus

Experimental evidence is not wholly in support of the arteriole as the site of the primary determinant in initiation or maintenance of filtration failure in ARF. The preceding theories are considered untenable because of two major observations. There is much data which indicates that RBF may be at least one half of normal when there is filtration failure in established AORF both in human and in experimental models (Conn, et al., 1954; Goldberg, 1962; Braun & Lilienfield, 1963; Walker, et al., 1963; Reubi, et al., 1966; Jaenike, 1967; Retik, 1967; Biber, et al., 1968; Mende, et al., 1972; Chedru, et al., 1972; Steinhausen, et al., 1973; Arendshort, et al., 1974; Eisenbach, et al., 1974; Gottschalk, et al., 1974; Lavender, et al., 1975; Oken, 1975a; Blantz, 1975; Best, et al., 1976; Kurtz, et al., 1976; Oken, 1976; Hsu, et al., 1976a; Ishikawa & Hollenberg, 1976; Churchill, et al., 1977). Churchill, et al. (1977) have suggested that this may be a species difference which could render any rat model inappropriate in relation to clinical renal failure. However, not all the preceding observations pertained to rats.

RBF can be increased with pharmacological agents like acetylcholine, furosemide or prostaglandin E, but these did not improve filtration (Mende, et al., 1972; Levy, 1973; Eliahou, et al., 1973a; Lavender, et al., 1974; Newhouse &

Hollenberg, 1974; Greven & Klein, 1976; Moskowitz, et al., 1975). These data could be explained hemodynamically by selective vasodilation of efferent over afferent arterioles, thereby decreasing transcapillary hydrostatic pressure, or by an abnormality in the glomerulus which would reduce filtration independent of blood flow effects (Oken, 1975a).

The rate of filtration through the glomerular capillary bed is the product of the ultrafiltration coefficient, K_f, and the differences in pressures supporting (P_{GC}) and opposing (P_T & π_{GC}) solute transport. This can be represented by:

$$\text{Filtration} = K_f \left(P_{GC} - P_T - \pi_{GC} \right)$$

where P_{GC} is the glomerular capillary hydrostatic pressure, P_T is the proximal tubule hydrostatic pressure and π_{GC} is the oncotic pressure of the blood in the capillary. K_f is the product of the surface area of the glomerulus and a permeability factor, the hydraulic conductivity (Gertz, 1966; Cox, et al., 1974). Flanigan & Oken (1965) theorized that a decrease in glomerular permeability could not produce a significantly reduced SNGFR without damage that would be visible under electron microscopy, and they knew of no evidence to indicate any morphological changes in the glomerulus. This was corroborated with clinical biopsy material (Dalgaard & Pedersen, 1959) and rat histological material (Dach & Kurtzman, 1976). However, recent transmission electron microscopy evidence indicates that the normal epithelial foot processes

are disrupted (Cox, et al., 1974). Scanning electron microscopy indicates that the podocytes are disrupted and cell integrity appears lost in glomeruli from dog kidneys in failure (Cox, et al., 1974; Stein & Sorkin, 1976). These changes, however, are not consistently seen in the clinical situation (Meyers, et al., 1977).

Cox, et al. (1974) suggested that their data could be best explained on the basis of a decrease in K_f, or, less likely, an extreme reduction in the mean glomerular capillary hydrostatic pressure. Kurtz, et al. (1976) studied rats in glycerol-induced renal failure and came to the same conclusion.

The mechanism by which a decrease in the glomerular ultrafiltration coefficient could be effected is to date not established. A decrease in surface area would be possible if afferent-to-efferent arteriolar shunts exist. Retik (1967) uses this unsubstantiated hypothesis to explain his data of decreased GFR. Hobbs, et al. (1976) give direct evidence that in glycerol-induced renal failure in rabbits there is vasoconstriction of the artery and arteriole. They looked at renal tissue grafts established in the rabbit ear chamber. When angiotensin II, noradrenaline or adrenaline were infused into the tissue graft, the renal vasculature, but not the ear chamber vasculature responded. Angiotensin first constricted the arterioles, and then the larger arteries. Cate-

cholamines caused arteriolar vasoconstriction with venous stasis. They saw no evidence of thrombi or intrarenal edema. They show photographs in which it can be seen that blood was shunted through the base of the glomerular tuft so that red blood cells passed directly from the afferent to the efferent arteriole. Ljungqvist and Wagermark (1970) on the contrary saw no anatomical connection from the afferent to efferent arteriole in normal rat kidney after fixation. O'Dorisio, et al. (1973) say that there is no shunt in dogs because they injected 15 μ microspheres, and even with acetylcholine or hemorrhage, none of the microspheres travelled further than the glomerulus (95% in glomerulus, 4% in the afferent arteriole). Balint and Szocs (1976) report evidence of a shunt in dogs after failure was induced by ischemia.

To date, support for a pathological decrease in glomerular permeability is based on the exclusion of the other parameters of glomerular capillary filtration. Either a contraction or proliferation of the mesangium may be involved. Glomeruli have been isolated from rat and rabbit kidneys (Brown, et al., 1965; Hornych & Richet, 1977). The glomeruli appear to contract in response to exogenous application of angiotensin, presumably by a direct effect on the glomerular capillary or contraction of the mesangium. Blantz (1975) came to the same conclusion in a micropuncture study in rats, but said that there was no smooth muscle in the glomerulus

to produce this contraction. This may not be correct. Michielson (1961; cited by Galbraith, 1971) said the mesangial cells in the glomerulus were similar to smooth muscle. Galbraith (1971) found that mesangial mass increased in the frog, Rana catesbiana, given uranyl nitrate. The vascular loops were occluded, both in this study, and in that reported in rats by Suzuki and Mostofi (1970a; 1970b; 1970c).

II. Statement of the Problem

Human acute oliguric renal failure is unpredictable in its occurrence. Because of this, as well as uncertainties in early diagnosis and lack of accurate non-invasive techniques for human studies, numerous experimental models have been developed. Results from investigative studies in animal models have yielded conflicting findings, and, to date, there is still no consensus about the pathophysiology of acute renal failure.

In the current study an attempt has been made to develop a system for the isolated perfusion of kidneys which would be stable in a cardiovascular sense, would demonstrate physiologic responses to vasoactive agents and would lend itself to the study of the pathogenesis of renal failure. The isolated perfusion system, separating the kidney from concurrent neural and hormonal influences, would possibly distinguish intra- from extra-renal factors pertaining to the filtration failure and the increased vascular resistance.

III. Choice of Experimental Model

A. The isolated perfusion system.

Under ideal conditions an isolated perfused kidney might be expected to maintain a vascular resistance within the physiological range, show evidence of maintained tubular integrity, and be capable of resuming homeostatic function when a donor's blood supply is re-established. Unfortunately, there are few studies reported which even approximate these ideal requirements. Blood perfused, isolated kidneys have a high resistance to flow (Bainbridge & Evans, 1914; Starling, et al., 1961) which may increase (Belzer, et al., 1968; Starling, 1973) or decrease (Nizet, et al., 1967; Gagnon, et al., 1974) with time.

As well, an early loss of tubular function with associated tubular necrosis occurs (Bahlman, et al., 1967). In spite of less than optimum conditions, Barkin and coworkers (1968) developed a blood perfusion system that they reported could be used for physiological studies, as did many others including Nizet, whose classic papers on the physiology of the isolated dog kidney were reviewed in 1975. Nizet, to date, has the greatest success in perfusing kidneys with blood.

Blood perfused kidneys are not completely isolated from extrarenal factors, and various studies have been directed towards perfusion with a fluid that has a more defined composition. The first of these, recently purported for physiological and not preservation purposes, was that of Belzer, et al. (1968) who used a plasma perfusate with or without dilution with an electrolyte solution.

With this perfusate they report a stable resistance to flow, unlike most of the earlier studies. Their perfusate has become a standard, and has been used in hypo- and normothermic studies such as those by Kulatilake (1967) and Starling, et al. (1973). The resistance to flow, although stable, is high and variable among the studies.

Numerous attempts have been made to develop a totally cell free perfusate. The most definitive study to date is that reported by Pegg and coworkers in a series of papers (Pegg, 1970; 1971; Pegg & Farrant, 1969; Pegg & Green, 1973; Fuller & Pegg, 1976; Fuller, et al., 1977). They have reported the development of a reliable isolated perfusion system for rabbit kidney perfusion at 37^{o}C for 120 minutes. They used an electrolyte solution similar to that of rabbit plasma, and varied the colloids to determine which resulted in the most acceptable glomerular filtration rates. In their preparation, the histological integrity was maintained, and the performance in general, was reproducible. Failure

to survive with extended perfusion has been suggested to be due to inadequate oxygen supply or accumulation of metabolic products (Rosenfield, et al., 1959; Pegg & Green, 1973), damage to the vascular system because of lack of platelets (Danielli, 1940; Gimbron, et al., 1969; Wexler, et al., 1971), or DNA autolysis (Belzer, et al., 1968).

In the current study both blood perfusion and cell-free perfusion were performed. Dogs were chosen because their overall renal function resembles that of man, because laboratory personnel were familiar with canine surgical procedures, and because a reproducible model of in vivo acute renal failure had previously been developed in the laboratory (Fung, 1972).

B. The model of noradrenaline-induced acute renal failure in the dog.

Intravenous infusions of noradrenaline in man have been associated with increases in blood pressure, and decreases in renal plasma flow, GFR, and urine flow rate (Marson, 1956). The first indication that an exogenous catecholamine could produce renal failure experimentally was reported in 1940 by Penner and Bernheim. They found that intraperitoneal adrenaline injected in dogs produced functional and histologic changes consistent with ARF in man.

Intra-arterial infusions in the dog (Hatcher, 1962; Fung, 1972; Knapp, et al., 1972) also produce renal lesions similar to those seen clinically. The latter studies, in which noradrenaline was given into a single renal artery, resulted in the production of a unilateral model of acute renal failure, with the contralateral kidney continuing to function within normal limits. Knapp, et al. (1972) followed dogs through to recovery and reported that the model resembled the human situation. The model, therefore, appeared appropriate for further studies into the mechanisms of acute renal failure.

IV. Purpose and Scope of the Study

In summary, the purpose and scope of this study has been:

a) to test the feasibility of studying the physiological responses of the isolated, blood perfused dog kidney;

b) to design a 37°C isolated, cell-free perfusion system with which dog kidneys would function within acceptable physiological limits for a restricted period of time;

c) to test pharmacologically the reactivity of the vascular smooth muscle in the isolated kidney;

d) to determine if the hemodynamic and pharmacologic performance of the isolated kidney in which noradrenaline-induced renal failure had been produced varied from that of its contralateral control;

e) to suggest the possible mechanisms of maintenance of AORF on the basis of the experimental findings.

MATERIALS AND METHODS

I. Surgical Removal of Kidneys from the Donor Dog

A. Preparation of the animal.

Mongrel dogs of either sex, weighing between 15 and 30 kg, which were fasted for 24 hours but allowed free access to water, were used. The dogs were anaesthetized with sodium pentobarbital (33 mg/kg) administered intravenously, and were intubated with a cuffed McGill endotracheal tube. Each was ventilated mechanically throughout the experimental time period with a Harvard^{R} constant volume respirator.

The left brachial artery was cannulated and the arterial blood pressure monitored with a Statham^{R} P23A pressure transducer. Cannulation of the left brachial vein allowed for fluid replacement, additional anaesthetic delivery and constant infusion of 19.4 mg/min creatinine. The cannulae were kept patent with isotonic heparinized saline. Cardiac rhythm was monitored using a bipolar electrocardiogram. All data were recorded with a Grass^{R} Model 5D Polygraph.

B. Flank removal of kidneys.

The kidneys were approached retroperitoneally in the initial experiments. The ureter was cannulated and the renal vessels freed. Immediately prior to renal manipulation, the dog received 10 g mannitol and 5,000 I.U. heparin intravenously. The vessels were ligated and cut distal to the ligation. The kidneys were freed, trimmed of excess perirenal fat and each renal artery cannulated. They were then flushed with 100 ml of either 4° C or 37° C perfusate, and attached to the perfusion apparatus. Kidneys with multiple renal arteries or with early bifurcations were not included in this study.

This procedure resulted in considerable manipulation of the renal vessels and associated nerves prior to the excision of the kidney. The warm ischemic time was from three to eight minutes. The manual flush with saline or buffer appeared to successfully remove all blood from the kidney, but the relatively low flow rates regardless of type of perfusate necessitated an alteration in procedure.

In ten early experiments the renal vein was cannulated. This procedure increased venous pressure, the kidneys became grossly swollen, and the renal vascular resistance was extremely high. When the procedure was stopped the venous outflow drained by gravity and the preparation was stable

although flow rates remained low.

C. Abdominal removal of kidneys.

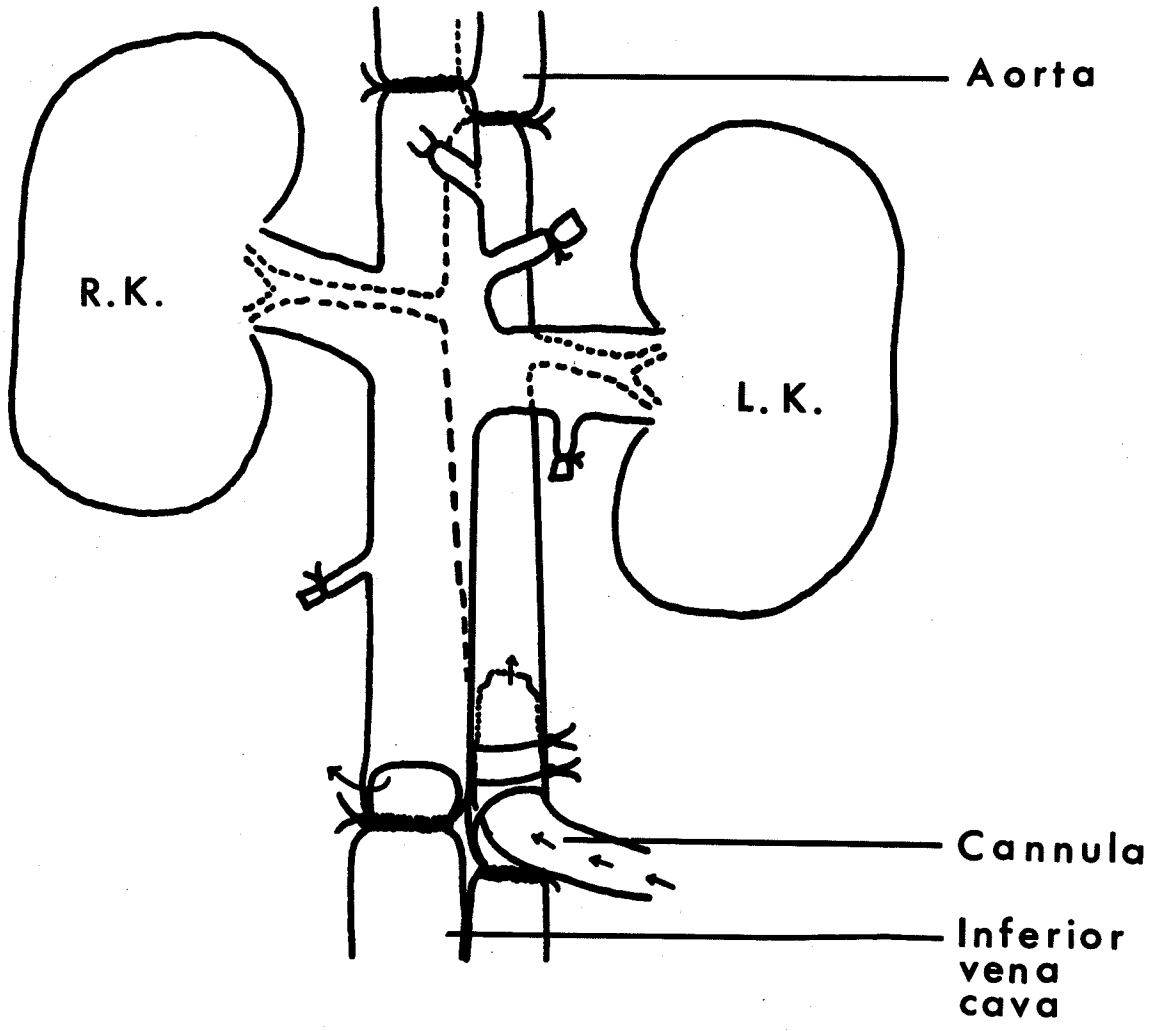
In 64 experiments the kidneys were exposed by a mid-abdominal incision. The inferior vena cava and abdominal aorta were exposed above and below the level of the kidneys. Ligatures were placed around the vessels. The gonadal veins, the left phrenico-abdominal vein and the mesenteric artery were ligated and cut. The renal vessels were not manipulated. Prior to occlusion of the major vessels the dogs received 10 g mannitol and 5,000 I.U. heparin intravenously. The distal end of the aorta was then cannulated and the proximal aorta was ligated between the coeliac and superior mesenteric arteries. The proximal vena cava was ligated at the level of the aortic ligature, then the distal vena cava was ligated and cut. At the time that the vena cava was opened 4^{o} C saline flowed by gravity into the aorta via the aortic cannula. The warm ischemic time was less than two minutes. Figure 1 schematically illustrates the *in situ* flush.

After one litre of saline had flowed through the renal beds the left renal artery and vein were clamped and the left kidney was removed. Saline continued to flow to the right kidney while the artery and ureter were cannulated and the kidney placed in the perfusion apparatus. The right kidney was subsequently removed from the dog, the vessels cannulated and then also placed in the perfusion system.

Figure 1. Schematic diagram of the *in situ* flush with 4° C saline.

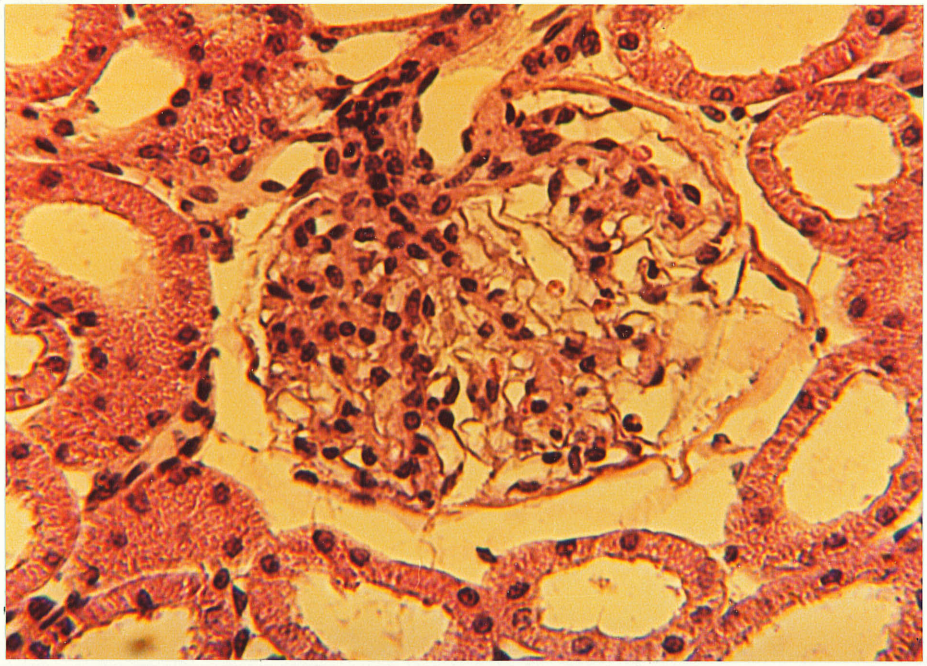
L.K. = left kidney

R.K. = right kidney


Aorta
R.K.
L.K.
Cannula
Inferior
vena
cava

The technique of abdominal removal of the kidneys reduced both the amount of trauma to the kidney and the warm ischemic times. The flush with 4^{o} C saline effectively removed all the blood from the renal circuit as well as cooling the kidney prior to severe manipulation. Plate I is a photomicrograph of a kidney taken immediately after being flushed with saline. Blood has effectively been removed from the glomerulus and the vasculature. The tubules appear normal.

Plate I. Histological section of a kidney which was flushed with 4^{o} C saline. Blood is effectively removed from the glomeruli and vasculature (H & E; 500X).



II. An in vitro System for Renal Perfusion at 37° C

A. Perfusion apparatus.

1. In vitro Perfusion using Autologous Whole Blood

Initial experiments (N = 26) utilized the donor dog as part of the perfusion system. The kidney was removed following the technique described earlier (see I. B and I. C). The right femoral artery and right jugular vein of the donor dog were cannulated. Blood flowed from the femoral artery into the renal artery and through the kidney. The blood drained by gravity from the uncannulated renal vein into a silastic funnel. The funnel was connected to silastic tubing which was emptied by a Travenol^{R} blood pump with a variable speed control. The blood pump forced blood through a warming coil which was placed in a water bath at 37° C, then the blood was returned to the dog via the jugular vein. The dog's blood pressure, body temperature and cardiac electrical activity were continuously monitored. The experiment was terminated after three to five hours of perfusion and sections of the kidneys were excised for subsequent histological examination.

2. In vitro Perfusion using a Modified Gambro^{R} Perfusion Apparatus

A Gambro^{R} perfusion apparatus (Type PF2-C #88) was modified for the purpose of perfusing kidneys at 37^{o} C. The apparatus was designed to perfuse two kidneys simultaneously from a common reservoir using separate pumps. The design of the system is illustrated schematically in Figure 2.

The kidney rested on its side in a closed chamber with cannulae in the renal artery and ureter. Venous and ureteral effluent flowed freely and bathed the kidney, then flowed by gravity to a reservoir. The fluid in the reservoir was aerated with 95% O_2 - 5% CO_2 resulting in a stable pH of 7.35 to 7.40. The pO_2 levels in the perfusate which reached the kidney were 460 - 480 mm Hg. A pulsatile pump forced the perfusate from the reservoir through a Swank^{R} transfusion filter. Insertion of the filter in the circuit ensured that debris larger than 10 μ would be removed effectively, as indicated by the stable pressure-flow relationships. From the filter, the fluid passed through a heating coil placed in a constant temperature bath. The temperature of the bath was regulated so as to maintain the perfusate at 37^{o} C. The fluid then passed through a bubble trap and returned to the kidney via the renal artery. A Statham^{R} P23A pressure transducer was used to monitor the perfusion pressure. A multi-injection rubber connector was placed in the circuit distal

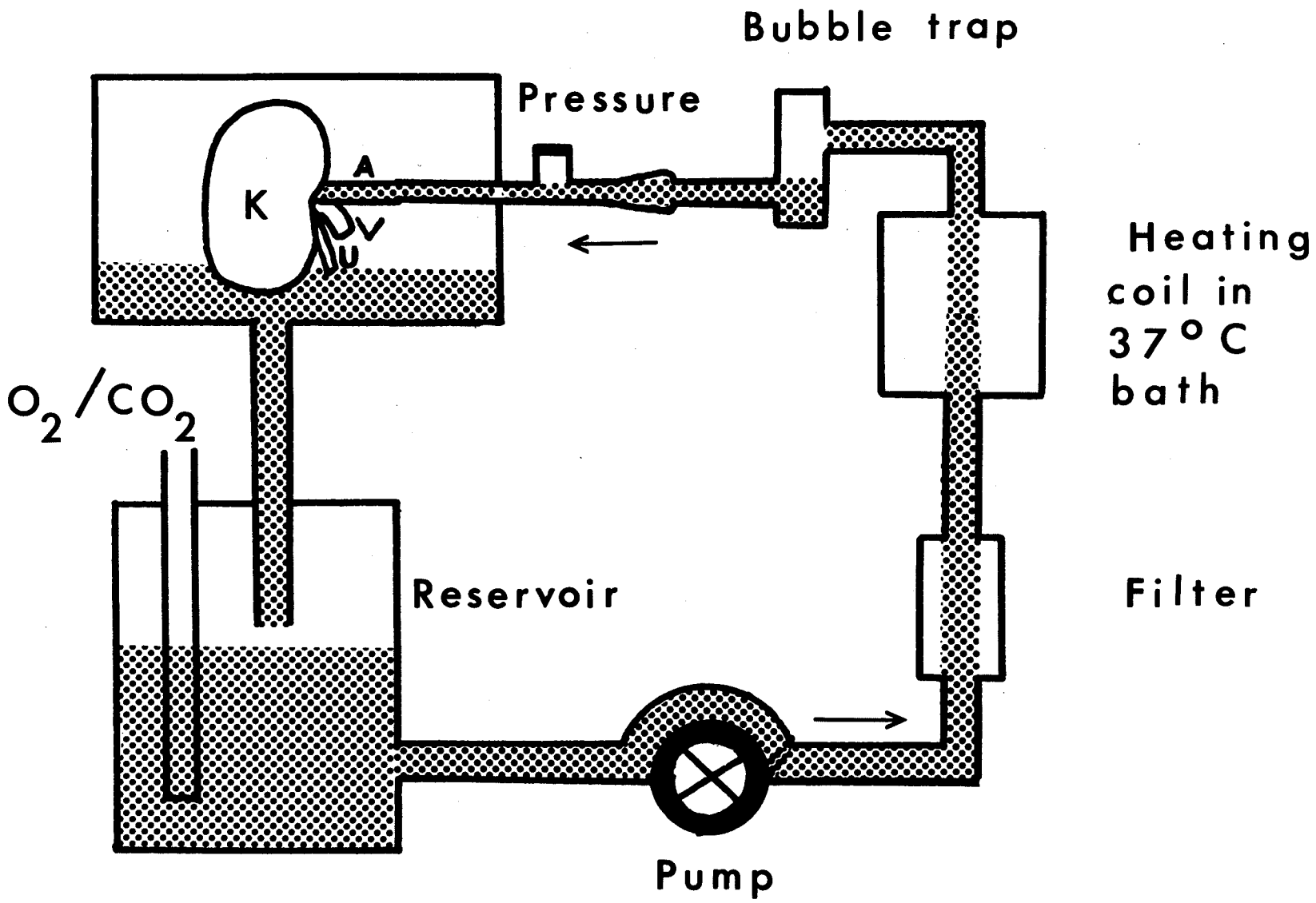
Figure 2. The experimental perfusion system. The arrows indicate the direction of perfusate flow.

K = kidney

A = renal artery

V = renal vein

U = ureter


Bubble trap
Pressure
K
A
V
U
Heating coil in 37°C bath
O_2/CO_2
Reservoir
Filter
Pump

to the pressure transducer. It was possible to repeatedly inject solutions into the artery through this connector.

The circulating fluid volume was 2 litres and the circuit time was 2½ minutes. By manipulating the pump it was possible to vary the pulsatile flow from 50 to 475 ml/min.

The technique of inert gas washout (Thorburn et al. 1963) was used to measure the relative rate of blood flow through the kidney. Xenon was radiolabelled, and injected in a bolus into the renal artery. A scintillation probe, placed directly above the kidney measured the exponential decrease in radioactivity as the gas travelled through the kidney. The disappearance curve was separated into a series of exponentials that have been correlated by autoradiography to anatomical sites in the kidney (Carlsson and Sparks, 1970; Goluboff et al., 1969; Pedersen et al., 1969; Jones and Herd, 1974; Ladefoged et al., 1975). Components I, II, III, and IV are generally accepted as representing flow to the outer cortex, inner cortex, inner medulla and perirenal fat, respectively.

B. Artificial perfusates.

1. Phosphate Buffer

Preliminary experiments were conducted using a modification of the phosphate buffer system described by Cross and Taggart (1950). The buffer contained 151 mEq/l sodium, 4.0 mEq/l potassium, 130 mEq/l chloride, 40.4 mEq/l phosphate, 5.0 mEq/l calcium and 2.0 g/l glucose. The perfusate was oxygenated with 100% oxygen.

2. Bicarbonate Buffer

Experiments were routinely done using a modified Krebs-Henseleit bicarbonate buffer (K.H.B). This contained: 154 mEq/l sodium; 4.2 mEq/l magnesium; 4.0 mEq/l potassium; 26.3 mEq/l bicarbonate; 130 mEq/l chloride; 8.3 mEq/l HPO_4; 5.0 mEq/l calcium; 0.05 g/l creatinine; 10.0 g/l mannitol; 2.0 g/l glucose.

In calcium free experiments the calcium chloride was omitted and 1.14 g/l [Ethylenebis(oxyethylenenitrilo)] tetra-acetic acid (EGTA) was added to the perfusate.

High molecular weight additives were included in the perfusate of forty-one experiments. In nineteen experiments dextrans were added. We used: 4 g% dextran of high molecular weight (200,000 to 245,000); 4 g% dextran of molecular weight 70,000 (D T 70); 4 g% dextran of molecular weight 75,000 (6 % s). In five experiments 37.5 g/l human serum albumin (outdated for clinical use) was included instead of the dextrans. Bovine serum albumin (Fraction V), 37.5 g/l was used in seventeen experiments.

C. Performance of kidneys *in vitro*.

1. General Protocol

The kidney was placed in the perfusion apparatus and the flow rate of the perfusate to the kidney was adjusted so that the resulting perfusion pressure was 100 ± 5 mm Hg. The preparation was then allowed to stabilize for fifteen to thirty minutes. If the perfusion pressure could not be stabilized or if the flow rate was less than 100 ml/min the kidney was discarded from the study. Once the preparation was stable the flow rate was not again mechanically altered so that all further measurements were conducted in a constant flow system. In preliminary experiments ^{133}Xe washout analysis was used to measure the perfusate flow distribution. This procedure was eliminated from the study because of progressive accumulation of background activity.

After the stabilization period urine samples were collected and aliquots of perfusate and urine were saved for analysis. The responses of the renal vasculature to various chemicals were then studied.

Neither steroids nor antibiotics were added to the perfusate, and the experimental time period was limited to a maximum of 120 minutes. The kidney was weighed at the termination of the experiment. Ink was injected into the renal

artery to assess uniformity of perfusion. Renal tissue was excised for light microscopal examination.

Kidneys from twenty-seven dogs that were perfused initially *in vitro* with phosphate buffer were returned to the dog for blood perfusion. The clinical procedure has been described earlier (see II.A.1.). Times for blood perfusion varied from 60 to 240 minutes.

2. Analytical Procedures

Renal vascular resistance was calculated as the ratio of mean perfusion pressure (mm Hg) to flow rate (ml/sec/g kidney weight). Flow rates to the kidney were determined from direct measurements of delivery to the renal artery.

Urine output (ml/min) was determined directly and corrected for kidney weight. pO_2 and pH of the perfusate were monitored at random intervals.

Perfusate and urine samples were analysed for: creatinine (mg/100 ml); sodium (mEq/l); potassium (mEq/l); calcium (mEq/l); osmolality (mOsm/kg). Creatinine concentration was determined by the procedure of Folin and Wu using a Technicon^R Auto Analyzer^R and the automated assay developed by Stevens and Skeggs (1961). Sodium and potassium concentrations were determined by flame photometry using the Instrumentation Laboratory Inc. Flame Photometer (Model 343). Osmolality was measured by freezing point depression with a Fiske Mark III osmometer. Calcium levels were determined on a Perkin Elmer 303 Atomic Absorption Spectrophotometer.

Sodium excretion rate ($U_{Na}\dot{V}$, μEq/min/100 ml GFR), potassium excretion rate ($U_K\dot{V}$, μEq/min/100 ml GFR), creatinine clearance (C_{Cr} ml/min/100 g kidney weight) and per cent tubular sodium reabsorption of filtered sodium (% $Reab_{Na}$)

were calculated by conventional formulae. Statistical analyses used were as indicated in the individual experimental results.

3. Vascular Responses

i) Initial experiments with phosphate buffer

Initial experiments were designed to study the viability of the kidney in vitro using the phosphate buffer system. Noradrenaline, to a final bath concentration of 10^{-7} g/ml was added to the stable preparation and allowed to circulate. Phenoxybenzamine, 10^{-5} g/ml, was used as a noradrenergic antagonist.

ii) Experiments with bicarbonate buffer without high molecular weight additives

Preliminary experiments were designed to determine the possible improvement in this buffer system over the phosphate buffer system. Once the renal preparation was stable in the perfusion system, noradrenaline, to a total concentration of 10^{-7} g/ml, was added and allowed to circulate. After fifteen minutes of circulation phenoxybenzamine (10^{-5} g/ml) was added to the perfusate and the response noted.

In five experiments the kidney was perfused in a calcium-free buffer containing 3 mM EGTA. Noradrenaline, 4 mM calcium and phenoxybenzamine were added sequentially to determine the vascular requirement of calcium for contraction.

In all subsequent experiments the dose-response relationship to various agonists which act on the renal vasculature was then determined. The drugs were injected intra-arterially in graded doses through the multiple injection connector. The resultant change in pressure in response to the drugs was monitored on the Grass^{R} polygraph. Pressure returned to 100 mm Hg between each bolus.

The drugs tested in the system were: noradrenaline (NORA, l-noradrenaline bitartrate); angiotensin II (A II, Hypertensin^{R}, CIBA); acetylcholine (ACh, acetylcholine chloride); dopamine (DA, dopamine hydrochloride); parathyroid hormone (PTH, Parathormone^{R}, Lilly); serotonin (5-HT, 5-hydroxytryptamine creatinine sulphate); histamine (Hist., histamine acid phosphate); phenoxybenzamine (POB, phenoxybenzamine hydrochloride).

iii) Experiments with bicarbonate buffer containing 3.75 g% bovine serum albumin

The dose-response relationship to the same drugs as listed previously was determined in kidneys perfused with this buffer system, with the exception of parathyroid hormone and dopamine.

III. The Noradrenaline-induced Model of Acute Renal Failure

A. Animal preparation.

Mongrel dogs of either sex, which weighed between 15 and 30 kg, and which had been denied food for 24 hours but allowed free access to water, were used. The dog was anaesthetized with sodium pentobarbital (33 mg/kg) administered intravenously and was intubated with a cuffed McGill endotracheal tube. It was ventilated mechanically during the experiment with a Harvard^{R} constant volume respirator.

The left brachial artery was cannulated and the arterial blood pressure monitored with a Statham P23A pressure transducer. The left brachial vein was cannulated as a site for fluid replacement, anaesthetic addition and for infusion of 19.4 mg/min creatinine. Cardiac rhythm was monitored with an electrocardiogram.

An incision in the right flank was made and the ureter cannulated retroperitoneally. The incision was closed, and the animal placed on its right side.

The left renal vessels were exposed via an incision in the left flank. The ureter was cannulated and a Statham^{R} non-cannulating flow probe of the appropriate diameter was

placed around the renal artery. Total renal blood flow was measured by a Statham^{R} square-wave electromagnetic flowmeter (Model 0-5000). Calibration of the flow probes was effected using heparinized whole blood and a segment of common carotid artery or femoral artery in a bath of saline.

A polyvinyl catheter was inserted into the renal artery distal to the flow probe using a modification of the technique developed by Herd and Barger (1964). Blood flow determinations before and after placement of the catheter were identical.

The animal preparation was allowed to stabilize for thirty minutes before control blood and urine samples were collected.

B. General experimental protocol.

Control hemodynamic data were collected and blood and urine samples obtained for analysis. Noradrenaline, at an infusion rate of 0.4 to 4.0 μg/kg/min, calculated as the weight of the base, was introduced by a Harvard^{R} constant infusion pump to the left kidney via the polyvinyl catheter. The amount of noradrenaline required to keep total renal blood flow at zero was infused for 120 minutes. This quantity of noradrenaline also produced hypertension and, in most dogs, cardiac arrhythmias.

Urine samples were continuously collected over the two hour time period. Blood samples were analysed for hematocrit, and both were analysed for sodium, potassium and creatinine concentrations, and for osmolality. Analytical procedures, stated earlier in the methodology section (II.C. 2.) were used on these samples.

The noradrenaline infusion was terminated after 120 min. The animal was then monitored during four hours of stabilization. Blood and urine samples were collected and analysed.

After four hours of recovery, the kidneys were removed from the animal using one of the procedures outlined earlier.

IV. Performance of the Renal Vasculature in Acute Renal Failure

A. In situ.

In single experiments, the in situ vascular reactivity of the renal bed to three agonists was tested prior to and after acute renal failure was established. Parathyroid hormone was infused via the renal artery in an increasing rate from 0.97 to 9.7 U/min. Histamine was infused in increasing rates from 9.7 to 97.0 µg/min. Serotonin was infused at rates of 9.7 to 194 µg/min.

B. In vitro

After acute renal failure had been established in the experimental animals, the kidneys were removed in accordance with the protocol discussed earlier (Section I). The physiological status of the kidney in renal failure was compared to its contralateral control, and differences in the vascular reactivity studied, following the methodology discussed in Section II.C. The paired-t test was used for the statistical analysis.

R E S U L T S

I. Development of the Model

A. *In vitro* performance of isolated kidneys.

1. Autologous Whole Blood Perfusion

Initial experiments used autologous whole blood as the perfusate. Blood circulated between the dog and kidney by an extracorporeal circuit. This had no significant effect on the dog's blood pressure which remained at a comparable value prior to, and during, the extracorporeal circuiting of the blood. Table 2 lists these results as well as *in vivo* data from the *in situ* kidney and data from the kidney in the extracorporeal circuit after thirty and sixty minutes of perfusion. Mean renal blood flow fell progressively during blood perfusion. Flow was 317 ± 23 ml/min *in vivo*, 202 ± 18 ml/min *in vitro* at 30 minutes and 164 ± 42 ml/min at 60 minutes. These kidneys initially produced urine, but were anuric within sixty minutes.

There were no observable hemodynamic changes in the donor dog during the time of perfusion. Blood pressure and EKG patterns remained stable throughout. Body temperatures of two dogs monitored during the course of the experiments were maintained at 36^{o} and 37.5^{o} C respectively.

Table 2. Comparison of *in vivo* data to *in vitro* data from five kidneys perfused with autologous whole blood. Samples were collected *in vitro* thirty and sixty minutes after the beginning of the perfusion. N = 5.

	In vivo	*In vitro*	
		30 min	60 min
Dog mean Arterial Pressure, mm Hg	124 ± 2.8	123 ± 2.0	123 ± 2.0
RBF, ml/min	317 ± 23*	202 ± 18**	164 ± 42**
$\dot{V}$, ml/min	0.87 ± 0.11	0.60 ± 0.12	0.0
$U_{Na}\dot{V}$, μEq/min	76.2 ± 17.3	21.5 ± 4.2	0.0
$U_{K}\dot{V}$, μEq/min	26.3 ± 8.4	21.5 ± 1.3	0.0
U/P Osm, mOsm	2.1 ± 0.6	1.3 ± 0.1	0.0

Values are mean ± s.e.

* - flow measured by the electromagnetic flow probe.

** - flow measured by direct measurement.

The mean RBF determined from Xenon washout analysis (Table 3) was lower than that from direct measurement (188 ± 22 ml/min compared with 202 ± 18 ml/min after 5 minutes of perfusion, and 136 ± 38 ml/min compared with 156 ± 31 ml/min after ninety minutes of perfusion).

Within ninety minutes of autoperfusion the blood flow to Compartment I decreased from 57.0 ± 2.1 to 37.8 ± 4.6 % while the per cent flow increased in Compartment II from 38.5 ± 1.4 % to 57.4 ± 3.9 %.

Light microscopy of renal tissue obtained after the blood perfusion in vitro showed extensive tubular necrosis. There were no changes in the glomeruli and no evidence of intravascular clotting.

Because autologous blood perfusion was associated with early and progressive hemodynamic deterioration and with the development of tubular autolysis, the protocol was abandoned in favor of one using artificial perfusates.

Table 3. Intrarenal blood flow distribution in the isolated kidney perfused with autologous dog blood. N = 5. For comparison, values obtained *in vivo* from five dogs are also given.

	Time of Autoperfusion		*In vivo* for reference
	5 min	90 min	
Mean RBF, ml/min	202 ± 18	156 ± 31	317 ± 23
Hematocrit, %	44	44	43 2
Data derived from ^{133}Xenon washout analysis:			
Mean RBF, ml/min	188 ± 22	136 ± 38	352 ± 34
Per cent RBF:			
to C I	57.0 ± 2.1	37.8 ± 4.6	71.6 ± 2.8
to C II	38.5 ± 1.4	57.4 ± 3.9	24.3 ± 2.3
to C III and C IV	4.5 ± 0.5	4.8 ± 0.8	4.1 ± 0.3

Values are mean ± s.e.

2. Perfusion with a Phosphate Buffer

Kidneys from twenty-one dogs were removed via a retroperitoneal approach, flushed with 4^{o} or 37^{o} C buffer, then perfused for three to five hours *in vitro* with phosphate buffer. The kidney was then transferred back to the dog for autologous blood perfusion.

Table 4 shows that at similar perfusion pressures, renal perfusate flow was approximately one-half that of the same kidney *in vivo*, and was stable over ninety minutes. The initial *in vitro* urine flow rate was greater than *in vivo*, but was not sustained. Returning the kidney to blood perfusion did not change the anuric condition.

In initial experiments we tried to determine blood flow and intrarenal blood flow distribution using ^{133}Xenon washout while the kidneys were being perfused *in vitro*. Increasing background counts occurred with repetitive Xenon injections thus rendering this method unsuitable for the serial measurement of blood flow distribution.

In five kidneys the addition of a bolus of 10^{-5} g/ml noradrenaline to the phosphate buffer after thirty minutes of relatively stable perfusion produced a temporary increase in perfusion pressure. The pressure increased from 111 ± 1.7 mm Hg prior to noradrenaline addition to 218 ± 2.5 mm Hg

Table 4. Kidneys perfused with a phosphate buffer followed by autologous whole blood. Kidneys were perfused *in vitro* for 150 to 300 minutes, then blood perfused via the dog's femoral artery for 60 to 120 minutes. N = 21. The control values listed here were obtained from the kidney prior to its removal from the dog.

	Control	*In vitro*		*In situ*
	in vivo	30 min	90 min	
Mean arterial pressure of dog, mm Hg	127 ± 4*	-	-	90 ± 8*
Mean perfusion pressure, mm Hg	-	125 ± 3	125 ± 5	-
Mean blood flow, ml/min	321 ± 72	125 ± 10	150 ± 35	180 ± 120
$\dot{V}$, ml/min	1.1 ± 0.07	3.2 ± 1.0	0.0	0.0
$U_{Na}\dot{V}$, μEq/min	104.2 ± 67.2	654.1 ± 45.3	0.0	0.0
$U_{K}\dot{V}$, μEq/min	37.2 ± 8.1	29.9 ± 7.6	0.0	0.0

Values are mean ± s.e.

* - electromagnetic flow probe measurement.

within two minutes of the addition. The increase in pressure was sustained for more than ten minutes but after 120 minutes the average pressure had dropped to 124 ± 1.8 mm Hg. Noradrenaline assay indicated an average of 37.6 ± 1.4 µg/ml after 15 minutes and 29.3 ± 1.2 µg/ml after 120 minutes.

This protocol resulted in a preparation which had stable hemodynamics although the resistance to flow was high. Tubular integrity was not maintained but there was evidence for an intact and responsive vasculature. These functional observations were supported by light microscopy. The tubules, but not the vasculature, appeared necrotic after the six hours of perfusion.

3. Perfusion with a Modified Krebs-Henseleit Bicarbonate Buffer (K.H.B.)

Use of a Krebs-Henseleit buffer (K.H.B.) with or without calcium was associated with a stable pressure-flow relationship. In Table 5 the *in vitro* values are compared to *in vivo* values from 25 dogs that had been anaesthetized and had their ureters cannulated. The pressure-flow relationship was stable for 120 minutes of *in vitro* perfusion whether or not calcium was present. The resistance to flow, calculated as the ratio of pressure to flow, was consistently less *in vivo* than *in vitro*.

Kidneys were polyuric *in vitro* (11.5 ± 2.2 ml/min in K.H.B., 5.3 ± 1.4 ml/min in calcium-free K.H.B.) when compared to *in vivo* values of 0.78 ± 0.1 ml/min. GFR, measured by creatinine clearance was 30.1 ± 3.7 ml/min *in vivo*, and was reduced by one-quarter to one-half *in vitro*. Kidneys in K.H.B. had a C_{Cr} of 17.7 ± 5.1 within the first thirty minutes. This decreased slightly but not significantly over the next ninety minutes to 11.2 ± 3.6 ml/min. C_{Cr} in the calcium-free perfusate was 9.3 ± 1.5 ml/min in the first thirty minutes and did not change significantly in the next ninety minutes.

The urinary excretion rates of both sodium and potassium were significantly greater *in vitro* than *in vivo*. In K.H.B. the $U_{Na}\dot{V}$ was 1225 ± 266 μEq/min and stable, and the $U_{K}\dot{V}$

Table 5. *In vitro* performance of kidneys perfused with a modified Krebs-Henseleit buffer with or without calcium. 3 mM EGTA was added to the Ca^{++}-free perfusate.

	Control N = 25	*In vitro*, minutes of perfusion with Ca^{++} (N = 24)			*In vitro*, minutes of Ca^{++}-free perfusion (N = 13)		
		30	60	120	30	60	120
Mean perfusion pressure, mm Hg	139.3 ± 1.4	118.5 ± 3.7	124.4 ± 10.0	110.2 ± 3.0	109.5 ± 1.7	110.0 ± 4.9	110.3 ± 5.2
Mean flow rate, ml/min	390.5 ± 2.74	170.9 ± 13.6	176.9 ± 11.9	174.7 ± 14.0	157.4 ± 9.7	159.5 ± 10.3	159.4 ± 10.7
RVR, mm Hg - min ml^{-1}	0.36 ± 0.10	0.78 ± 0.08	0.70 ± 0.14	0.71 ± 0.14	0.72 ± 0.16	0.73 ± 0.13	0.74 ± 0.14
$\dot{V}$, ml/min	0.78 ± 0.1	11.5 ± 2.2	9.2 ± 0.9	6.6 ± 1.1	5.3 ± 1.4	6.8 ± 1.4	6.7 ± 2.3
C_{Cr}, ml/min	30.1 ± 3.7	17.7 ± 5.1	15.4 ± 2.9	11.2 ± 3.6	9.3 ± 1.5	10.1 ± 2.1	8.0 ± 2.1
$U_{Na}\dot{V}$, µEq/min	81.2 ± 12.5	1225 ± 266	991.8 ± 145	957.3 ± 147	650.7 ± 167	854.6 ± 183	967.8 ± 280
$U_K\dot{V}$, µEq/min	26.7 ± 2.7	56.9 ± 8.9	58.9 ± 6.8	43.2 ± 4.5	36.4 ± 9.4	45.1 ± 9.4	47.7 ± 14.0
% $Reab_{Na}$	98.0 ± 0.3	53.7 ± 5.8	52.2 ± 6.8	40.3 ± 5.4	45.8 ± 4.8	42.0 ± 4.2	28.8 ± 2.3
U/P Osm	2.2 ± 0.3	0.92 ± 0.03	0.93 ± 0.03	0.95 ± 0.02	0.98 ± 0.12	0.92 ± 0.03	1.22 ± 0.2

Values are mean ± s.e.

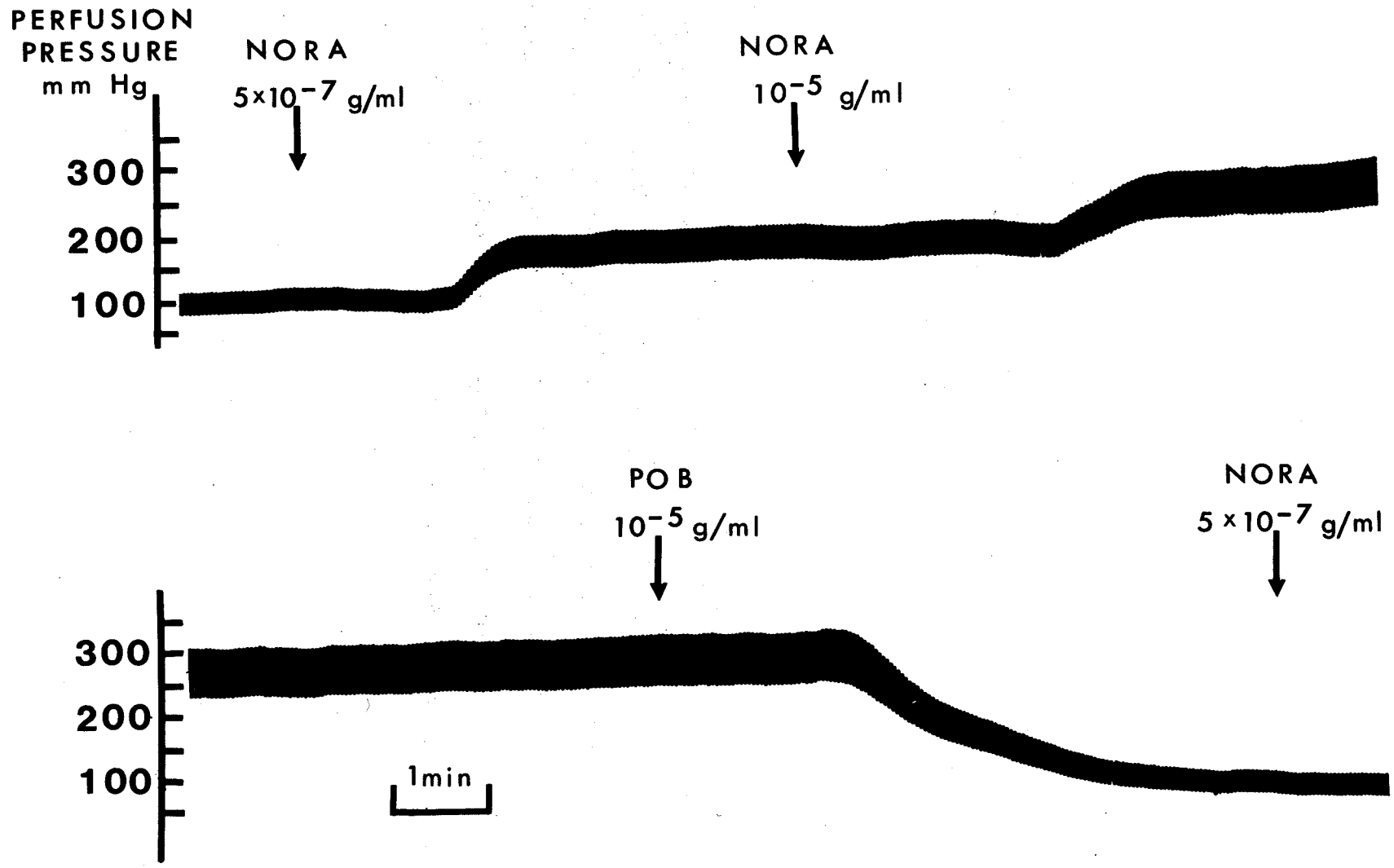
was 56.9 ± 8.9 μEq/min and stable. These values are much greater than the *in vivo* values of 81.2 ± 12.5 and 26.7 ± 2.7 for sodium and potassium respectively. Removal of the calcium from the perfusate did not affect the sodium and potassium excretion rates. They remained high when compared to the *in vivo* values. The $U_{Na}\dot{V}$ was 650.7 ± 167 μEq/min after thirty minutes and rose slightly in the subsequent ninety minutes. $U_K\dot{V}$ was 36.4 ± 9.4 μEq/min after thirty minutes and stable for 120 minutes of perfusion.

The kidney in the control situation was capable of reabsorbing 98.0 ± 0.3 % of the filtered sodium. The kidney in K.H.B. was capable of reabsorbing 53.7 ± 5.8 % of its filtered sodium, and when calcium was not present the capability of the kidney to conserve sodium was further reduced (45.8 ± 4.8 % to 28.8 ± 2.3 %).

The ratio of urine to plasma osmolality was 2.2 ± 0.3 in the control situation and near unity *in vitro*.

The vasculature of the kidney *in vitro* responded to noradrenaline with an increase in resistance which could be blocked by phenoxybenzamine. A typical recording is reproduced in Figure 3. Perfusion pressure was stable at 100 mm Hg at a flow rate of 185 ml/min. Addition of noradrenaline to the perfusate reservoir to a final concentration of 5×10^{-7} g/ml produced a rise in perfusion pressure to 170 mm

Figure 3. A portion of a continuous tracing which illustrates the change in pressure in the kidney *in vitro* in response to noradrenaline (NORA) and to phenoxybenzamine (POB). The drugs were added to the perfusate reservoir, and took two minutes to reach the kidney.


PERFUSION
PRESSURE
mm Hg
300
200
100
NORA
5×10⁻⁷ g/ml
NORA
10⁻⁵ g/ml
POB
10⁻⁵ g/ml
NORA
5×10⁻⁷ g/ml
1min

Hg. Further addition of noradrenaline to 10^{-5} g/ml produced a further increase in perfusion pressure to 275 mm Hg. Addition of 10^{-5} g/ml phenoxybenzamine blocked the pressor response to noradrenaline. The perfusion pressure returned to pre-noradrenaline values. Subsequent addition of noradrenaline had no effect. This response was typical of 8 kidneys tested (Table 6). The pressure rose to 172.5 ± 3.0 mm Hg from 114.0 ± 1.3 mm Hg when 5×10^{-7} g/ml noradrenaline was added to the perfusate. The pressure increased to 272.5 ± 3.0 mm Hg with a further addition of noradrenaline. The pressure dropped to 96.7 ± 2.3 mm Hg when phenoxybenzamine was added. There was no further response to noradrenaline.

Calcium was necessary for production of the vasoconstriction in response to noradrenaline as shown in Figure 4. This illustrates the typical response recorded in one of four kidneys tested. The perfusion pressure, stable at 100 mm Hg with a flow rate of 238 ml/min, did not change when noradrenaline was added to the perfusate which contained no calcium but which contained 3 mM EGTA. Addition of excess calcium chloride (4 mM) resulted in an increase in perfusion pressure in the presence of noradrenaline that could be blocked by phenoxybenzamine. Further additions of noradrenaline had no effect on the perfusion pressure. The average response of four kidneys is listed in Table 7. The initial pressure was 106.3 ± 1.4 mm Hg. After the addition of up to 10^{-5} g/ml NORA the pressure remained at 106.3 ± 1.4 mm Hg. After the

Table 6. Changes in perfusion pressure (mm Hg) in response to noradrenaline (NORA) and to phenoxybenzamine (POB). N = 8.

Control	Noradrenaline, final concentration, g/ml		POB, g/ml	NORA, g/ml
	5×10^{-7}	10^{-5}	10^{-5}	10^{-5}
114.0 ± 1.3	172.5 ± 3.0	272.5 ± 3.0	96.7 ± 2.3	96.7 ± 2.3

Values are mean ± s.e.

Figure 4. A portion of continuous tracing which illustrates the requirement of calcium for renal vasoconstriction *in vitro*. Noradrenaline (NORA), calcium chloride ($CaCl_2$) and phenoxybenzamine (POB) were added to the perfusate reservoir.

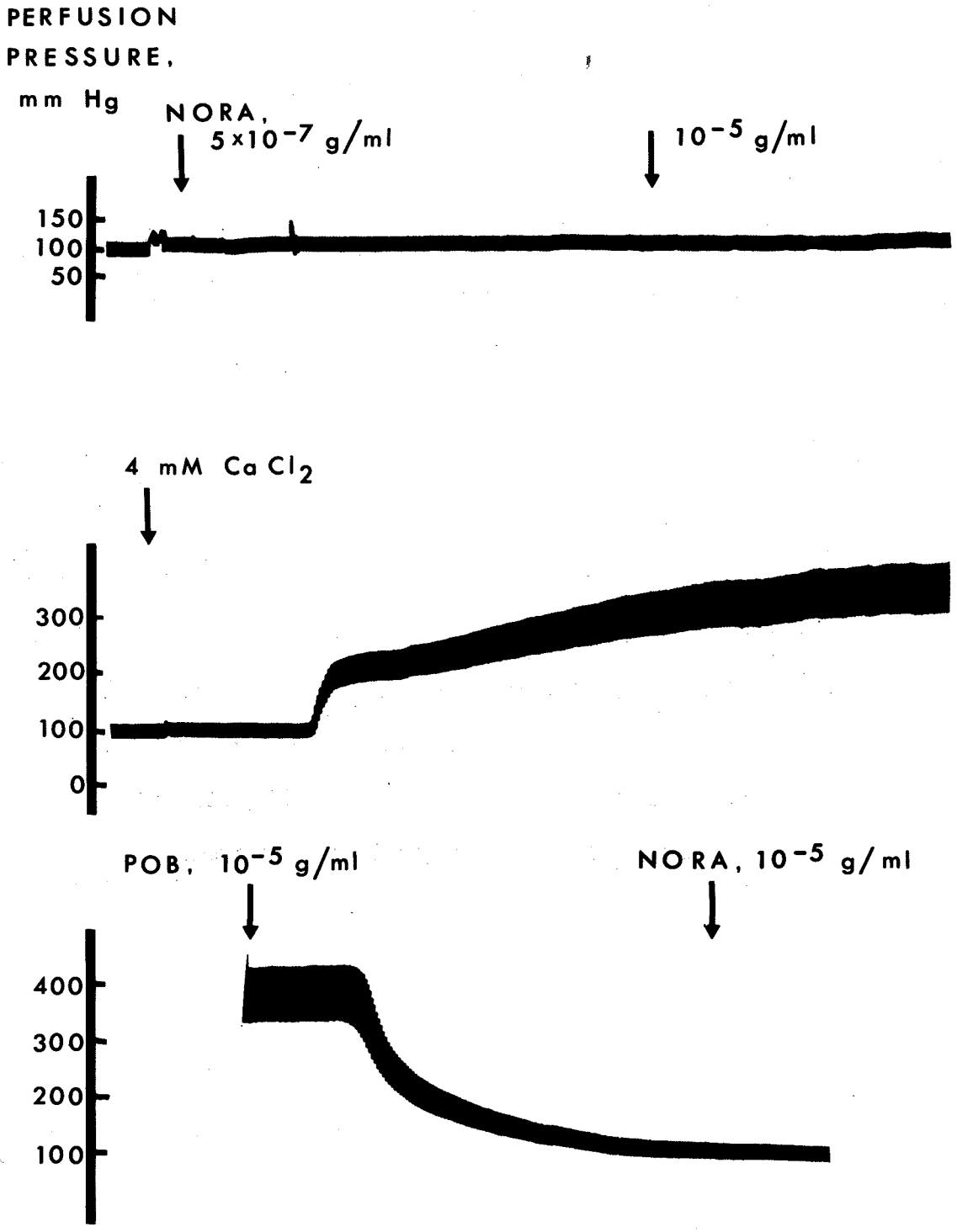

PERFUSION
PRESSURE,
mm Hg
NORA,
5x10⁻⁷ g/ml
10⁻⁵ g/ml
150
100
50
4 mM Ca Cl2
300
200
100
0
POB, 10⁻⁵ g/ml
NORA, 10⁻⁵ g/ml
400
300
200
100

Table 7. Changes in perfusion pressure (mm Hg) in response to NORA and phenoxybenzamine when the perfusate was calcium free. Calcium to a final concentration of 4 mM was added followed by 10^{-5} g/ml POB. N = 4.

Control	NORA, final concentration, g/ml		Calcium	POB,	NORA,
			mM	g/ml	g/ml
	5×10^{-7}	10^{-5}	4	10^{-5}	10^{-5}
106.3 ± 1.4	106.3 ± 1.4	106.3 ± 1.4	285 ± 4.9	105 ± 2.4	105 ± 2.4

Values are mean ± s.e.

addition of 4 mM calcium the pressure rose to 285 ± 4.9 mm Hg. This rise in pressure was reversed with the addition of phenoxybenzamine to 105 ± 2.4 mm Hg. The perfusion pressure did not respond to additional noradrenaline.

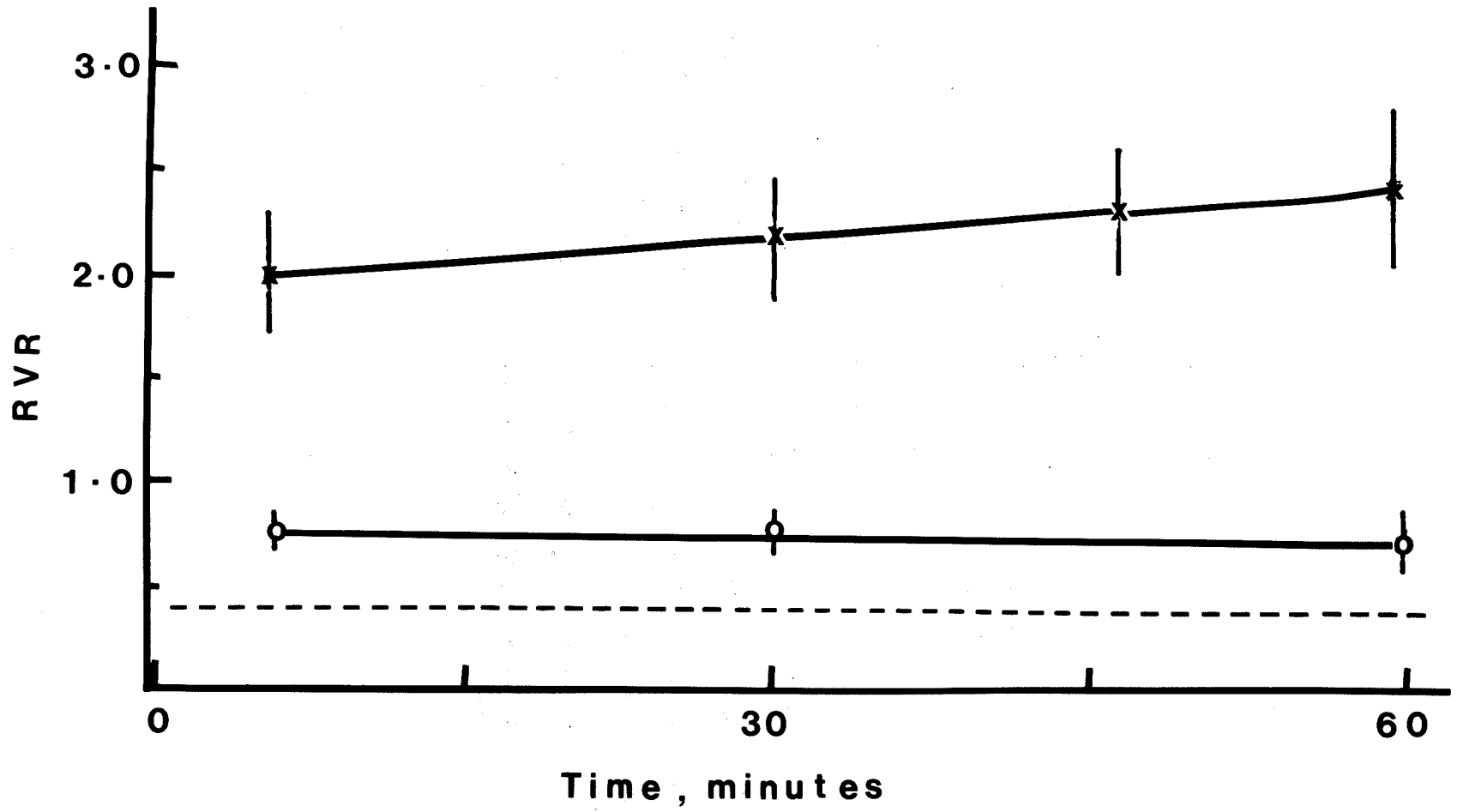
To determine whether it was the calcium and not the noradrenaline that effected the rise in perfusion pressure, three kidneys in calcium-free perfusate were exposed to incremental increases in the amount of calcium in the perfusate. The perfusion pressure was 108.3 ± 1.6 mm Hg initially. After the addition of 1 mM calcium chloride, the pressure was 108.3 ± 1.6 mm Hg. Addition of another 1 mM calcium did not change the pressure (106.7 ± 1.9 mm Hg). When the final concentration of calcium in the perfusate was 3 mM, the pressure was 106.7 ± 1.9 mm Hg, and when the final concentration was 4 mM, the pressure was 102.5 ± 2.3 mm Hg.

4. Krebs-Henseleit Buffer with High Molecular Weight Additives

i) Dextrans

Dextrans were added to the perfusate in an attempt to decrease renal vascular resistance. However, the resistance was high and remained high throughout the perfusion (Figure 5). The RVR that was associated with each dextran solution tried was at least twice that seen when no oncotic agent was present. The commercially prepared 6% dextran in saline (Dextran 6%, Abbott) had a calcium concentration of 38-49 mEq/l. When the D 6% S solution was dialysed against water, both Na^{+} and Ca^{++} were effectively removed, but renal performance was not changed. The use of dextrans as high molecular weight albumin substitutes was therefore discarded.

Figure 5. Comparison of renal vascular resistance in *in vitro* kidneys perfused with or without dextran in the perfusate. (**X–X**) indicates RVR with dextran; (**O–O**) indicates RVR without dextran; dotted line shows *in vivo* value.


3·0
2·0
1·0
RVR
0
30
60
Time, minutes

ii) Albumin

Compared to Krebs-Henseleit buffer without an oncotic agent, the presence of 3.75 g% albumin was associated with a decrease in resistance to flow. Table 8 compares hemodynamics, urine flow and salt excretion in 7 kidneys perfused with Krebs-Henseleit buffer in the absence of albumin to 5 kidneys perfused with K.H.B. containing albumin. Resistance, calculated as mean perfusion pressure/flow rate, ml/sec/g, was 3441.9 ± 317.5 without albumin and 1338.7 ± 123.4 when albumin was present. Omitting the renal weight from the calculation results in ratios of pressure/flow, ml/min of 0.6 when albumin was absent and 0.3 when albumin was present. Renal vascular resistance, therefore, was similar to that of the kidney in vivo when albumin was present (see Table 5).

GFR was significantly less when albumin was present in the perfusate (9.8 ± 1.5 ml/min/100 g without albumin, 5.3 ± 0.5 ml/min/100 g with albumin; $p < 0.05$). Urine flow rate was significantly less when albumin was present (6.3 ± 0.6 ml/min and 0.59 ± 0.2 ml/min; $p < 0.05$). The osmolality of the urine was not greater than that of the plasma, as indicated by U/P Osm values of 0.8 ± 0.1 and 0.95 ± 0.03. The urinary excretion rates of sodium were significantly less when albumin was present (585.8 ± 69.1 μEq/min and 29.5 ± 4.1 μEq/min; $p < 0.05$). The potassium excretion rates were similar and low. Without albumin the kidney excreted 1.2 ±

Table 8 . A comparison of hemodynamics, urine flow rate and salt excretion in kidneys perfused with a Krebs-Henseleit buffer in the presence or absence of 3.75 g% albumin (Fraction V). All kidneys were flushed with 0.9 % NaCl at 4°C prior to removal.

	Lacking Albumin N = 7	3.75 g% Albumin N = 5
Dog weight, kg	25.4 ± 2.8	19.6 ± 1.7
Kidney weight, g	81.5 ± 7.0	71.1 ± 4.5
RVR, $\frac{\text{mm Hg}}{\text{ml/sec/g}}$	3441.9 ± 317.5	1338.7 ± 123.4[1]
GFR, ml/min/100 g	9.8 ± 1.5	5.3 ± 0.5[1]
$\dot{V}$, ml/min	6.3 ± 0.6	0.59 ± 0.2[1]
$U_{Na}\dot{V}$, µEq/min	585.8 ± 69.1	29.5 ± 4.1[1]
$U_{K}\dot{V}$, µEq/min	1.2 ± 0.2	3.65 ± 0.8
% $Reab_{Na}$	61.9 ± 9.7	95.4 ± 2.5[1]
U/P Osm	0.8 ± 0.1	0.95 ± 0.03

Values are mean ± s.e.

[1] = $p < 0.05$

0.2 μEq/min K^+; when albumin was present the kidney excreted 3.65 ± 0.8 μEq/min K^+.

Per cent sodium reabsorption, an indicator of tubular function, was significantly higher when albumin was present. Without albumin the kidney could reabsorb only 61.9 ± 9.7 % of its filtered load. When albumin was present the kidney reabsorbed 95.4 ± 2.5 % of its filtered load.

Figure 6 shows the dose-related response to noradrenaline in this preparation. The threshold for response was 0.3 μg noradrenaline delivered in a bolus to the kidney in both albumin-free and albumin buffer. The presence of albumin in the buffer was associated with an increase in the response of the vasculature to the agonist at 3 and 10 μg when compared to responses obtained from albumin-free perfusion.

The responses to acetylcholine and angiotensin are shown in Figure 7. Acetylcholine, 1.0 μg, decreased the perfusion pressure by 10 ± 3 mm Hg when albumin was absent, and by 20 ± 4 mm Hg when albumin was present. Angiotensin, 0.3 μg, increased the perfusion pressure to 126.4 ± 4.9 mm Hg in the kidneys perfused with the albumin-free buffer and to 165.2 ± 6.2 mm Hg when albumin was present. A complete dose-response curve to angiotensin was attempted, but the data was of questionable value because there was a decrease in the response when repetitive boluses were injected. This is

Figure 6. Log dose-related noradrenaline-induced vasoconstriction of the renal bed. The closed squares (■–■) represent the mean response of the vasculature of five kidneys when albumin was present in the perfusate. The open squares (□–□) represent the mean response of the vasculature of five kidneys when there was no albumin in the perfusate. Mean ± s.e.

* - significant difference, $p < 0.05$.

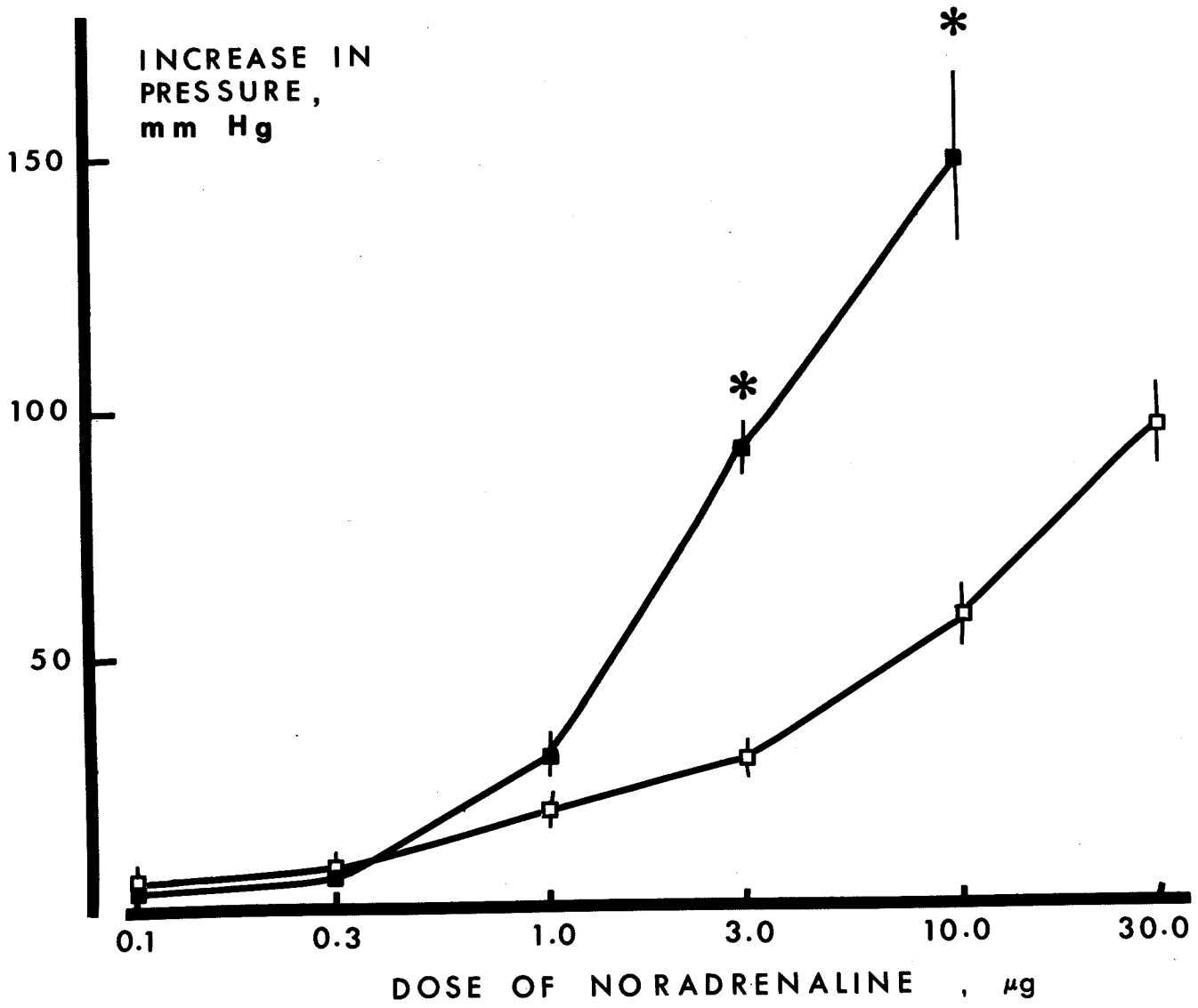
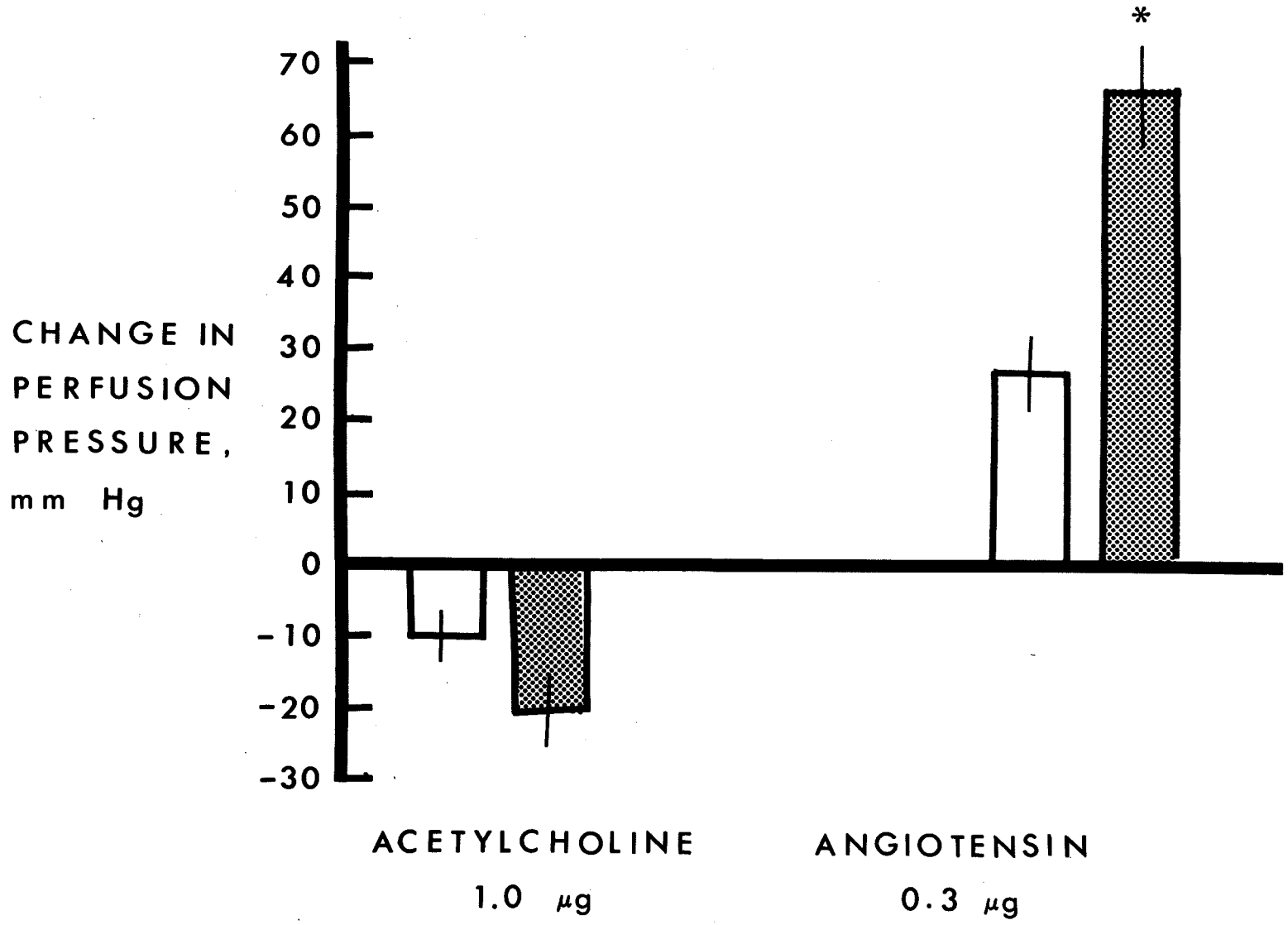

INCREASE IN
PRESSURE,
mm Hg
150
100
50
*
*
0.1
0.3
1.0
3.0
10.0
30.0
DOSE OF NORADRENALINE , µg

Figure 7. Response of the renal vasculature to acetylcholine and angiotensin. The open bar graphs represent values from five experiments without albumin. The stippled bar graphs represent the response when albumin was present. Mean ± s.e.


CHANGE IN PERFUSION PRESSURE, mm Hg
70
60
50
40
30
20
10
0
-10
-20
-30
*
ACETYLCHOLINE
1.0 μg
ANGIOTENSIN
0.3 μg

illustrated in Figure 8. A bolus of 0.3 µg angiotensin repeated at five minute intervals produced a decreased response with time. The response to 1.0 µg noradrenaline was not diminished, and in fact was enhanced after the angiotensin administration.

The gross appearance of the kidney after being perfused for 120 minutes is illustrated in Plate II. Blood was effectively removed. There was no evidence of edema. Plate III indicates the histological appearance of the kidney after two hours of perfusion. Plate III a) illustrates a relatively normal cortical region with a normal glomerulus and tubules. Plate III b) shows that the structural integrity of the medulla has been retained. No blood remains.

Figure 8 . The response of the isolated kidney to repeated administration of 0.3 µg angiotensin. This is from portions of a continuous tracing. 1.0 µg noradrenaline was added before and after the angiotensin. The arrows indicate the times of addition of the angiotensin. A II = angiotensin; NORA = noradrenaline. The asterisk (*) indicates $p < 0.05$.

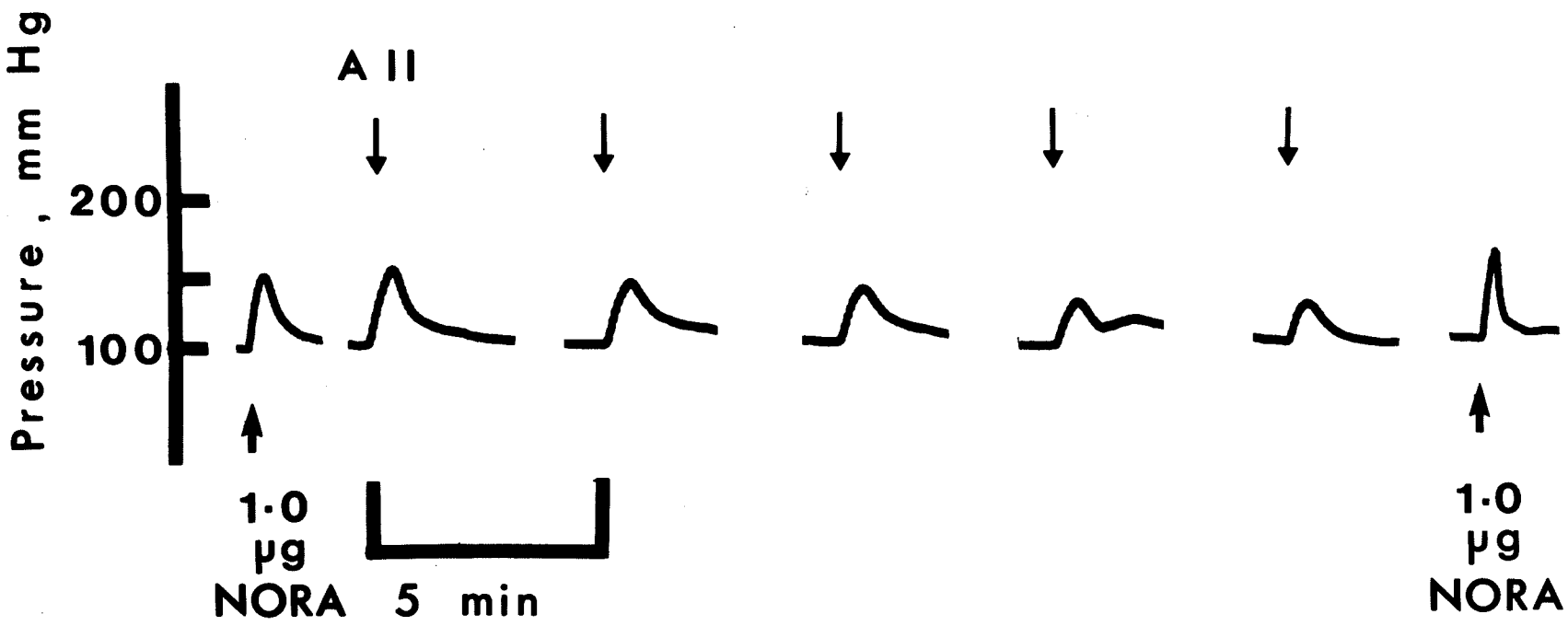

Pressure, mm Hg
200
100
A II
1·0
µg
NORA
5 min
1·0
µg
NORA

Plate II. Gross appearance of the kidney after 120 minutes of perfusion with Krebs-Henseleit buffer at 37° C. The capsule has been removed.

Left: outer surface is well cleared of blood.

Right: longitudinal section through the kidney. It is completely cleared of blood.

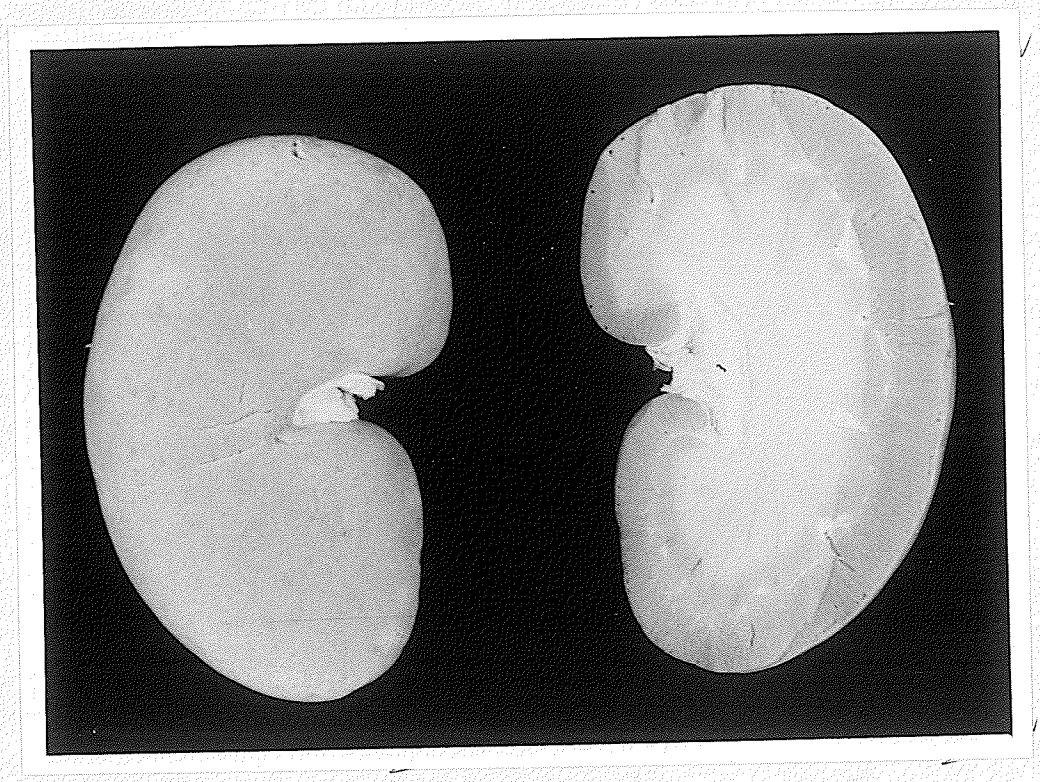
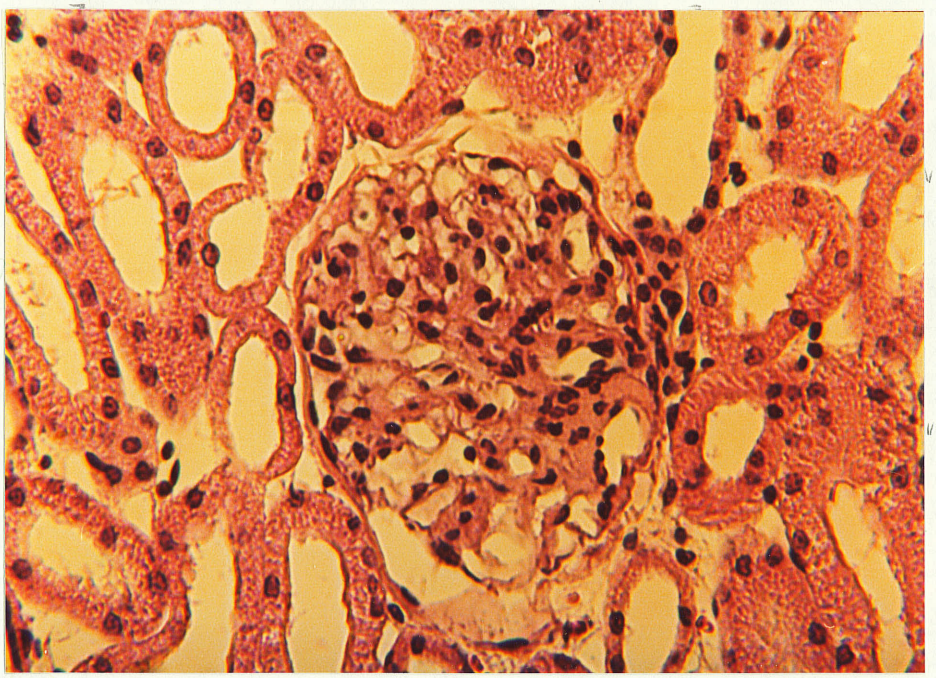


Plate III. Histological sections of the kidney perfused for 120 minutes in Krebs-Henseleit buffer. Note the relatively normal glomeruli and tubules.

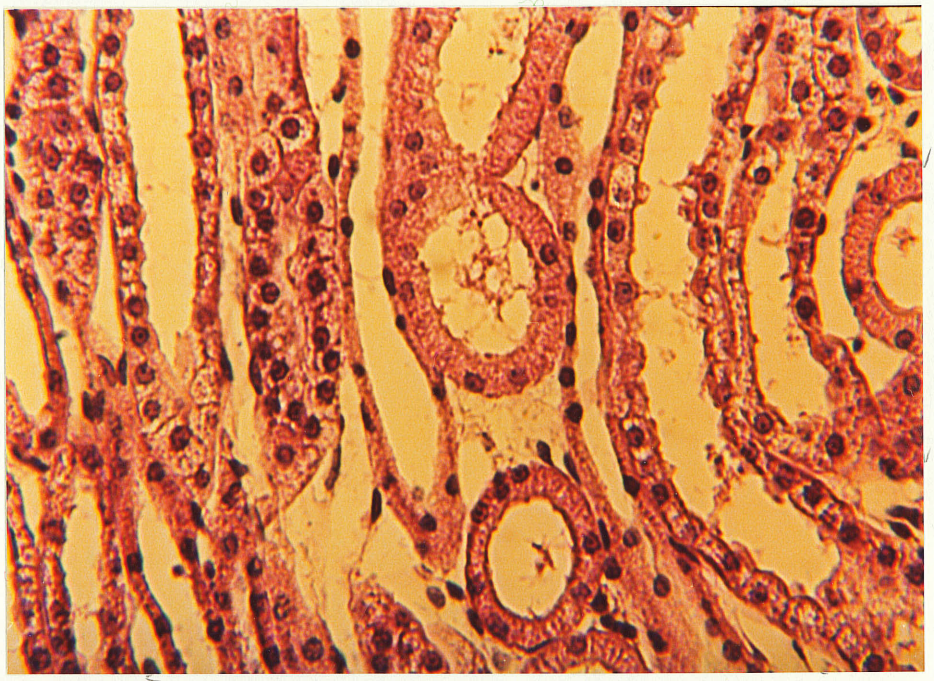
a) cortex

b) medulla

(H & E; 500X)



a)



b)

5. Summary

Use of the Krebs-Henseleit bicarbonate buffer resulted in an *in vitro* preparation which most closely resembled that of the *in vivo* situation. Hemodynamics were stable, and resistance to flow approximated that seen *in vivo* when 3.75 g% bovine serum albumin was present in the perfusate. GFR was less than *in vivo*, but stable, and the kidney in the presence of albumin was capable of conserving sodium. The vasculature responded to noradrenaline in a dose-related manner. The threshold of 0.3 μg noradrenaline is the same as the threshold needed to decrease renal blood flow in the dog (Fung, *et al.*, pers. comm.). Perfusion pressure could be decreased by acetylcholine and increased by angiotensin. We therefore considered the model acceptable for studying *in vitro* responses of the acute renal failure kidney.

B. The model of unilateral noradrenaline-induced acute oliguric renal failure.

1. General Protocol

Figure 9 indicates the in vivo responses of both kidneys to an infusion of noradrenaline into one renal artery. Mean systemic blood pressure was elevated to near 200 mm Hg during the period of infusion and returned to control values when the infusion stopped. The urine flow rate ceased in the experimental kidney and was increased in the control during the noradrenaline infusion. After the noradrenaline infusion ceased, the kidney exposed to the noradrenaline remained oliguric while the urine flow rate in the contralateral control returned to pre-noradrenaline values. Creatinine clearance in the experimental kidney was zero during the infusion period and remained very low. After four hours of stabilization it was 3 % of its contralateral control. The GFR of the control kidney did not significantly change throughout the six hour time period. Cumulative data from 86 dogs are listed in Table 9 .

Figure 9. Changes in creatinine clearance, blood pressure, urine flow rate and renal blood flow during and following a two-hour infusion of noradrenaline into one renal artery.

(▲—▲) kidney receiving noradrenaline

(●—●) contralateral control

(■—■) dog blood pressure

B.P. = blood pressure

C_{Cr} = creatinine clearance

$\dot{V}$ = urine flow rate

RBF = renal blood flow

NORA = noradrenaline

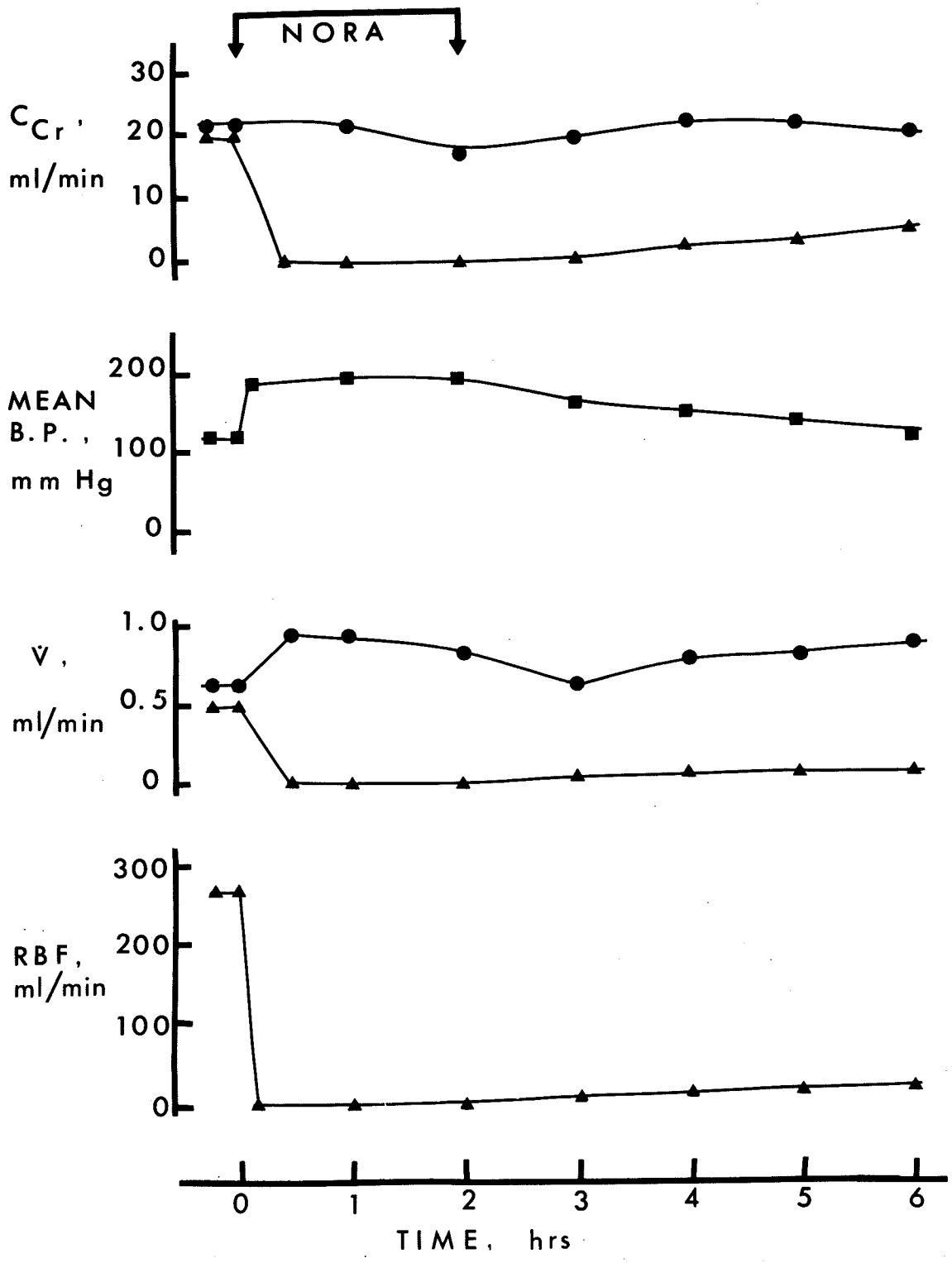

NORA
30
20
10
0
C_{Cr}, ml/min
200
100
0
MEAN B.P., mm Hg
1.0
0.5
0
$\dot{V}$, ml/min
300
200
100
0
RBF, ml/min
0
1
2
3
4
5
6
TIME, hrs

Table 9. Cumulative data of hemodynamics and renal function during induction of AORF by noradrenaline infusion.

	Control	Noradrenaline 60	Noradrenaline 120	Minutes post-Noradrenaline 60	Minutes post-Noradrenaline 120	Minutes post-Noradrenaline 180	Minutes post-Noradrenaline 240
Hemodynamics							
Mean arterial pressure, mm Hg	127 ± 4	182 ± 3	189 ± 3	130 ± 4	125 ± 3	123 ± 4	121 ± 3
Mean blood flow to experimental kidney, ml/min	321 ± 72	0	0	50 ± 6	83 ± 10	87 ± 9	93 ± 16
Heart rate, beats/min	140 ± 10	Arrhythmic		140 ± 6	161 ± 8	171 ± 12	163 ± 15
Renal Excretion							
a) Experimental kidney:							
$\dot{V}$, ml/min	1.1 ± 0.1	0	0	0	0.003 ± 0	0.01 ± 0	0.01 ± 0
$U_{Na}\dot{V}$, µEq/min	104 ± 67	0	0	0	0 to 2.6	0 to 9.8	0 to 13
U/P Osm	1.9 ± 0.4	0	0	-	-	-	-
% $Reab_{Na}$	97 ± 0.6	-	-	-	-	-	-
C_{Cr}, ml/min/100 g	30 ± 10	-	-	-	0.1 ± 0	0.1 ± 0.01	0.9 ± 0.1
b) Contralateral control kidney:							
$\dot{V}$, ml/min	1.0 ± 0.1	0.5 ± 0.03	0.2 ± 0.05	0.3 ± 0.08	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
$U_{Na}\dot{V}$, µEq/min	120 ± 58	51 ± 17	10 ± 1	9.2 ± 2	13 ± 2	23 ± 8	33 ± 6
U/P Osm	2 ± 0.4	2 ± 0.1	3 ± 0.4	2 ± 0.1	2 ± 0.4	2 ± 0.3	2 ± 0.3
% $Reab_{Na}$	98 ± 0.4	99 ± 0.3	99 ± 0.5	99 ± 0.4	98 ± 0.3	98 ± 0.3	98 ± 0.3
C_{Cr}, ml/min/100 g	30 ± 6	29 ± 4	16 ± 3	12 ± 2	13 ± 2	16 ± 3	19 ± 3

2. *In situ* Sensitivity of the Renal Vasculature

Single experiments were conducted to determine the *in vivo* sensitivity of the renal vasculature to parathyroid hormone, serotonin and histamine both in the control and in the kidney in renal failure. These data are shown in Table 10 . Parathyroid hormone (PTH) was associated with an increase in renal blood flow in a dose-related fashion in the kidney both before and after renal failure was produced by noradrenaline. The per cent increase in RBF was comparable under both conditions. Incremental doses of histamine produced increases in renal blood flow of similar magnitude both before and after the kidney was in established renal failure. In contrast, serotonin was associated with a modest increase in blood flow to a maximum of 19 % prior to noradrenaline induced renal failure, but was associated with a decrease in flow when the kidney was in failure.

Table 10. Effect of parathyroid hormone, histamine, and serotonin on *in vivo* renal blood flow, ml/min, before and after unilateral AORF was produced by noradrenaline infusion. RBF was measured with an electromagnetic flow probe. Per cent change in blood flow is relative to the control flow rate. An - indicates a decrease in blood flow, an + indicates an increase in blood flow.

		Before induction of AORF		After induction of AORF	
		ml/min	% change	ml/min	% change
Parathyroid hormone U/min:	0.0	315		90	
	0.97	425	+35	90	0
	1.94	530	+68	140	+56
	3.88	575	+83	175	+94
	9.70	600	+90	200	+122
Histamine, µg/min:	0.0	204		66	
	9.7	204	0	75	+13
	19.4	251	+23	83	+26
	38.8	264	+29	96	+45
	97.0	311	+52	109	+65
Serotonin, µg/min:	0.0	221		79	
	9.7	230	+4	57	-29
	19.4	242	+10	83	+5
	38.8	242	+10	75	-5
	97.0	255	+15	75	-5
	194.0	264	+19	75	-5

II. In vitro Responses of the Kidney in Acute Oliguric Renal Failure

A. Kidneys perfused with albumin-free K.H.B.

Table 11 compares the renal function of kidneys *in situ* prior to noradrenaline infusion into one renal artery, *in situ* prior to removal 4 hours after noradrenaline infusion ceased, and *in vitro* after thirty minutes of perfusion. The contralateral control kidney had a V of 0.81 ± 0.3 ml/min before noradrenaline was infused to the opposite kidney, 0.47 ± 0.2 ml/min after four hours of stabilization, and 3.6 ± 0.1 ml/min *in vitro*. Renal excretion of sodium was 99.9 ± 49.7 μEq/min initially, 26.6 ± 11.5 μEq/min prior to removal, and 326.9 ± 92.6 μEq/min *in vitro*. Renal excretion of potassium was 34.8 ± 8.0 before noradrenaline infusion, 30.2 ± 6.0 μEq/min after stabilization and 17.5 ± 4.1 μEq/min *in vitro*. The ratio of urine to plasma osmolality was 2.7 ± 0.9 initially, 2.2 ± 0.5 prior to removal and 0.9 ± 0.1 *in vitro*. GFR, determined by C_{Cr} was 38.7 ± 6.7 ml/min before noradrenaline infusion, 28.4 ± 5.3 ml/min after four hours of stabilization and 5.7 ± 2.3 ml/min *in vitro*. The kidneys reabsorbed 98.4 ± 0.8 % of their filtered load initially, 99.1 ± 0.6 % after four hours of stabilization, and 66.5 ± 4.1 % *in vitro*. The perfusate flow rate was 156.8 ± 16.8 ml/min which indicates the renal vascular resistance was 3667.7 ± 429.0 PRU.

Table 11. A comparison of the kidney *in vivo* prior to NORA, four hours post - noradrenaline infusion, and *in vitro* in Krebs-Henseleit buffer. N = 12.

	Before NORA infusion	Stabilization: 4 hr. post-NORA	*In vitro*
Control kidney:			
$\dot{V}$, ml/min	0.81 ± 0.3	0.47 ± 0.2	3.6 ± 0.1
$U_{Na}\dot{V}$, µEq/min	99.9 ± 49.7	26.6 ± 11.5	326.9 ± 92.6
$U_{K}\dot{V}$, µEq/min	34.8 ± 8.0	30.2 ± 6.0	17.5 ± 4.1
U/P Osm	2.7 ± 0.9	2.2 ± 0.5	0.9 ± 0.1
C_{Cr}, ml/min	38.7 ± 6.7	28.4 ± 5.3	5.7 ± 2.3
% $Reab_{Na}$	98.4 ± 0.8	99.1 ± 0.6	66.5 ± 4.1
Weight, g			84.3 ± 6.0
Flowrate, ml/min	-	-	156.8 ± 16.8
MAP, mm Hg	131.5 ± 4.4	125.0 ± 6.1	100
Experimental kidney:			
$\dot{V}$, ml/min	0.63 ± 0.3	0.28[1]	0.3 ± 0.2
$U_{Na}\dot{V}$, µEq/min	69.2 ± 36.6	1.7[1]	30.5 ± 19.9
$U_{K}\dot{V}$, µEq/min	28.4 ± 7.0	1.2[1]	1.6 ± 1.3
U/P Osm	2.5 ± 0.9	1.2[1]	0.9 ± 0.1
C_{Cr}, ml/min	35.2 ± 6.6	0.3[1]	1.2 ± 0.8
% $Reab_{Na}$	98.7 ± 0.73	96.5[1]	48.2 ± 20.5
Weight, g			75.3 ± 4.4
Flowrate, ml/min	215.6 ± 26.0	76.5 ± 10.0	167.2 ± 12.4
MAP, mm Hg	131.5 ± 4.4	125.0 ± 6.1	100

[1] = single result

The experimental kidney had a urine flow rate of 0.63 ± 0.3 ml/min prior to noradrenaline infusion. After four hours of stabilization, only one kidney was not anuric. It had a $\dot{V}$ of 0.28 ml/min. However, in vitro each kidney produced urine (0.3 ± 0.2 ml/min). Renal excretion of sodium was 69.2 ± 36.6 µEq/min initially. The $U_{Na}\dot{V}$ of the oliguric kidney after four hours of stabilization was 1.7 µEq/min. In vitro the kidneys excreted 30.5 ± 19.9 µEq/min. Renal excretion of potassium was 28.4 ± 7.0 µEq/min prior to noradrenaline infusion. The kidney that was not anuric after four hours of stabilization excreted 1.2 µEq/min. In vitro the $U_K\dot{V}$ rate was 1.6 ± 1.3 µEq/min. The U/P Osmolality ratio was 2.5 ± 0.9 initially and 0.9 ± 0.1 in vitro. C_{Cr} was 35.2 ± 6.6 ml/min before noradrenaline infusion. The single oliguric kidney had a C_{Cr} of 0.3 ml/min. In vitro the C_{Cr} was 1.2 ± 0.8 ml/min. The kidneys reabsorbed 98.7 ± 0.7 % of their filtered load initially, and 48.2 ± 20.5 % of the filtered load in vitro. The single kidney after four hours of stabilization could reabsorb 96.5 % of its sodium load. The renal blood flow was 215.6 ± 26.0 ml/min initially, and 76.5 ± 10.0 ml/min just prior to removal. When these kidneys were perfused in vitro, the flow rate showed an increase of greater than two-fold. The in vitro flow rate was 167.2 ± 12.4 ml/min, not significantly different from their contralateral controls.

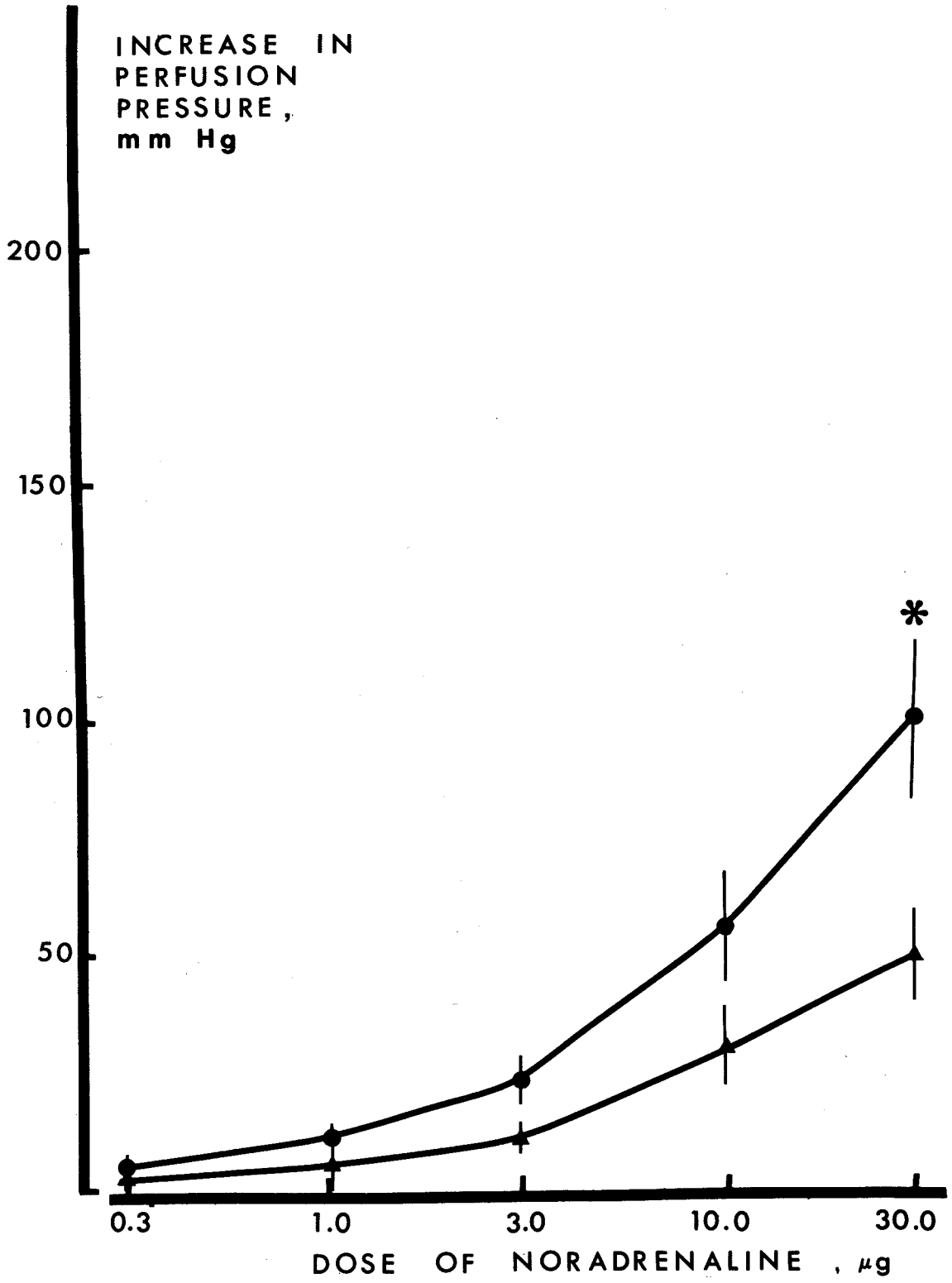
The vasculature of both groups of kidneys in vitro responded to noradrenaline as shown in Figure 10. The threshold

Figure 10. Dose-related increases in perfusion pressure in response to noradrenaline in the control kidney and the kidney in AORF. No albumin was present in the buffer. Five dogs were used. Mean ± s.e.

(●–●) = control

(▲–▲) = AORF

* $p < 0.05$


INCREASE IN
PERFUSION
PRESSURE,
mm Hg
200
150
100
50
*
0.3
1.0
3.0
10.0
30.0
DOSE OF NORADRENALINE , µg

for response was shifted ½ log dose to the right. The kidneys in AORF had a quantitatively reduced response that was significantly different ($p<0.05$) when a bolus of 30.0 µg was administered.

The renal vasculature was exposed to acetylcholine, dopamine and angiotensin (Table 12). Acetylcholine, 10 µg, decreased the perfusion pressure in each group of kidneys slightly (15.0 ± 4.0 mm Hg drop in the control and 15.0 ± 5.5 mm Hg drop in the kidney in AORF). Dopamine, 300 µg, increased the perfusion pressure to a variable amount. The pressure increased 38.5 ± 21.6 mm Hg in the control and 23.7 ± 21.2 mm Hg in AORF. Angiotensin, 0.3 µg, also produced an increase in pressure, and as indicated earlier (Figure 8), a dose-related vasoconstriction could not be studied. However, there was a tendency for vasculature to respond less in the kidneys in AORF (37.0 ± 12.5 mm Hg and 22.0 ± 9.6 mm Hg).

Table 12. Changes in perfusion pressure (mm Hg) in response to various agonists in the kidney in the control situation and when in AORF. The kidneys were perfused in albumin-free buffer (N = 6). A decrease in perfusion pressure is indicated by a -; an increase in perfusion pressure is indicated by a +. Mean ± s.e.

	Control	AORF
Acetylcholine, 10 μg	-15.0 ± 4.0	-15.0 ± 5.5
Dopamine, 300 μg	+38.5 ± 21.6	+23.7 ± 21.2
Angiotensin, 0.3 μg	+37.0 ± 12.5	+22.0 ± 9.6

B. Kidneys perfused with K.H.B. containing albumin.

Table 13 compares renal function of kidneys *in situ* before noradrenaline was infused into one renal artery, *in situ* after the four hour period of stabilization, and *in vitro* after thirty minutes of perfusion with a buffer that contains 3.75 g% albumin. The contralateral control kidney had a V of 1.1 ± 0.3 ml/min initially, 0.4 ± 0.2 ml/min after the period of stabilization, and 0.5 ± 0.01 ml/min *in vitro*. Renal excretion of sodium was 126.0 ± 38.6 µEq/min initially, 33.4 ± 12.6 µEq/min just prior to removal, and 38.5 ± 8.4 µEq/min *in vitro*. Renal excretion of potassium was 48.2 ± 13.1 µEq/min before noradrenaline infusion, 20.6 ± 6.3 µEq/min prior to removal, and 11.8 ± 2.5 µEq/min *in vitro*.

The U/P osmolality ratio was 1.9 ± 0.4 initially, 1.8 ± 0.3 after stabilization and 0.9 ± 0.01 *in vitro*. GFR was 29.2 ± 4.1 ml/min prior to noradrenaline infusion to the contralateral kidney, 21.2 ± 6.8 ml/min after stabilization, and 3.0 ± 0.4 ml/min *in vitro*. The kidneys reabsorbed 96.3 ± 1.2 % of their filtered load initially, 98.2 ± 0.8 % of the load just prior to their removal, and 90.3 ± 2.5 % of the load when perfused *in vitro*. The perfusate flow rate was 271.9 ± 25 ml/min which indicates the renal vascular resistance *in vitro* was 1710 ± 164 PRU, less than half of that seen without albumin in the buffer.

Table 13. A comparison of the kidney *in vivo* prior to NORA, four hours post - noradrenaline infusion, and *in vitro* in Krebs-Henseleit buffer that contains albumin. N = 8.

	Before NORA infusion	Stabilization: 4 hr. post-NORA	*In vitro*
Control kidney:			
$\dot{V}$, ml/min	1.1 ± 0.3	0.4 ± 0.2	0.5 ± 0.01
$U_{Na}\dot{V}$, µEq/min	126.0 ± 38.6	33.4 ± 12.6	38.5 ± 8.4
$U_{K}\dot{V}$, µEq/min	48.2 ± 13.1	20.6 ± 6.3	11.8 ± 2.5
U/P Osm	1.9 ± 0.4	1.8 ± 0.3	0.9 ± 0.01
C_{Cr}, ml/min	29.2 ± 4.1	21.2 ± 6.8	3.0 ± 0.4
% $Reab_{Na}$	96.3 ± 1.2	98.2 ± 0.8	90.3 ± 2.5
Weight, g			74.3 ± 3.6
Flowrate, ml/min	-	-	271.9 ± 25.0
MAP, mm Hg	131.0 ± 4.0	120 ± 6.0	100
Experimental kidney:			
$\dot{V}$, ml/min	1.1 ± 0.2	0.02 ± 0.01	0.02 ± 0.02
$U_{Na}\dot{V}$, µEq/min	119.1 ± 34.9	9.6 ± 7.7	5.1 ± 4.0
$U_{K}\dot{V}$, µEq/min	37.2 ± 6.7	0.3 ± 0.3	0.3 ± 0.2
U/P Osm	1.9 ± 0.4	0.1 ± 0.1	-
C_{Cr}, ml/min	30.4 ± 6.9	1.1 ± 1.0	0.002 ± 0.0
% $Reab_{Na}$	96.0 ± 0.9	3.9 ± 3.9	-
Weight, g			66.7 ± 4.4
Flowrate, ml/min	304.4 ± 42.6	90.0 ± 33.4	229.5 ± 28.6
MAP, mm Hg	131.0 ± 4.0	120 ± 6.0	100

The experimental kidney had a urine flow rate initially of 1.1 ± 0.2 ml/min. This decreased to 0.02 ± 0.01 ml/min after four hours of stabilization. This value was the same when the kidneys were perfused *in vitro* (0.02 ± 0.02 ml/min). Renal excretion of sodium was 119.1 ± 34.9 µEq/min initially, 9.6 ± 7.7 µEq/min after stabilization, and 5.1 ± 4.0 µEq/min *in vitro*. Renal potassium excretion was 37.2 ± 6.7 µEq/min before it was exposed to noradrenaline, 0.3 ± 0.3 µEq/min just prior to removal, and 0.3 ± 0.2 µEq/min *in vitro*. The U/P osmolality ratio was 1.9 ± 0.4 initially and 0.1 ± 0.1 after 4 hours of stabilization. The kidney could reabsorb 96.0 ± 0.9 % of its filtered load of sodium before it was placed in failure. The renal blood flow decreased from 304.4 ± 42.6 ml/min before noradrenaline to zero during the noradrenaline infusion. The blood flow recovered to 90.0 ± 33.4 ml/min during stabilization. The *in vitro* flow rate was 229.5 ± 28.6 ml/min, not significantly different from their contralateral controls.

The vasculature of the kidneys *in vitro* responded to noradrenaline (Figure 11). The threshold of 0.3 µg is similar to that seen *in vivo*. The kidneys in AORF were less sensitive to noradrenaline. The increase in pressure produced by 1.0, 3.0 and 10.0 µg bolus doses of the agonist was significantly less ($p < 0.05$) in the kidney in AORF.

The kidneys *in vitro* responded to other agonists (Table

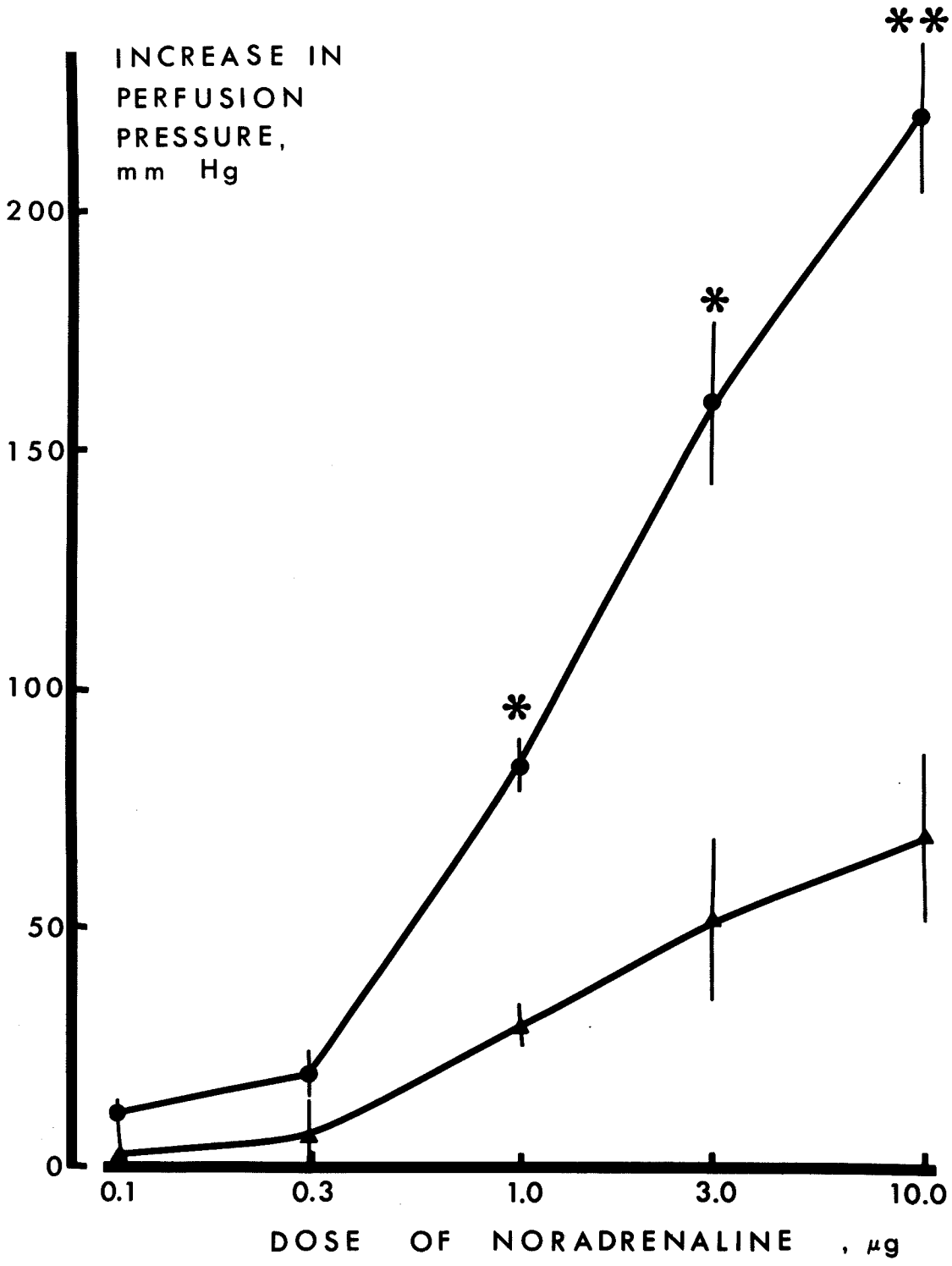
Figure 11. Dose-related increases in the perfusion pressure in response to noradrenaline in the control and the kidney in AORF. Albumin was present in the perfusate. N = 7. Mean ± s.e.

* $p < 0.05$

** $p < 0.01$

(●—●) = control

(▲—▲) = AORF


INCREASE IN
PERFUSION
PRESSURE,
mm Hg
200
150
100
50
0
**
*
*
0.1
0.3
1.0
3.0
10.0
DOSE OF NORADRENALINE , µg

14). Acetylcholine, 10 µg, decreased the perfusion pressure slightly in both groups of kidneys (14.1 ± 1.5 mm Hg and 10.0 ± 2.5 mm Hg). Histamine, 10 µg, produced a slight decrease in pressure (8.5 ± 1.0 and 7.5 ± 0.8 mm Hg). Serotonin, 10 µg, increased the perfusion pressure in both groups of kidneys. The response was significantly greater in the control kidney (19.5 ± 1.8 mm Hg and 12.5 ± 2.0 mm Hg; $p < 0.05$). Angiotensin, 0.3 µg, increased the perfusion pressure (70.3 ± 10.3 mm Hg in the control kidneys and 36.0 ± 6.4 mm Hg in the kidneys in AORF; $p < 0.05$).

Figure 12 illustrates that the vasculature responded to noradrenaline with a greater increase in perfusion pressure when it had been previously exposed to angiotensin, 0.3 µg. The control kidneys have an enhanced response. The slopes were not different (144.5 and 151.2), but after exposure to the amide, the curve shifted to the left. The kidneys in AORF do not show this response.

The kidney in failure also appeared grossly and histologically different from its contralateral control. Plate IV illustrates the gross appearance of the kidney after being perfused for 120 minutes in an artificial buffer. On the left, the outer surface of the decapsulated kidney exhibits many small patches of blood. On the right, the appearance of the inner tissues are shown. The patchy areas of blood seen on the cortical surface extend into the cortex. The

Table 14. Changes in perfusion pressure, mm Hg, in response to various agonists when kidneys, either control or in AORF were perfused with K.H.B. plus 3.75 g% albumin (N = 8). A decrease in perfusion pressure is indicated by a -; an increase in perfusion pressure is indicated by a +. Mean ± s.e.

	Control	AORF
Acetylcholine, 10 µg	-14.1 ± 1.5	-10.0 ± 2.5
Histamine, 10 µg	-8.5 ± 1.0	-7.5 ± 0.8
Serotonin, 10 µg	+19.5 ± 1.8	+12.5 ± 2.0*
Angiotensin, 0.3 µg	+70.3 ± 10.3	+36.0 ± 6.4*

* $p < 0.05$

Figure 12. Responses of the renal vasculature to noradrenaline before and after exposure to angiotensin.

Control kidney:

(●–●) Pre- A II r = 0.91

B = 144.5

(o–o) Post-A II r = 0.80

B = 151.2

AORF kidney:

(▲–▲) Pre- A II r = 0.49

B = 26.2

(Δ–Δ) Post-A II r = 0.37

B = 22.4

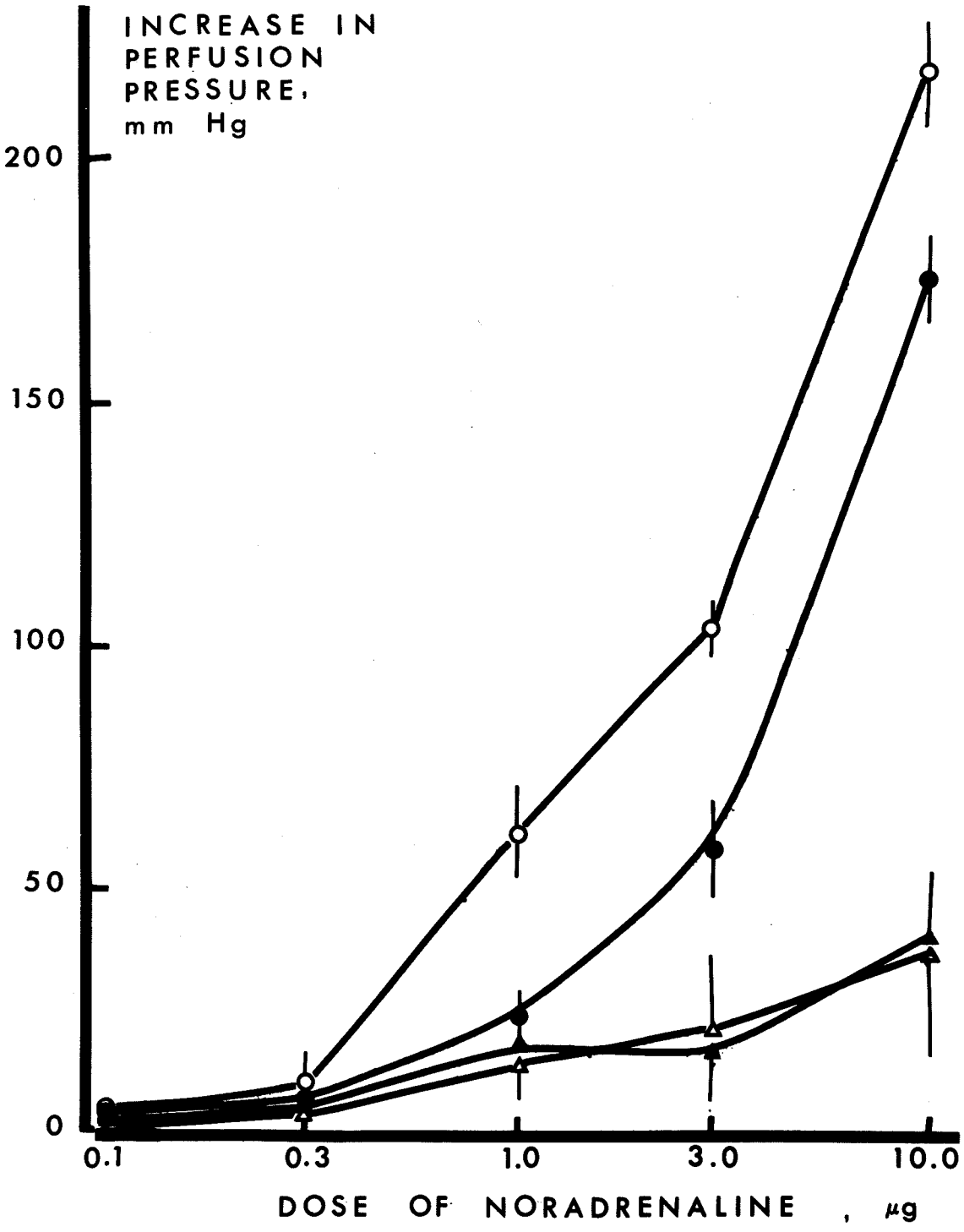
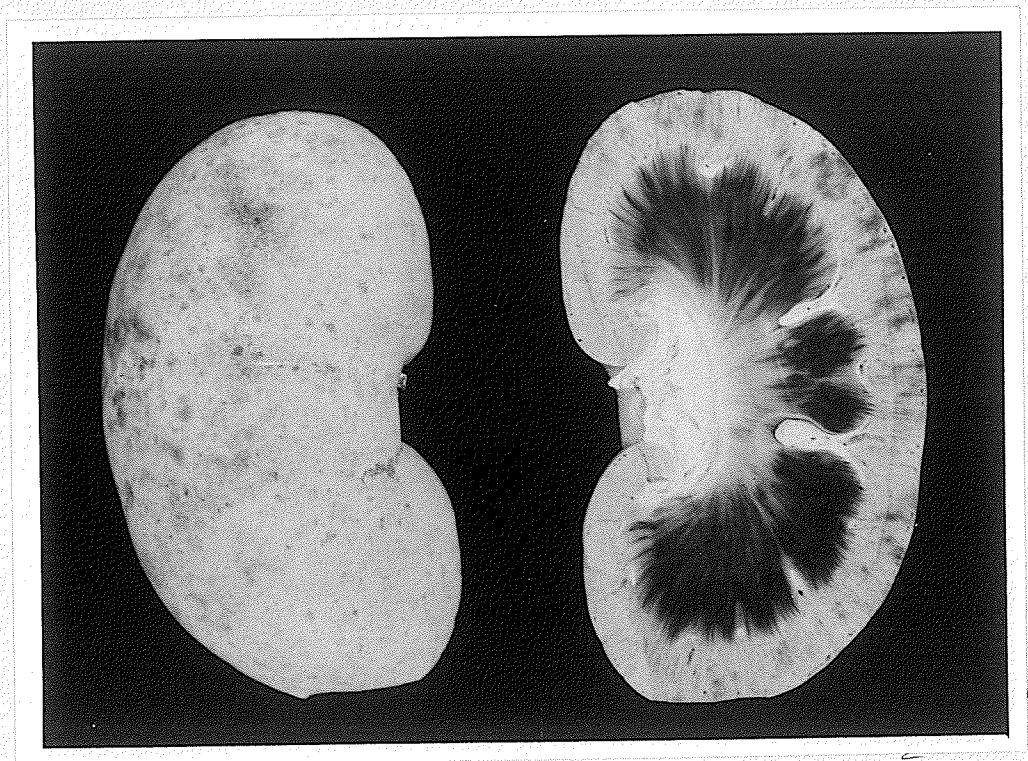

INCREASE IN
PERFUSION
PRESSURE,
mm Hg
200
150
100
50
0
0.1
0.3
1.0
3.0
10.0
DOSE OF NORADRENALINE , µg

Plate IV. Gross appearance of the kidney in AORF after being perfused for 120 minutes.

Left: appearance of the outer surface after the capsule has been removed. The outer surface contains numerous patches of blood.

Right: longitudinal section of the kidney. There are patchy areas of blood in the cortex; the medulla contains much blood.



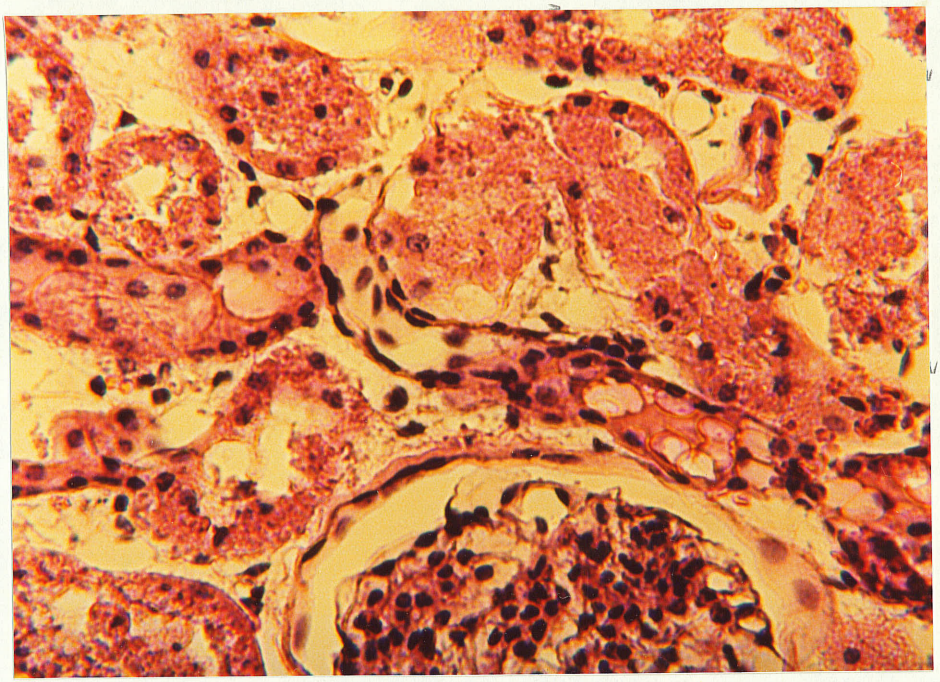
medulla also has not been well cleared of blood.

The histological appearance of the kidney in failure is typical of that seen in other studies in which noradrenaline is used to precipitate the failure. Plate V illustrates the features of acute tubular necrosis. The upper plate V a) is a section through the cortex. The glomerular capillaries are slightly abnormal, but the most evident damage is that seen in the proximal tubules which are dilated, have damaged basement membranes and which contain remnants of red blood cells. Plate V b) is a section of the medulla illustrating tubular damage and tubules filled with casts.

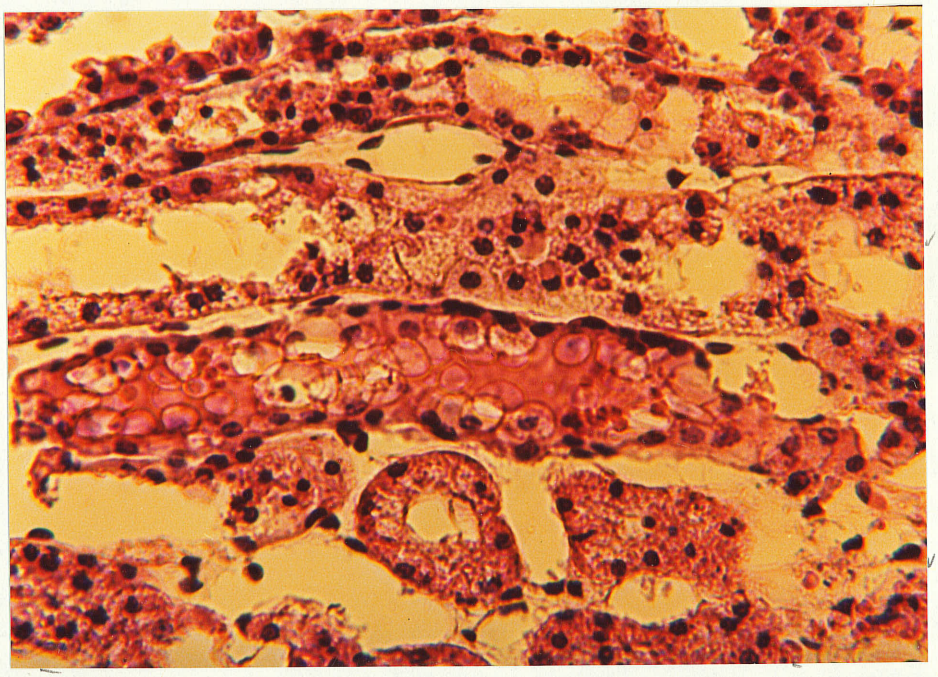
Plate V. Histological sections of the kidney in AORF after being perfused for 120 minutes. Note evidence of ATN: glomerular capillaries slightly abnormal, dilated tubules with damaged basement membrane, red blood cells in tubules, tubules in the medulla contain numerous red blood cell casts. (H & E; 500X)

a) cortex

b) medulla



a)



b)

D I S C U S S I O N

I. Perfusion of Isolated Kidneys

A. Use of whole blood as a perfusate

In this study, when isolated kidneys were perfused with autologous blood, total renal blood flow was lower than that found in intact animals (Table 2). The flow, corrected for kidney weight, was 2.5 ml/min/g kidney. This value is similar to that reported in other studies for isolated, blood perfused dog kidneys. When similarily corrected for kidney weight, the literature values range from 1.2 ml/min/g (Dunham & Zimmerman, 1970), 2.1 ml/min/g (Berkowitz et al., 1968), 2.2 ml/min/g (Kaloyanides et al., 1974) to 3.0 ml/min/g (Vanherweghem et al., 1975). These are all lower than the 4 ml/min/g value reported by Smith (1951) in the intact dog. In the present experiments the blood flow fell within ninety minutes to approximately 80% of the initial value. By this time the kidneys were anuric. As the blood for the extra-corporeal perfusion was taken from, and returned to the donor animal, who showed stable hemodynamics during the period of observation, it is unlikely that a circulating vasoconstrictor, such as that described by Nizet et al., (1967; 1975) was responsible for the initial high RVR or for its maintenance. Fat emboli have been suggested as a causal factor in the high resistance to flow in blood perfused organs (Ross & Nizet, 1978). However, histological examination of these kidneys showed no

evidence of emboli.

^{133}Xenon washout was used to monitor intrarenal blood flow distribution in the extracorporeal circuit. The relative amount of blood flow to the outer cortex, CI, the fastest flowing compartment, was initially less than that seen *in vivo*, but was greater than that flowing to the inner cortex. After ninety minutes of perfusion, the total flow decreased, but the fast flowing compartment still remained, although the percent of blood flowing through this compartment had decreased markedly (Table 3). The initial low total blood flow and low proportion of flow to the outer cortex seen in this study may be a reflection of high levels of angiotensin or catecholamines to the superficial cortex, or of increased intrarenal medullary prostaglandin synthesis. The latter could result from surgical stress (Itskovitz *et al.*, 1973b) or occur secondarily to angiotensin release (McGiff and Wong, 1979). A persistent, high level of circulating vasoactive material is not supported in these experiments, as the donor dogs' hemodynamics were stable throughout the period of study. The increase in relative blood flow to the inner cortex by 90 minutes *in vitro* is therefore most likely a reflection of intrarenal events, either a prolonged prostaglandin-mediated medullary vasodilation or a loss of angiotensin-mediated vasoconstriction of the same vessels. Prostaglandins were measured in the renal venous effluent of the autoperfused kidney by Dunham and Zimmerman (1970), and were suggested to be released in response to an

increased RVR. Prostaglandins have been reported to enhance relative blood flow to the inner cortex (C II) (Larsson and Angaard, 1974; Chang et al., 1975; Lonigro et al., 1978) and intrarenal prostaglandin synthesis has resulted from increased angiotensin levels (Lonigro et al., 1978; McGiff and Wong, 1979). Redistribution of intrarenal blood flow has been reported in isolated kidneys by others (Berkowitz et al., 1968; Itskovitz et al., 1973a; Gagnon et al., 1974; Kaloyanides et al., 1974) and has been linked to the renin-angiotensin system. Itskovitz et al., (1973a) reported that in the isolated dog kidney, initial blood flow to the outer cortex was 79% and to the inner cortex was 21%. After 150 minutes of perfusion the inner cortical flow had increased to 34% and intrarenal renin was depleted. Infusion of a synthetic renin substrate, but not A II, re-established the initial blood flow distribution. They suggested that intrarenal A II produced in the medullary vasculature normally vasoconstricted the vasa recta and medullary efferent arterioles. Depletion of renin resulted in reduction of A II and loss of the preferential medullary vasoconstriction. Circulating angiotensin, shown by Carriere and Fryberg (1969) to constrict C I, was suggested by Itskovitz to vasoconstrict the afferent cortical vessels, thereby indirectly increasing the flow to the inner cortex.

Ideally, perfusion with whole blood should closely simulate physiological conditions, and yet, even in the most

successful laboratories the functional parameters undergo progressive change such as a gradual loss of vascular tone, redistribution of intrarenal blood flow and a gradual reduction of GFR (Nizet, 1975; Ross and Nizet, 1978). Our protocol differed from that of Nizet, et al. (1967) in that we recirculated heparinized blood through the donor, oxygenating the blood via the donor's lung, whereas Nizet started the perfusion with heparinized blood that subsequently was oxygenated with a membrane oxygenator. Their preparation was associated with stable RBF, $\dot{V}$ and GFR for sixty minutes. The reasons for the temporary stability of Nizet's preparation and the lack of stability of our preparation is not evident.

B. Use of artificial perfusates

1. Phosphate buffer

When kidneys were perfused with a cell-free phosphate buffer renal vascular resistance was approximately double that seen in vivo and it remained stable throughout ninety minutes of study. These kidneys initially produced urine but flow progressively fell and the kidneys were anuric by ninety minutes. The anuria persisted when the kidney was reperfused with autologous whole blood (Table 4).

A phosphate buffer was used in early studies of renal

tubular transport of p-aminohippurate (Cross and Taggart, 1950; Taggart et al., 1953) and potassium and sodium (Mudge, 1953). These studies were done with rabbit kidney slices in vitro, and Mudge and Taggart (1953) reported a good correlation between the in vitro results and in vivo results from normal, unanaesthetized dogs. As the PAH was transported equally well in the dog kidney slices in phosphate buffer and the intact dog, this buffer was chosen as a perfusate in this study.

The perfusion was associated with a stable vasculature, but because the RVR was much greater than that seen in vivo, and because anuria quickly developed, it appeared that this artificial buffer was not acceptable for physiological studies.

The vasculature constricted in the presence of 10^{-5} g/ml noradrenaline, as indicated by a rise in perfusion pressure when the catecholamine was added to the buffer. The elevated pressure was not sustained over 120 minutes. This could not be accounted for simply on the basis of a decrease in circulating noradrenaline as bioassays of NORA from the perfusate indicated that the concentration had not changed appreciably with time. Although glucose utilization was not examined, Ross et al. (1973) reported that the glucose requirement of the isolated perfused rat kidney was low, much less than that supplied by our buffer. There was no histological evidence of smooth muscle damage, but there was evidence of tubular

necrosis. This suggested that the preparation may not have stayed viable. An alternative explanation for the kidneys' inability to remain vasoconstricted is that the adrenergic receptors may have become tolerant to the continued presence of the agonist. The relationship between the receptor and the adrenergic agonist is discussed in more detail in a later section of the discussion (see I. B.2. iii).

These experiments were useful as a positive indication that it was possible to study the dog renal vasculature in a completely isolated, whole organ situation, and that a stable pressure:flow relationship could be obtained for a limited time. However, as there remained a high resistance to flow, anuria within ninety minutes, a response to noradrenaline which could not be sustained, and histological evidence of tubular necrosis, another possibly more physiological buffer system was tried.

2. Bicarbonate buffer

i) Presence versus absence of calcium

When Krebs-Henseleit buffer was used as the perfusate the renal vascular resistance was stable during 120 minutes of perfusion and, like the kidneys perfused with the phosphate buffer, the RVR was approximately twice that of the dog kidney in vivo. The buffer is composed of ions similar to those in

mammalian plasma and was the perfusate chosen for all subsequent experiments. It is now acknowledged as the perfusate of choice in the isolated perfusion of rat kidneys (Ross and Nizet, 1978; Maack, 1980).

Calcium, (essential for smooth muscle contraction, Mellander and Johansson, 1968; Somlyo and Somlyo, 1970) was deleted and the chelator EGTA was added to the buffer in one set of experiments. This was done to determine the role of this ion in the maintenance of renal function and normal vascular tone. The removal of calcium had no affect on any of the hemodynamic or tubular parameters measured. This suggests that either calcium was not effectively chelated by EGTA, that tightly bound calcium remained and was sufficient for the requirements of the vasculature and tubular function, or that calcium is not a significant ion in the maintenance of renal smooth muscle tone and tubular function.

Unlike the kidneys perfused with blood or phosphate buffer, these kidneys were polyuric, and had a measureable GFR that was approximately one-half of the rate in vivo. This is noted in most reports and is considered to be a defect of isolated perfusion systems. Ross and Nizet, in their 1978 review of isolated perfusion systems, suggest that the cause of the low GFR could be related to the loop of Henle; Maack, in his 1980 review, offers no explanation.

The kidneys in the presence or absence of calcium were natriuretic. This observation is consistent with other studies. Maack (1980) has recently suggested that this is due to an as yet undefined defect in the distal tubule. The kidneys in the present study excreted approximately 50% of the filtered load of sodium and water. Reabsorption of sodium is an index of tubular function, and on the basis of these data, it appeared that tubular function was not within physiological limits. Within the body, reabsorption of sodium primarily occurs in the proximal tubule, and is effected by active transport out of the tubule, and by transfer of solute and water from the tubular lumen to the peritubular space as a result of favourable oncotic and osmotic gradients (Pitts, 1974; Lassiter, 1975). Active sodium transport was not studied here, but isolated, perfused rat kidneys have been shown to have a functional Na-K ATPase system (see Nizet, 1975; Ross and Nizet, 1978). Our initial bicarbonate buffer contained no high molecular weight additives to provide an effective oncotic pressure in the post-glomerular capillaries. The addition of albumin did improve sodium reabsorption to 95% (Table 8).

The U/P osmolality ratio of unity was anticipated as antidiuretic hormone has a very short half-life and none was added to the buffer. The observation that urine was isosmolar to the perfusate was consistent with what would be expected

in vivo in the absence of ADH, and with data from other isolated perfused kidneys (Bahlman *et al.*, 1967; DeMello and Maack, 1976; Ross and Nizet, 1978). Even with addition of exogenous ADH the rat isolated kidney does produce concentrated urine, possibly due to the high perfusate flow through the vasa recta, or due to abnormalities in the distal tubule (Ross and Nizet, 1978; Maack, 1980). Changes in medullary blood flow may reduce the medullary solute gradient and thus result in isothenuria (Thurau, 1964; Goldberg and Ramirez, 1967).

When noradrenaline was added to the perfusate the kidneys responded with an increase in pressure that was dose-related and could be eliminated by the addition of phenoxybenzamine (Figure 3: Table 6). Phenoxybenzamine, an alkylating agent, specifically blocks noradrenergic receptors (Furchgott, 1966). It effectively counteracted the adrenergic response and inhibited further action of the agonist. This response was consistent in eight kidneys, thus, we were satisfied that a drug-receptor interaction could be reproduced in the *in vitro* kidney.

Calcium, although apparently not required for production or maintenance of the renal vascular tone *in vitro*, was required for the contractile response elicited by noradrenaline. (Figure 4: Table 7). Kidneys perfused with calcium-free

buffer did not respond to NORA until calcium was added. To eliminate the possibility that the increase in pressure after the addition of calcium was due to the calcium, and not the noradrenaline, a separate set of experiments was done. Sequential addition of calcium to the calcium-free buffer did not alter the perfusion pressure. The role of calcium in smooth muscle contraction has been well documented (Somlyo and Somlyo, 1970), and has been shown to be required for contraction of isolated vascular smooth muscle (for example, see Seidel and Bohr, 1971; Godfraind and Koba, 1972).

This perfusion system, in which the renal vasculature could respond to both an agonist and antagonist, fulfilled one of the criteria upon which as acceptable isolated perfusion system was based. However, because only 50% of its sodium was reabsorbed, its tubular function did not resemble its status in vivo. Therefore either dextran or albumin was added to increase the reabsorption of sodium.

ii) Dextran as an osmotically active solute

The presence of 3.75g% dextran in the bicarbonate buffer was associated with an RVR that was extremely high, and increased with time of perfusion (Figure 5). Addition of an osmotically active solute, like dextran, to a cell-free perfusate should have provided a driving force to move water

from the tubular lumen through the intercellular space and into the post-glomerular capillaries. This effect has been reported by Gazitua et al. (1969) when dextran was infused intra-arterially into dog kidneys. Although dextrans have been used in in vitro perfusion studies (Murphy et al. 1968; Welbourne, 1974; Jeske et al., 1974; Franke and Weiss, 1976), Pegg and Farrant (1969) found that dextran increased RVR. Both Ross and Nizet (1978) and Maack (1980) have also found dextrans to be unsatisfactory.

One type of dextran that we used had a calcium concentration of over 40 mEq/l, and this may have produced precipitates with carbonate and phosphate ions, and subsequently led to microvascular obstruction that could have caused instability in the preparation. Alternatively, there is the possibility that calcium itself was involved in excessive and irreversible vasoconstriction of the renal bed. Removal of the calcium from the dextran solution did not result in any modification of the high resistance to flow seen with the other dextran supplemented perfusates.

iii) Albumin as an osmotically active solute

The presence of 3.75 g% albumin in the bicarbonate buffer resulted in a significant increase in sodium reabsorption (Table 8). It also was associated with a RVR that was significantly lower than when kidneys were perfused without the

additive; it was the same as that seen in vivo (Table 5). Urine flow rate was similar to that in vivo and GFR was low. Natriuresis was not apparent. Thus, with the exception of the low GFR, the hemodynamic and tubular parameters of function that were measured resembled those of the intact kidney in the dog.

Bovine serum albumin, Fraction V, is the oncotic agent that has been added to cell-free perfusion in the rat (Nishiitsutsuji-Uwo et al., 1973; Bowman and Maack, 1974; Little and Cohen, 1974) and rabbit (Pegg and Green, 1973); Fuller and Pegg, 1976; Pegg, 1977). Its presence is associated with an increase in sodium reabsorption (Besarab et al., 1975; Little and Cohen, 1974) and it has been reported to be more effective at concentrations higher than are found in vivo (Bowman and Maack, 1974; Little and Cohen, 1974; Ross and Nizet, 1978; Maack, 1980).

Low GFR in isolated kidneys is a consistent observation both in our experiments and in other perfusion systems (Besarab et al., 1975; Bahlman, et al., 1967; Nizet, 1975; Maack, 1980). The reason for the low GFR is as yet undetermined. Perhaps it is the expected response of a kidney that has been removed from vasular, hormonal and neural influences. The normal kidney can adjust its GFR in response to both hemodynamic changes such as alterations in BP, RBF, RVR, IRBFD,

effective filtration pressure, and to permeability changes at the level of the glomerulus or tubule (Tost & Nizet, 1973). Wright and Briggs, (1973) have suggested that low rates of glomerular filtration are the result of feedback from the high solute volume or concentration presented to the early distal tubule, and hence the juxtaglomerular apparatus. Subsequent renin release and angiotensin generation would vasoconstrict the pre- and post-glomerular arterioles. Alternatively, the direct effect of angiotensin could be on the mesangial tissue, resulting in a change in surface area or permeability of the glomerular capillary.

When dose-related responses to noradrenaline were determined, in the presence or absence of albumin, the threshold was similar but the slopes were different, as was the maximum pressure that could be attained (Figure 6). The threshold is an index of the affinity of the agonist for the receptor whereas the significant difference in response at 3 and 10 µg indicates a difference in intrinsic activity. That is, the presence of albumin was associated with an increase in intrinsic activity or, with the biological effectiveness of the drug-receptor complex. The role of albumin in determining intrinsic activity is unknown. Gazitua et al. (1969) suggested that the presence of a hyperosmolar solution would reduce the water content of smooth muscle cells. If the cells in this study that were perfused with albumin-free buffer, contained excess

water, perhaps the contractile mechanism would be less biologically effective. The receptor, on the cell surface, would not be affected by the swollen state.

The affinity of the adrenergic receptor for noradrenaline appears to be the same in vitro as in vivo. Fung (pers. comm.) monitored the change in renal blood flow in response to NORA infusion in normal dogs and found that the threshold for decreasing RBF is approximately 200 ng/min (2×10^{-7} g/min). Our threshold, as a bolus into the renal artery, was 0.3 µg (3×10^{-7}g).

We could not obtain dose-related responses to acetylcholine and angiotensin. Acetylcholine would dilate only slightly at all doses administered. However, the kidneys were slightly more sensitive to acetylcholine when albumin was present in the buffer (Figure 7). Angiotensin, too, elicited a greater response when albumin was a constituent of the perfusate. However, the kidneys exhibited acute tolerance to the effects of the angiotensin, so we could not obtain a valid dose-response curve for this agonist. As shown in Figure 8, when bolus doses of A II were introduced, the change in pressure became less. Noradrenaline did not have this affect. The phenomenon of acute tolerance has been reported with sympathomimetic amines (eg. tyramine, ephedrine, dopamine, adrenaline, noradrenaline), and has been explained

as a progressive loss of noradrenaline from vesicles in adrenergic nerve endings (Burn and Rand, 1958). Acetylcholine (Paton, 1954), histamine (Ambrus et al., 1951) and morphine (Schmidt and Livingston, 1933) are some other agonists that have produced acute tolerance, though not via depletion of stored noradrenaline. Similarily, acute tolerance to A II has been documented but the mechanism of tolerance has not been defined (Peach, 1977), although prostaglandin has been suggested to be involved (Gryglewski and Ocetkiewicz, 1974).

Gross morphology and light microscopy of these kidneys did not reveal any structural damage. The initial saline flush and subsequent perfusion was not hampered by clotting (Plate II). Cellular integrity was maintained and no debris or casts were observed (Plate III). The time of perfusion was important as Malanin et al. (1974) found that glomerular endothelium and vascular endothelium were damaged after four hours of perfusion. DNA autolysis had begun which would make subsequent transplantation ineffective.

C. Validity of the isolated perfusion system

In this study a great amount of time and effort was taken to develop an isolated dog kidney perfusion system that functioned as closely to in vivo as possible although we had no a priori reason to assume the kidney in vitro would mimic

its actions in vivo. This meant that although the vasculature, even in the phosphate buffer was responsive, we extensively refined the technique until adequate tubular function was demonstrated. Pegg and co-workers (Pegg and Green, 1973; Pegg, 1970; Fuller and Pegg, 1976; Fuller, et al., 1977) also had comparable difficulties in their quest for a reliable rabbit kidney perfusion system. The ultimate test of viability of the isolated perfused kidney is transplantation.

The isolated perfusion system has both advantages and disadvantages (Nizet, 1975; Fuller et al., 1977; Ross and Nizet, 1978; Maack, 1980). Two of the advantages that are important for the subsequent studies on acute renal failure kidneys are: two kidneys can be perfused simultaneously and one kidney can serve as the reference for the experimental kidney: physiological and pharmacological procedures can be assessed without interference from vascular, nervous and extrarenal hormonal factors.

II. Experimental Model of Acute Renal Failure

Noradrenaline, when infused into one renal artery in vivo, produced changes in C_{Cr}, BP, $\dot{V}$ and RBF consistent with development of ARF (Figure 9) (Knapp et al., 1972; Fung, 1972; Cox et al., 1974). Four hours after the infusion of NORA ceased,

the kidney was anuric, RBF had returned to 30% of the control, the kidney was oligoanuric and GFR was very low (Table 9). These hemodynamic abnormalities are similar to those seen clinically (Hollenberg et al., 1968). Infusion of NORA into one renal artery produces hemodynamic changes of acute renal failure without sustained hemodynamic or tubular functional changes in the contralateral kidney, which was a definite practical advantage for subsequent in vitro studies (Cox et al., 1974; Moskowitz et al., 1975; Mauk et al., 1977; de Torrente et al., 1978; Schrier et al., 1978; Cronin et al., 1978a; 1978b; Patak et al., 1979).

In this in vivo preparation three agonists were infused intra-arterially both before and after NORA was used to produce ARF in an attempt to monitor in situ differences in the reactivity of the vasculature (Table 10). Parathyroid hormone dilated the renal vasculature in a step-wise manner both before and after the induction of failure. Vasodilation in response to parathyroid hormone administration has been reported clinically (Massry et al., 1975), and is similar to the effect of acetylcholine (Thomson & Fung, 1973). Histamine dilated the renal vasculature in a dose-related fashion, and the vasculature was slightly more sensitive when the kidney was in ARF. Histamine acts via specific H_2 receptors to produce renal vasodilation (Banks et al., 1978; McGrath and Shepherd, 1978), and the present data suggest that

the H_2 receptor is not altered by the induction of ARF. Serotonin, which dilated the renal bed prior to its exposure to noradrenaline, constricted the vasculature post-noradrenaline. Serotonin dilates vessels, presumably by inhibiting neurogenic vasoconstriction, but can exert a direct excitatory action of vascular smooth muscle cells (McGrath and Shepherd, 1978). Thus the dilatory but not the contractile response to serotonin is inhibited in the vasculature of the kidney in renal failure.

Since these studies have been completed it has been suggested that 120 minutes of infusion of noradrenaline produces irreversible renal damage. The period of infusion has been shortened to 40 minutes in an attempt to produce a reversible lesion (Mauk et al., 1977; Schrier et al., 1978; de Torrente et al., 1978; Cronin et al., 1978a; 1978b; Patak et al., 1979). The change in protocol is awaiting confirmation.

III. The ARF Kidney In Vitro

A. Renal function

When the kidney that was in ARF was transferred to the perfusion system, its RVR was no different from the RVR of its contralateral control in vitro. This was seen when the kidney was perfused with either of the bicarbonate buffers.

It is possible that the stimulus for *in vivo* vasoconstriction is blood-borne, either as a hormone or some vasoactive material, and that removal of the kidney from the circulating stimulus enables the resistance to return to normal. As the contralateral kidney was exposed to the same blood *in vivo* and yet does not succumb to ARF, these results suggest that some intrarenal difference exists in the two kidneys, most likely at the level of the vascular smooth muscle. This difference allows the ARF kidney but not the control kidney to maintain a high RVR when exposed to the common blood-borne stimulus. The nature of the blood borne stimulus is unknown.

These kidneys were definitely in ARF *in vivo*. The experimental kidney but not the contralateral kidney was oligo-anuric, C_{Cr} was severely reduced and blood flow rate was approximately 30% of its initial value (Table 11 and Table 13). There also was no evidence to suggest that the contralateral control was compromised as a result of the surgical procedure. Although the donor dog had been anaesthetized for 8 to 10 hours before the contralateral control kidney was transferred to the perfusion system, the performance of these kidneys, when compared to those of kidneys from dogs that had not been exposed to this long surgical procedure, was similar. This pertained both to kidneys perfused in the absence (Table 8; Table 11) or in the presence (Table 8; Table 13) of albumin. In albumin-free buffer the pressure:

flow relationship was stable, urine was produced, C_{Cr} was approximately 15% of the *in vivo* kidney, and the kidney reabsorbed only about 65% of its filtered load. When albumin was present the perfusate flow rate was higher than that when albumin was absent, clearance of creatinine was approximately 10% of its *in vivo* value and the kidney reabsorbed 90% of its filtered load of sodium. Therefore we were confident that the differences seen between members of each pair of kidneys after they were transferred from the donor dogs to the perfusion system would be a reflection of the differences between a normal and an ARF kidney.

Although the perfusate flow rates in the ARF and its contralateral control were comparable, the GFR of the ARF kidney was significantly less ($p<0.05$) than its contralateral control. The persistence of a low GFR in the absence of high RVR suggests that a defect at the level of the glomerulus may not be wholly dependent on sustained high RVR. A low GFR *in situ* in both experimental models and the clinical situation without a concomitant low RBF has been reported and provides the basis for the postulate that the primary defect in ARF is within the glomerulus. This was discussed in an earlier section of this thesis (Historical Review). Vasoconstriction may be a secondary phenomenon seen in maintenance of renal failure, as it is well known that renal blood flow returns to normal before GFR during the recovery phase of ARF. Acetylcholine,

dihydralazine, dopamine and bradykinin were infused in patients with ARF and all increased RBF without increasing the low GFR (Moskowitz et al., 1975). It is tempting to suggest that the severely reduced GFR in the ARF kidney in vitro in spite of high perfusate flow is evidence in support of a reduced ultrafiltration coefficient (K_f). This has been suggested to be the mechanism of reduction of GFR by uranyl nitrate in rats (Blantz, 1975; Stein et al., 1975) and the mechanism by which ultrafiltration can decrease although renal plasma flow is constant (Ichikawa and Brenner, 1976; Baylis et al., 1977). K_f can be reduced by altering surface area or glomerular permeability. A II contracts isolated glomeruli (Sraer et al., 1974) and micropuncture studies indicate that it reduces glomerular permeability (Blantz, 1976). Located within the glomerulus are mesangial cells which are contractile (Hobbs et al., 1976), are sensitive to A II and NORA (Sraer et al., 1974; Hobbs et al., 1976) and perhaps are the site of A II receptors (Sraer et al., 1974). A reduction in glomerular ultrafiltration by sustained mesangial contraction could reduce GFR without affecting RBF, and it is tempting to suggest that this is the location of the primary defect in this model of ARF.

B. The vasculature

1. Response to noradrenaline

The biological effectiveness of noradrenaline was reduced in the ARF vasculature. This response was seen both in the absence (Figure 10) or the presence (Figure 11) of albumin. The threshold, and hence the affinity of the receptor for the agonist was no different but the slope and maximum response was less in the kidney in ARF than its contralateral control. The differences could be post-receptor events within the vascular smooth muscle or, alternatively, could be the result of acute tolerance to noradrenaline. The latter is unlikely because repeated doses of NORA did not elicit diminished responses. It is noteworthy that the long experimental protocol used to induce ARF did not alter the dose-response relationship of the control kidney to NORA (Compare Figure 6 with Figures 10 and 11).

2. Response to other agonists

Results of bolus injections of vasodilators and vasoconstrictors are listed in Tables 12 and 14. Acetylcholine slightly dilated the vasculature of both the control and ARF kidneys. Thomson and Fung (1973) infused incremental doses of acetylcholine into the renal artery before and after renal failure was induced by noradrenaline infusion in vivo. Their data suggested that ACh was less biologically effective in the ARF kidney. This finding was not corroborated in the present study. Histamine also vasodilated the kidneys whether

or not ARF had been induced. This suggests that the H_2 receptor responsible for histamine-induced vasodilation was viable; it also contradicts the report that isolated tissue vasoconstricts in response to histamine (Banks et al., 1978; McGrath and Shepherd, 1978).

Serotonin, reported to exert a direct excitatory action on vascular smooth muscle cells (McGrath and Shepherd, 1978), constricted the renal vasculature equally in the control and the ARF kidney. Dopamine, in a high bolus dose, constricted the vasculature of both the control and experimental kidney. This agonist, in low doses (5-50 µg) dilates vessels by a direct action on specific dopamine receptors whereas doses larger than 100 µg have a predominantly alpha adrenergic effect (Goldberg, 1972; Bell et al., 1974; 1975; Pendleton and Setler, 1977). Angiotensin, as discussed earlier, produced acute tolerance and so could not be used to study dose-related changes in pressure. This tachyphylactic phenomenon was discussed by Thomson and Fung (1973) in their study of adrenergic and cholinergic mechanisms in ARF. However, the response to a single bolus dose of 0.3 µg resulted in a reproducible response that was less in the ARF kidney than its contralateral control. This reached a significant level when albumin was present in the buffer.

We had observed that prior exposure of the kidney to A II

increased vascular activity in response to NORA. This effect was seen in the control, but not the kidney in ARF (Figure 12). A potentiation of intraarterially administered noradrenaline to intact vascular beds by A II was reported by Zimmerman (1973) and Ichikawa et al., (1978). The potentiation of noradrenaline by angiotensin has been suggested to be due to increased noradrenaline released from nerve endings (Zimmerman et al., 1972), or blockade of noradrenaline reuptake (Panisset et al., 1968). A II has also been shown to inhibit sodium efflux or enhance influx into smooth muscle causing a nonspecific sensitization (Day and Moore, 1976; Zimmerman, 1978). Since the isolated kidney exhibits the same response seen in situ, it can be suggested that neural factors may play only a small part in potentiation, and that the primary mechanism is at the level of the vascular smooth muscle cell. The lack of potentiation by angiotensin is further evidence that the vascular smooth muscle is altered.

Because the ARF kidney, but not the contralateral control has a high RVR in vivo, it is tempting to postulate that some alteration in the vascular smooth muscle results in its enhanced response to normal levels of circulating vasoactive agents. However, this enhanced responsiveness was not observed in the ARF kidney when it was exposed to any of the agonists and, indeed was diminished relative to its contralateral control when exposed to noradrenaline and angiotensin.

C. The possible defects of the ARF kidney

As the RVR of the *in vitro* kidney was comparable to its contralateral control while its GFR was severely reduced, it can be postulated that the primary defect involved in maintenance of acute renal failure lies within the glomerulus. There are, however, altered vascular smooth muscle responses in these kidneys. They are severely vasoconstricted *in vivo* although this vasoconstriction is not observed *in vitro*. Thus intrinsic vascular tone appears comparable. The response to noradrenaline, angiotensin, and angiotensin-potentiated noradrenaline is less than that of the contralateral control.

Infusion of noradrenaline into the renal artery could be constricting pre- and post-glomerular vessels to reduce RBF, as well as acting on the mesangium, or glomerular capillary to reduce GFR. Once the initial insult is removed the vascular smooth muscle is altered, as is the glomerulus. The defect in GFR remains and is independent of blood flow. The vascular smooth muscle appears to have a normal intrinsic tone although its responsiveness is changed. The biological effectiveness to agonists is reduced, and this suggests that some post-receptor event within the vascular smooth muscle is responsible for the hemodynamic differences seen between the kidney in ARF and its contralateral control.

IV. Summary

1. Isolated perfusion of dog kidneys with either autologous whole blood or phosphate buffer did not result in a stable, reproducible hemodynamics or tubular function.

2. Kidneys perfused at 37°C with a bicarbonate buffer for 90 minutes had a stable pressure:flow relationship, produced urine, and had a low but reproducible GFR. The $\%Reab_{Na}$ was 50% without albumin and 95% when albumin was present in the buffer. This model was considered to be acceptable for study of the kidney in ARF.

3. The vasculature *in vitro* responded in a dose-related fashion to noradrenaline. The response indicated the receptors *in vitro* had the same affinity to the agonist as *in vivo*.

4. The vasculature *in vitro* responded only slightly to the vasodilators acetylcholine and histamine, and responded to dopamine, serotonin, and angiotensin by vasoconstriction. Thus receptors for a variety of agonists were functional, and inability to obtain dose-response curves may be the result of post-receptor events.

5. ARF kidneys perfused with bicarbonate buffer had a significantly lower GFR than the contralateral control. But the high RVR and anuria found *in vivo* were not maintained when the kidney in ARF was removed from the dog. These data support the theory that the primary site of damage in ARF is the glomerulus.

6. The vasculature of the kidney in ARF was less responsive to noradrenaline and to angiotensin, but no differences in its response to histamine, acetylcholine, dopamine, or serotonin were observed. These suggest that the vascular smooth muscle of the ARF kidney could have been damaged by the induction of noradrenaline *in situ*.

7. Potentiation of noradrenergic response by prior exposure to angiotensin was seen in the control but not the kidney in failure.

8. The data suggest that the primary defect in ARF from noradrenaline is at the level of the glomerulus. The vasculature may be less capable of producing appropriate contraction when exposed to agonists, and this may be the reflection of damage incurred by sustained infusion of noradrenaline *in situ*.

9. The ultimate test of the validity of isolated perfusion will be associated with the ability to reperfuse these kidneys *in situ* with blood. This technique is not yet perfected.

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