MICROPUNCTURE STUDIES ON THE DOG'S REMNANT KIDNEY

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Micropuncture Studies on the Remnant Kidney of a Dog a Model of Chronic Renal Failure.

Department of Physiology

Ph.D.

ABSTRACT

Three phase recollection micropuncture studies were performed to assess the response of the remnant kidney in various stages of renal failure to furosemide administration (10 mg/kg) and graded volume expansion (3% and 10% body weight). Mean fractional excretion of sodium, potassium and water rose progressively in the normal dogs (Stage I) after the diuretic maneuvers, with a greater increase in the remnant kidneys in the presence (Stage II) and absence (Stage III) of the contralateral kidney. Proximal and distal TF/P inulin ratios were depressed after 3% volume expansion. However, proximal TF/P inulin was not further lowered after 10% volume expansion and furosemide administration, but distal TF/P inulin was further depressed. Control proximal and distal TF/P inulin ratios in Stage III were significantly lowered compared to the corresponding Stage II. Acute clamping of the contralateral control kidney in Stage I and II resulted in a marked reduction in the proximal TF/P inulin ratios in the experimental kidney. Mean fractional excretion of sodium and water increased significantly in Stage II following clamping but no significant change was observed in Stage I. Additional experiments were performed in an attempt to block the contralateral clamping response in normal dogs by reducing perfusion pressure, acute volume expansion (3% body weight) and denervation. Such maneuvers failed to block the contralateral clamping response. It was concluded, that factors other than perfusion pressure and neural mediation are required to explain the reduced proximal reabsorption of contralateral clamping.

MICROPUNCTURE STUDIES ON THE REMNANT KIDNEY OF A DOG A MODEL OF CHRONIC RENAL FAILURE

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Three phase recollection micropuncture studies were performed to assess the response of the remnant kidney in various stages of renal failure to furosemide administration (10 mg/kg) and graded volume expansion (3% and 10% body weight). Mean fractional excretion of sodium, potassium and water rose progressively in the normal dogs (Stage I) after the diuretic maneuvers, with a greater increase in the remnant kidneys in the presence (Stage II) and absence (Stage III) of the contralateral kidney. Proximal and distal TF/P inulin ratios were depressed after 3% volume expansion. However, proximal TF/P inulin was not further lowered after 10% volume expansion and furosemide administration, but distal TF/P inulin was further depressed. Control proximal and distal TF/P inulin ratios in Stage III were significantly lowered compared to the corresponding Stage II. Acute clamping of the contralateral control kidney in Stage I and II resulted in a marked reduction in the proximal TF/P inulin ratios in the experimental kidney. Mean fractional excretion of sodium and water increased significantly in Stage II following clamping but no significant change was observed in Stage I. Additional experiments were performed in an attempt to block the contralateral clamping response in normal dogs by reducing perfusion pressure, acute volume expansion (3% body weight) and denervation. Such maneuvers failed to block the contralateral clamping response. It was concluded, that factors other than perfusion pressure and neural mediation are required to explain the reduced proximal reabsorption of contralateral clamping.

ABBREVIATIONS

P - plasma concentration

U - urinary concentration

V - urine flow

C - clearance, as calculated by $C = UV/P$

TF - tubular fluid concentration

In - inulin

Na - sodium

K - potassium

GFR - glomerular filtration rate = C_{In}

SNGFR- single nephron glomerular filtration rate

PAH - para-aminohipurrate

RPF - renal plasma flow = C_{PAH}

RBF - renal blood flow = RPF (1/1-Hct)

FE - fractional excretion, as calculated by $FE = UV/P.GFR = C/GFR$

C_{osm} - osmolar clearance

C_{H_2O} - free water clearance, as calculated by $C_{H_2O} = V - C_{osm}$

T^cH_2O- negative free water clearance, as calculated by $T^cH_2O = C_{osm} - V$

$(TF/P_{Na})/(TF/P_{In})$ - fraction of filtered sodium remaining

$(TF/P_K)/(TF/P_K)$ - fraction of filtered potassium remaining

P/TF - fraction of filtered water remaining

FF - filtration fraction, as calculated by C_{In}/C_{PAH}

SEM - standard error of the mean

p - significance

INTRODUCTION

Many chronic renal disorders are characterized by a progressive loss of functional nephrons and hypertrophy of the surviving nephrons, as pointed out by Oliver as early as 1924 (71). He and his co-workers also demonstrated that 80% of the hypertrophy is confined to the proximal convoluted tubules (71, 72). Thus, during the course of chronic renal disease, the nephrons continue to decrease in number and at the same time the remaining nephrons show hypertrophy of various degree. One would not be surprised to find alterations in the functional aspect of the surviving nephrons in chronic renal disease. The residual nephrons enlarge and take on an additional work load in order to maintain excretory function, and to preserve as long as possible the composition of the body fluids with regard to sodium, potassium and other substances essential to survival.

Renal function in chronic renal disease is known to follow certain characteristic patterns (28, 29). The diseased kidney excretes a greater fraction of its filtered solute, particularly sodium (87), and exhibits concentrating and diluting defects (54, 77). Two different theories have been offered to explain the observed defects in chronic renal disease. One view attributes these abnormalities to damage to specific sites in the nephrons and assumes that urine was produced by partially damaged nephrons.

For example, it had been suggested that the inability of the diseased kidney to concentrate and to dilute urine was due to damage to the loop of Henle, which impairs the counter-current mechanisms (47). The alternative view is that the damaged nephrons do not participate in urine formation (34) and virtually all the urine is produced by the surviving nephrons, which have become adapted to the increased work load. The observed defects are due to the adaptation of the remaining 'intact nephrons'. The latter thesis has been derived from an animal model of unilateral pyelonephritis and remnant kidney, developed mainly by Bricker and his associates.

The precise mechanism responsible for this functional adaptation is still unclear. To obtain further information on the possible mechanism at the tubular level, micropuncture studies were performed on hemi-infarcted kidneys of dogs, using the model described by Bricker. The experimental animals were subjected to volume expansion (3% and 10% body weight) and furosemide (10 mg/kg) administration, in the hope that under conditions of stress the abnormalities that exist in chronic renal failure can more readily be detected.

HISTORICAL BACKGROUND

Earlier Works (1892-1939)

A. Earlier attempts to investigate the minimum amount of renal mass necessary to sustain life.

The primary interest of the earlier investigators was to establish the amount of kidney necessary to maintain life. Paoli in 1892 (74) resected portions of the kidney in dogs, cats and rabbits, and observed that the largest quantity of kidney substance that could be removed without endangering life was one-half of one kidney, or one quarter of the total kidney mass. Tuffier in 1899 (85) performed unilateral nephrectomy in dogs and later removed half of the remaining kidney. He found that life was possible with only 1.5 gm of kidney substance per kilogram of body weight.

Bradford in 1899 (15) found that animals deprived of three quarters or more of their total kidney weight, wasted rapidly and died within two or three weeks. Pilcher in 1913 (76) using both cats and dogs, reduced the kidney mass by ligating branches of the renal artery. He found that one-quarter of the total kidney mass was sufficient to sustain life. Allen, Scharf and Lundia in 1925 (4) found that dogs died if more than 75% of the kidney tissue was removed but goats survived in apparently good health with one-fifth of one kidney.

B. Hypertrophy following reduction of kidney mass.

Evidence for renal hypertrophy as demonstrated by increased in urea clearance was shown in 1924 by Addis, Meyer and Oliver (2) who performed unilateral nephrectomies on rabbits. Fifteen to thirty-three days after the operation, they noted that the average urea clearance was 63% of its pre-operative value. Subsequently, the average clearance gradually rose, and four months after nephrectomy attained 79% of the pre-operative value. There was also anatomical hypertrophy of the remaining kidney. Anderson in 1926 (16) found that removal of 70% of the rabbit kidney produced anatomical hypertrophy in the remnant kidney.

Renal hypertrophy following unilateral nephrectomy as measured by kidney weight, was demonstrated in young rats by Mackay, Mackay and Addis in 1932 (68). Maximum hypertrophy was attained by the fortieth post-operative day and was more marked in younger rats.

In 1934 Karsner, Hanzel and Moore (59) followed the urea clearance of four mature dogs before and after unilateral nephrectomy. Their data indicated a decrease in urea clearance after the operation and a more or less complete restoration of its normal value in the course of six months. The remaining kidney weighed 3 to 27% more than the removed kidney, indicating that some hypertrophy had taken place.

Rhoads, Alving, Hiller and Van Slyke (1934) (79) repeated Karsner's experiment. Explantation of one of the kidneys to the exterior with the other left in situ, caused no change in urea clearance (49.8 cc/m^2 of body surface). Subsequent removal of the non-explanted kidney caused a 36% decrease in urea clearance. The fact that the clearance fell only by 36% instead of 50% indicates an increase in functional activity of the remaining kidney. This increase was apparent during the first week following the unilateral nephrectomy. Subsequently the clearance of the single, explanted kidney, during periods of observation extending up to two years, either remained constant or showed a tendency to increase.

C. Early reports on the physiological function of the remnant kidney.

Bradford (1899) (15) is credited with being the first investigator to report the inability of the kidney to concentrate urine following the reduction of renal mass. He observed that after removal of a portions of the dog's kidney, the urine became more abundant and more dilute and the dogs were apparently unable to excrete a concentrated urine; this effect was intensified by the subsequent removal of the opposite kidney. However, Bainbridge and Beddard (1907) (8) using cats and Pearce (1908) (75) using dogs did not confirm Bradford's results.

Karsner (1915) (58) found that excision of one-half or more of the total kidney substance was followed by a slight increase in urine output. Reduction of renal mass by one-third or less produced no change in urine quantity or concentration. A decade later, Allen et al. (1925) (4) found that sheep and goats deprived of 75% or more of their renal mass exhibited marked polyuria. Verney (1929) (87) working with a perfused heart-lung double kidney preparation obtained an immediate increase in urinary flow after ligation of a branch of the renal artery. The reduced kidney tissue responded at a constant blood pressure as if it were subjected to an increased perfusion pressure.

Allen et al. (1925) (4) found elevation of blood pressure from 20 to 30 mm Hg in dogs on a normal diet following partial nephrectomy. These animals also showed a retention of nitrogenerous materials and following a water load, an impaired water excretion with scanty urine output of relatively high specific gravity. However, Anderson (1926) (6) found that removal of 70% of the rabbit kidney did not produce hypertension even when prolonged renal insufficiency resulted. An increase was noted in the blood urea, which rose as high as 80-100 mg%, whereas creatinine was up by 3-3.5mg%. The specific gravity of urine remained unchanged between 1.012 and 1.018.

Apfelbach and Jensen (1931) (7) injected charcoal granules into the renal arteries to occlude the glomerular tufts. The following

changes were noted: decreased ability to concentrate and dilute urine, nitrogenous retention in the blood, polyuria, decreased body weight and metabolic acidosis. Edema and arterial hypertension did not occur.

Clearance Studies (1939-1956)

Increase in kidney weight as an indication of renal hypertrophy following unilateral nephrectomy was demonstrated by Addis in 1948. He showed that in animals subjected to unilateral nephrectomy, the remaining kidney increased in weight by 75%. This was also demonstrated by Platt and his associates in 1952 (78), when they removed half of one kidney in the first operation and the remaining whole kidney about ten days later. They succeeded in showing a 66% increase in kidney weight. The functional unit of the kidney, the nephrons also showed hypertrophy of various degree. Platt observed that the most striking change is one of tubular hypertrophy, which was most obvious in the proximal tubule and the loop of Henle. The tubular lumen was dilated, and glomeruli were also enlarged. This confirmed the earlier morphological studies of Oliver (71, 72).

Hayman (53, 54) attempted to correlate function and structure in the diseased kidney. He and his associates counted the number of permeable glomeruli in the kidneys of patients in whom functional studies had been performed immediately before death. They found

that the reduction of both urea and creatinine clearances were not directly proportional to the reduction in renal mass, nor to the percentage of glomeruli remaining. The clearances were reduced less rapidly than the number of glomeruli, the differences being most marked with the smaller kidney remnant. There was a slight reduction in clearances when the number of glomeruli was reduced to 50% of the original, with no increase in blood urea nitrogen. This might be due to hyperfunction of the surviving nephrons. However, when the renal mass was reduced to a point at which blood urea nitrogen was increased, the decreases in clearance became greater than the reduction of glomeruli. Hayman et al. also correlated function and structure in healthy dogs whose nephron number was reduced by 66%. In these dogs clearances of urea and creatinine amounted to approximately 70% of normal. The results were in good agreement with earlier observations by Addis and co-workers of functional regeneration of 60% of normal in rats whose nephron number were reduced by 75%.

Evidence suggesting a tubular defect in hyposthenuria was presented for the first time by Earle et al. (1944) (40). They measured the GFR, RPF and maximal rate of tubular excretion of diodrast in 22 patients, in various phases of diffuse glomerulonephritis. As the disease advanced, there was a depression of all three parameters associated with marked distortion in their normal

relationship. This was reflected by a low filtration fraction and a low GRF/Tm diodrast ratio. As chronic glomerulonephritis progressed, the tubular function underwent relatively greater impairment than the glomerular filtration rate, as indicated by high GFR/Tm diodrast ratios and a relatively excessive lowering of diodrast clearances.

Platt and associates (77, 78) performed some clearance studies on Wistar rats in which the upper and lower pole of the left kidney was removed initially, followed by a right nephrectomy ten to fourteen days later. A significant reduction in creatinine clearances was observed following the reduction in renal mass. After the nephrectomy the fractional excretion of water, sodium and potassium increased.

Clearance Studies (1957-1965)

Up to 1957 the intrarenal mechanisms responsible for the impaired ability to concentrate and dilute the urine in chronic renal disease had not been adequately explained. Although the opinion was widely held that the specific tubular sites for the concentrating and diluting operations were destroyed by the underlying pathologic processes, no experimental data existed to support this thesis. However, earlier work done by Hayman, Platt and Welt and other associates (53, 54, 78, 91) favoured an alternative explanation. They attributed the impaired ability

to concentrate and dilute the urine in chronic renal disease to functional adaptation in the persisting nephrons of the diseased kidney. The two adaptations considered were: a continuing osmotic diuresis due to high filtered load of urea and an increased gomerular filtration rate per functioning nephrons.

A. Evidence for functional adaptation in the persisting nephrons of chronically diseased kidney. (Intact nephron hypothesis)

Bricker and his associates provided the major bulk of evidence in favor of the 'intact' nephrons in chronic renal failure. The three experimental models which he employed were as follows:

1) A chronic disease characterised by marked contraction of the involved kidney and histologic abnormalities of the persisting nephron. This model (22) was produced by perfusing an anoxic kidney with isotonic saline containing 6-dimethyl aminopurine 3-amino-D ribose and directing the venous outflow of the kidney during perfusion to the exterior through an inlying renal venous catheter to prevent recirculation.

2) Unilateral pyelonephritis was produced using E. Coli (21). This produced a decrease in renal mass, GRF and RPF. Histologically the diseased kidneys demonstrated changes characteristic of both acute and chronic pyelonephritis.

3) A reversible lesion induced by rendering the experimental kidney anoxic for thirty minutes and then perfusing it with a saline

solution under moderate pressure. The histologic changes were limited to tubules and included vacuolated epithelial cells and intratubular casts (22).

Only unilateral renal disease was induced in the above mentioned models. Therefore BUN and other biochemical parameters within the animals are essentially within normal range.

In 1957 Bricker (20) and associates showed that when unilateral renal disease was induced in dogs, the C_{cr}/C_{PAH}, $C_{Cr}/C_{T_{PAH}}$ and CCr/Tm glucose ratios were unchanged. This was taken as evidence that the persisting nephrons in the chronically diseased kidney retained many normal functional characteristics.

I. Functional integrity of the concentrating and diluting mechanisms in the chronically diseased kidney.

Bricker et al. in 1959 (23) showed that the values for T^cH_2O for the experimental kidney, induced with amino nucleoside or pyelonephritis were appreciably below those of intact kidneys. The ratio of solute free water abstracted per 100 ml of GFR for the experimental kidney closely approximated the values for the intact organ. Despite marked reduction in GFR, values for the number of ml of free water in urine per 100 ml of glomerular filtrate were within the normal range for a diseased kidney. In all animals studied, regardless of the severity of the renal lesion in the diseased kidney, the concentrating and diluting capacities were within

the normal range when expressed as ml of $T^{C}H_2O$ or $C_{H_2}O$ per 100 ml of glomerular filtrate. These data served to document the essential integrity of concentrating processes in the persisting nephrons of the diseased kidney.

Bricker, in 1960 (25) performed experiments in dogs with one of three forms of unilateral parenchymal disease, as described earlier. He also made observations on a dog with unilateral hemi-infarcted kidney produced by ligating one of two major branches of the renal artery. The hemi-infarcted kidney is similar to the diseased kidney in that it has lost a significant fraction of its original nephron population and ultimately becomes contracted. However, it was different from the diseased kidney in one major respect; the residual nephrons were anatomically normal and uninvolved with the progressive renal disease. The data presented in this study were in full agreement with their earlier study. The concentrating ability of the experimentally diseased kidney was not markedly impaired when the contralateral kidney was intact. Comparison of the concentrating ability of the diseased kidney with that of the normal showed that in the hydropenic state, the maximal urinary osmolality in the diseased kidney was slightly lower than that of the normal kidney. During the high rate of urine flow induced by mannitol infusion, $T^{C}H_2O$/GFR was slightly less for the diseased kidney and was associated with the excretion of a greater fraction of filtered

solute (C_{osm}/GFR) and filtered sodium (C_{Na}/GFR). These differences were also noted between hemi-infarcted kidneys and the contralateral normal kidneys. Since the modest diminution in concentrating ability in the diseased kidney was present in the hemi-infarcted kidney whose surviving nephrons were anatomically normal, this led the author to suggest the observed differences in concentrating ability were due to at least in part, to functional adaptations in the residual nephrons.

In 1962, Bricker (61) designed a study to elucidate some of the factors responsible for the concentrating defect in uremia. Most of his earlier work in dogs was confined to animals with one of the three types of induced unilateral disease with a normal internal environment. In this study, he looked into the nature of concentrating defects in bilateral renal disease, in uremic patients with chronic renal disease, and also in dogs with severe pyelonephritis involving the inner medulla. After the lesion had stabilized, maximum Uosm and T^cH_2O/GFR were studied. The internal environment was rendered uremic by induction of pyelonephritis, hemi-infarction, ureteral ligation or removal of the contralateral kidney. After the contralateral kidney was damaged, Uosm decreased consistently with osmotic U/P ratio falling as low as 1.5; T^cH_2O/GFR also decreased to a lesser degree. These studies indicated that a diseased kidney which was able to concentrate urine effectively would exhibit an overt concentrating defect following deminution

in the function of the contralateral kidney with the attendant changes in body fluid.

Coburn et al. (33) studied the effect of osmotic load on the concentrating and diluting capacities in dogs before and after resection of five-sixth of the renal mass. During maximal antidiuresis and sustained water diuresis, graded infusions of urea were given to normal and 5/6 nephrectomised dogs. The GFR was taken as an indication of function at the same solute load per functioning mass. Urea was given to produce elevation of blood level to about four and then eight times normal. There was impairment of both maximal and minimal U/P osmolal ratios at basal and at increased solute excretion rates in the remnant kidney. During antiduresis, the maximal U/P osmolal ratio achieved at all observed solute excretion rates was greater in the normal than in the 5/6 nephrectomized animals; similarly, during sustained water diuresis, the 5/6 nephrectomized animals demonstrated an impaired ability to lower minimal U/P osmolal ratios at both basal solute excretion rate and at increased rates of solute excretion. However, when maximal and minimal U/P osmolal ratios of control and partially nephrectomized animals were compared at adjusted solute excretion rates (UosmV/GFR), the two groups were similar. These data confirmed earlier work of Bradford (1918), Hayman (1939), Platt (1952) and the more recent work of Bricker (1965) who demonstrated that subtotal

nephrectomized animals were unable to achieve maximal U/P ratios during antidiuresis. They concluded that factors other than increased solute load per residual nephron, such as an increment of renal tubular transport and changes in renal medullary blood flow might contribute to the altered concentrating and diluting ability seen in chronic renal disease.

In all these studies so far discussed, the effect of renal hypertrophy and possible hemodynamic changes on the renal concentrating mechanism following surgical induction of chronic renal disease could not be separated from the functional changes induced by an uremic environment. In the study of Morrin and Yulis (69), the effect of an uremic environment on the renal concentrating mechanism had been investigated using an experimental model in which a chronic, reversible, uremic state could be produced without a progressive disease in the population of the functioning nephrons. An infarct was produced in one kidney by ligation of renal arterial branches and the size of the infarction determined the severity of the uremia. The urine from the contralateral kidney was diverted to the duodenum and a nephrostomy tube was inserted on this side. When the nephrostomy was clamped, the blood urea rose until a steady state was obtained, and on re-opening the nephrostomy tube, the uremic abnormalities were restored quickly to baseline value. Maximum U/P osmolal ratio, $T^{c}H_{2}0$ and sodium excretion were measured in the uninfarcted renal segment in

uremic and nonuremic states. The BUN during the period of uremia was above 100 mg%. In the uremic state, GFR, T^cH_2O/GFR and the fraction of filtered sodium excreted were higher than the control values, but the U/P osmolal ratio were lower. When the external drainage was restored, the values returned towards the control level. The increase in T^cH_2O in a chronic uremic environment suggested that the medullary countercurrent mechanisms was not impaired by the sustained osmotic diuresis of the uremic state.

II. Residual nephrons capability to conserve sodium.

Bricker and associates (24), employing the experimental model just described, showed that the nephrons of the diseased kidney were capable of conserving sodium with extreme efficiency. Moreover, during mannitol diuresis, occlusion of the ureter of the diseased kidney for four minutes was associated with the delivery of urine virtually free of sodium from the region of the distal tubule. These phenomena indicated that the functioning nephrons were capable of reabsorbing sodium. Sodium excretion by the diseased kidney was also studied during 5% saline infusion, mercurial diuresis and osmotic diuresis induced by intravenous infusion of mannitol, urea, glucose, phosphate and para-aminohippurate. Natriuresis of the diseased kidney paralleled that of the contralateral normal kidney. This study indicated that the nephrons of diseased kidney essentially retain normal sodium transport mechanisms.

III. Functional homogenity of the chronically diseased kidney.

Bricker (26) evaluated the functional homogenity of the chronically diseased kidney in the dog using the glucose titration technique. The technique involved the measurement of glucose concentration in dogs with one of the three types of chronic unilateral renal disease that was described. The glucose titration curves for the diseased kidneys were compared with those simultaneously obtained from the contralateral intact organs. The mean GFR and Tm glucose were considerably lower in the diseased than in control kidneys. Glucose titration curves revealed no splay for either diseased or normal kidney. Within the limitations of the glucose titration method, the residual functioning nephrons of the experimentally diseased kidney in the dog would therefore appear to constitute a basically homogeneous population.

IV. Nephron adaptation to reduction in renal mass.

a) Proximal tubule transport of organic anion

In 1963 Bricker and Rieselbach (80, 81) studied the transport of PAH in the chronically diseased dog kidney in order to obtain further information on the adaptative capacity of the residual nephrons. Measurements were made at three successive stages. Stage I dogs possessed two normal kidneys, pyelonephritis was then induced in one kidney, and the contralateral kidney was left intact (Stage II studies). In Stage III the control kidney

was removed surgically. At each stage, the increment of Tm PAH produced by the infusion of acetate, a substance known to increase PAH transport in vitro and vivo was observed. In Stage I, Tm PAH/GFR values were equally bilaterally, and the increase following acetate averaged 82%. In Stage II, GFR and Tm PAH were greatly decreased in the diseased kidneys, but Tm PAH/GFR ratios before and after acetate remained equally bilaterally. The acetate induced increment averaged 83%. In Stage III, in the face of uremia, Tm PAH increased 195%, while GFR increased 35%. Hence Tm PAH/GFR was 103% greater in Stage III than for the same kidney in Stage II. Thus, despite a marked reduction in nephron population and the intervention of uremia functional organization as judged by PAH, transport showed marked adaptation in capacity.

b) Glomerular filtration rate

Although an increase in GFR has been postulated by Bricker (27) and Platt (77), technical difficulties have so far prevented its demonstration. The limitation of the earlier model was that comparison of GFR from different groups of animals did not provide the proper frame of reference, since the number of surviving nephrons in the diseased kidneys varied, hence the observed differences in GFR did not reflect changes in overall GFR but rather the number of functional nephrons remaining.

To overcome this difficulty, Bricker and Riesebach in 1964 (27) made successive measurements of GFR from the same kidney at the three stages of renal failure in 26 dogs. The average increment in GFR for the group between Stage II and III was 60.6%. The average value for PAH clearance value obtained in Stage III was 66.5% greater than in Stage II. The mechanisms responsible for the increase in filtration were poorly understood and still are. Hyperfiltration of the degree noted in these studies must lead not only to an increase in excretory capacity but to an increase in active transport of a number of solutes including sodium. These latter conclusion were confirmed in 1968 by Epstein with micropuncture studies in rat utilizing the split-oil droplet technique.

Bricker's experiments led him to make the "intact nephron hypothesis" in which he suggested that 1) the diseased kidney consists of a diminished number of nephrons, most of which retain essentially normal functional abilities; 2) certain of the apparent functional abnormalities in bilateral renal disease may relate to adaptive changes imposed by the decreased nephrons population and the attendant derangements of body fluids rather than to structural distortion of nephrons; 3) the overall flexibility of the diseased kidney decreases as the number of constituent nephrons decrease, but 4) there is an orderly and predictable pattern of excretion for all substances.

B. Evidence for tubular defect in the persisting nephrons of chronically diseased kidney.

In 1958, studies by Franklin, Niall and Merrill (47) on patients with chronic renal disease were not in general agreement with those of Bricker and associates. In the former studies, two experimental methods were used on patients with renal insufficiency; 1) Glomerular filtration was reduced by producing hypotension with ganglionic blockade. 2) Blood urea concentration was substantially lowered by hemodialysis and jejunal perfusion. Four patients with renal insufficiency were subjected to induced hypotension. Despite reduction in osmolar clearance, osmolar U/P ratio with maximum 'pitressin' stimulation showed either a slight or no significant increase. In five patients undergoing dialysis for decompensated renal insufficiency, serum urea was lowered by an average of 59%. Despite this lowering in the filtered urea load, osmolar U/P ratio with maximum 'Pitressin' stimulation was unchanged. These results suggested to the authors that the isosthenuria of renal disease might be due to an absolute decrease in free water reabsorption. The dissociation between nitrogen retention and the inability to concentrate urine in some early renal disease as well as the consistent depression of free water reabsorption in the most advanced renal disease may be explained by the malfunction of the medullary counter current system.

In 1964, Gonick (50) and associates studied the effect of 5/6 nephrectomy on sodium conserving ability. Gonick attempted to evaluate the ability of residual nephrons to conserve sodium following subnephrectomy and under conditions of increased osmotic load. Failure to conserve sodium is often an important functional abnormality of chronic renal disease. The major mechanisms responsible are thought to be the increased osmotic load per nephron, and the intrinsic ability of the diseased tubule to respond maximally to salt retaining stimuli. The effect of an increased osmotic load during maximal sodium reabsorption (DOCA treated, salt restricted animal) was compared in normal dogs and dogs that had undergone subnephrectomy. The salt retaining effect of the steroid and low sodium diet was not nullified until the solute load i.e. the plasma urea level, had exceeded five to six times the baseline; above this level, the increment of sodium excretion was comparable to tht seen in animals without salt restriction. The authors concluded, from these data that sodium lost at a severely depressed GFR was related to the increased osmotic load per nephron, whereas salt lost at a modest reduction in GFR could be attributed to a specific tubular defect.

The Third Factor

Thus far in the literature, the maintenance of sodium balance during progressive reduction of nephron mass was accounted for by the following factors. Disease of the nephron and decreased mineralocorticoid stimulation could restrict the capacity of the functioning nephrons to reabsorb filtered sodium. Sustained solute diuresis mediated by increased plasma concentrations of poorly reabsorbable solute such as urea could explain the natiuresis. An adaptive increase in GFR could enhance sodium excretory capacity by increasing filtered load. Finally, increased sodium excretion per nephron could result from a regulated decrease in fractional sodium reabsorption mediated by a control mechanism that is independent of any of foregoing factors.

Schultze (81a) and associates reduced the nephron population of one kidney approximately 85% by ligating most of the terminal branches of the renal artery. The residual nephrons retained their normal blood supply and remained free of anatomic abnormalities. The contralateral kidney was not altered initially. Each animal was maintained on a constant amount of sodium and 9-α-fluorohydrocortisone in a dosage of 1.0 to 0.2 mg per day. In the presence of the contralateral kidney, the sodium excretion rate was considerably greater in the control kidney, and both kidneys reabsorbed over 99% of the filtered sodium. The contralateral kidney was ultimately removed and the animals were left to survive with only the small

population of nephrons in the remnant kidney. On the same salt intake, sodium excretion rate by the remnant increased from 5.3 uEq/min to 84.6 uEq/min and the fraction of filtered sodium reabsorbed decreased from 99.4% to 92.3%. To rule out hyperfiltration as the factor responsible for the observed natiuresis, the renal artery of the remnant kidney was constricted and GFR was diminished to a level below that obtained before nephrectomy. The natriuresis was not abolished. Thus neither hyperfiltration nor mineralcortioid insufficiency could explain the enhanced rate of sodium excretion. Solute diuresis imposed by retention of impermeant anions and urea could not account for the natiuresis.

What then is the regulatory mechanism?

In 1961, de Wardener (36) rapidly expanded the extracellular fluid volume of dogs loaded with mineralcorticoid hormone. The influence of hyperfiltration was excluded since GFR did not increase or decrease following expansion. However, sodium excretion rose and de Wardener suggested the existence of another factor other than a mineralcorticoid hormone or GFR which could be responsible for the depressed sodium reabsorption. In 1965, Dirks (37) and associates utilizing micropuncture techniques in dogs demonstrated that the primary effect of saline loading was exercised in the proximal convoluted tubule. The increased sodium excretion in Schultze's study was independent of GFR and mineralcorticoid stimulation. Therefore, a 'third factor'

was implicated as the other agent responsible for sodium regulation in uremic animals.

Evidence supporting the role of the 'third factor' in renal failure was presented in a preliminary report by Kurtzman and associates (62). They obtained plasma from patients with chronic renal disease whose GFR were less than 5 ml/min. Plasma was dialysed against Ringers bicarbonate; the dialysate was then injected into the proximal tubule of an assay rat and reabsorption measured by the shrinking droplet technique of Gertz. The factor was not found in plasma of normal subjects on a 100 mEq/day sodium diet. It was detected in cases where plasma volume was expanded. It was concluded that sodium balance in such patients was mediated at least in part by the elaboration of a natriuretic hormone, presumably in response to a small increase in effective arterial volume.

A more recent preliminary report by Weber, Bourgoigne, Hwang, Klahr and Bricker (90) showed that a lower molecular weight fraction obtained from uremic serum when injected into rats with a reduced nephron population and on a high salt diet, reduced proximal reabsorption. The natriuresis induced by the uremic fraction was accompanied by phosphaturia. This supported the observed proximal effect of the fraction. However, experimental data supporting Weber et al. have not become available and the existence of such a hormone still requires further investigation.

Investigation of chronically diseased kidney using micropuncture technique.

The number of micropuncture experiments done to date is limited to studies in chronically diseased kidneys of rats.

A. Functional integrity of the proximal tubule.

The first micropuncture studies were done in 1966 by H. Lubowitz, Purkerson and Bricker (65). Unilateral pyelonephritis was induced in male rats. Experiments were performed from one to three months after the induction of the lesion. Their clearance data showed that the GFR for the diseased kidney averaged 35% of the control kidney. Their micropuncture data showed that the increase in TF/P inulin ratio along the course of the proximal tubule was identical in both diseased and control kidneys. The TF/P inulin ratio averaged 2.5 at the midpoint of the proximal tubule from both control and diseased kidney, indicating that 60% of the filtered water had been reabsorbed up to that point. From the TF/P inulin ratios and isotonicity of the tubular fluid, they concluded that sodium transport was unimpaired in the proximal tubules of surface nephrons of the chronically diseased pyelonephritis kidney.

This was confirmed by Bank and Aynedjan (9) who performed a micropuncture study in male rats made bilaterally pyelonephritic by injecting approximately 10^8 organisms of Proteus mirabilis into the exposed urinary bladder. The overall mean GFR was

significantly reduced in the pyelonephritic group, about 43% lower than the mean for the normal group. The clearance data also showed that the maximum urine concentration was markedly reduced and sodium excretion higher in the pyelonephritic rats as compared with the normal group. They failed to detect any difference in proximal TF/P inulin ratios between the two groups of animals.

In 1968, Hayslett, Kashgarian and Epstein (55) performed micropuncture studies in the proximal tubules of uninephrectomized rats in which the right kidney had been removed two to four weeks previously. The fraction of the glomerular filtrate absorbed remained unchanged in the proximal portions of the nephron. The mean TF/P inulin in the late proximal tubule was 2.8 in control animals and 3.1 in the experimental group. Also, in split droplet microperfusion studies, the mean reabsorptive half-time was unchanged in the proximal tubule. The $t_{1/2}$ was exactly the same in both groups, measuring 9.3 sec.

Lubowitz and his associates (65) measured the intratubular pressure of the proximal nephrons of the pyelonephritic rat kidneys and the contralateral control kidneys. The results obtained from the proximal tubules of the pyelonephritic and contralateral control kidneys were 13.5 mm Hg and 11.1 mmHg, respectively, which were not significantly different.

B. The behavior of the distal tubules in the chronically diseased kidney.

Bank and Aynedjian (10) performed a micropuncture study in male rats made bilaterally pyelonephritic. Both the concentrations of inulin and sodium in the distal tubular fluid were measured. Observations on the distal TF/P inulin ratios showed a marked reduction in the pyelonephritic rats. At the midpoint of the distal tubule the mean TF/P inulin in the pyelonephritic rats was 4.0 as compared to 6.6 observed in normal rats. Sodium concentration in the distal tubular fluid was well below plasma level in both the control (range 10-60 mEq/L) and pyelonephritic animals (range 10-50 mEq/L). In both groups sodium concentration fell progressively along the length of the distal nephron. Their data thus demonstrated that the sodium transport mechanism in the distal convoluted portion of the surviving nephrons was capable of establishing and sustaining a sizeable transtubular sodium concentration gradient.

In the split-droplet microperfusion study of Hayslett, Kashgarian and Epstein (55), the $t_{1/2}$ was significantly shortened from 39.3 sec in the normal rats to 22.4 sec after uninephrectomy in the distal convoluted tubule.

C. Adaptive increase in single nephron glomerular filtration rate.

Bank and Aynedjian (9,10) showed that the mean nephron GFR increased significantly from 104 mμl/min/kg in the normal rats to 160 mμl/min/kg in the bilaterally pyelonephritic rats.

This was confirmed by Hayslett and associates (56). They divided their rats into three groups; group A consisted of normal rats, group B rats had undergone uninephrectomy and group C had approximately 85% of renal tissue ablated. The mean blood urea nitrogen in groups A and B was 25 and 27 mg%, respectively, and group C was azotemic (BUN 52 mg%). The mean GFR in rats of group A with two normal kidneys was 820 μl/min/100 gm of body weight. The mean GFR in rats of group B with single kidney was 85% of normal. In group C the total GFR of the kidney remnant was reduced to 23% of the normal rats. GFR per nephron was 45 nl/min in group A, but following uninephrectomy, filtration in individual nephrons in group B increased by 76%. In group C the average single nephron GFR was not significantly different from group B. Although there was no difference in GFR per nephron in group B and C, the proportion of filtered sodium excreted increased in group C. The authors felt that hyperfiltration did not therefore, account for the stepwise increase in sodium excretion per nephron with progressive renal ablation. Since proximal tubular reabsorption, estimated by reabsorptive half-time was unchanged by renal insufficiency, therefore the data

suggested that as renal mass was reduced, sodium excretion was kept constant by reduction in reabsorption of sodium in the distal tubule. This is in full agreement with the earlier study of Bank and Aynedjian (9, 10).

Single nephron GFR was also measured by Lubowitz and his associates (67) as described below. In the first group of rats pyelonephritis was induced in one kidney with a control kidney in situ (Stage II). In a second group of rats, pyelonephritis was induced in one kidney, and two to four weeks later, the nephron population was reduced further by removing the contralateral kidney (Stage III). In the Stage II group, the mean GFR in the diseased kidney diminished by over 60% in relation to the contralateral control organs. The mean GFR per nephron in the diseased kidney of Stage II were not significantly different from the corresponding value for the nephrons of the contralateral control kidney. In animals in which the control kidney had been removed, GFR per nephron was 41% greater than the comparable value for the diseased kidney in Stage II. Thus, they demonstrated an adaptive increase in GFR per nephron, which could enhance the excretory capacity of surviving nephrons.

The Effect of Acute Reduction in Renal Mass on Proximal Reabsorption.

The study of the effect of acute reduction in renal mass on proximal reabsorption was reported in a preliminary form by

Gottschalk and associates (5). They showed that acute reduction of renal mass by uninephrectomy in nondiuretic rats led to a significant drop in proximal TF/P inulin ratios from 2.46 in the control to 1.88 two and a half hours after uninephrectomy. They also reported a rise in SNGFR from 62.5 nl/min to 73.9 nl/min following nephrectomy.

Giebisch (49) placed a silver clamp between the origin of the two renal arteries on the aorta of a rat. Twelve to fifteen days were allowed to elapse before the contralateral kidney was either removed or a sham operation performed. Micropuncture experiments were carried out 15 hours after contralateral nephrectomy. They observed that animals with a clamped kidney and unilateral nephrectomy had a significant elevation in the excretion of sodium. Also, their micropuncture data demonstrated a significant drop in TF/P inulin ratios from 1.87 to 1.62 following nephrectomy, confirming the observation of Gottschalk et al. However, in rats in which the aorta was ligated above the renal arteries to reduce mean arterial pressure to that of control, the TF/P inulin ratios did not change significantly. This was interpreted to mean that the observed reduced proximal reabsorption following acute reduction in renal mass is partly caused by an increase in perfusion pressure.

METHOD

Remnant Kidney Model

Three experimental stages were produced. Stage I consisted of normal dogs without any surgical manipulation prior to the study. Remnant kidney was induced in the left kidney through a flank incision by ligating 3/4 to 5/6 of the branches of the renal artery near the pelvis. This resulted in segmental infarction. These comprised the stage II animals. In order to make the kidney more accessible to micropuncture, the vessels were ligated in a fashion that the remaining renal mass would be located near the centre of the kidney. Two weeks were allowed to elapse following induction surgery before the micropuncture experiments were conducted. Stage III animals were prepared by removing the contralateral kidney two weeks after induction of Stage II. One more week was allowed to elapse following nephrectomy to permit the development of azotemia before the experiments were performed.

Animal Preparation for Micropuncture Studies

Micropuncture studies were performed on mongrel dogs weighing between 10 and 20 kilogram. The three stages described above were employed. The dogs were anesthetised with intravenous injections of pentobarbital (30 mg/kg) followed by injection of small doses of sodium pentobarbital as necessary to sustain

anesthesia. An endotracheal tube was inserted and adequate respiration was maintained with a Harvard respirator. Cannulae were placed in the jugular and foreleg vein for infusion of inulin, Ringer's solution and furosemide. The femoral artery was cannulated for blood pressure measurements and blood samples were obtained from a femoral vein cannula. The bladder was exposed through a suprapubic incision and both ureters were catheterized for urine collections. The left kidney was exposed by a flank incision and prepared for micropuncture.

Micropuncture Technique

The prepared dog was placed between two lucite covers, on one of which rested a Leitz manipulator. The kidney was lifted out of the body and mounted in a lucite kidney holder attached to a metal rod. The renal artery was cannulated with a 27 gauge needle connected to PE 20 polyethylene tubing for injection of F D and C green dye. About one square centimeter of the renal capsule was removed, utilizing a microcautery. This exposed the superficial cortical nephrons which were then illuminated by a fibre optic light source. Mineral oil was permitted to drop onto the decapsulted surface of the kidney to prevent dehydration of the cortex.

Recollection micropuncture was then performed. The site of puncture was selected by injection of 0.1 to 0.2 ml of 5%

F D and C green into the renal artery and timing the appearance of the dye to the surface tubules. This green dye when injected rapidly into the renal artery results in a wave of green colour first seen in the peritubular capillaries two to three seconds after injection. This flush of green disappears from the vessels and fills the proximal tubules after two to three seconds. On the average, the first phase of dye disappears from the proximal tubules after twenty seconds if the glomerular filtration rate is normal. In the dog, the dye reappears in the superficial distal tubules after sixty to seventy seconds and the kidney resumes normal colour after eighty to ninety seconds. Thus, by periodic injection of this dye, late proximal and distal tubules can be identified.

Tubule fluid samples were collected from the late or last accessible site of the proximal tubule as well as from the distal tubule. The collection of tubule fluid is facilitated by the insertion of an oil block into the tubule. This oil blocks was maintained immediately distal to the puncture site to prevent retrograde recollection. Approximately twenty to forty nanoliter tubule fluid samples were obtained and stored with the pipette tip under mineral oil to wait for the analysis of inulin, sodium and potassium.

Efforts was made to obtain recollected samples for all three phases from each puncture site. The re-identification

of the puncture site is facilitated by the injection of a small quantity of nigrosine dye on the surface of the cortex adjacent to the punctured tubule. Sketching the configuration of the tubule and its association with other puncture sites further aided identification. Thus, samples of tubule fluid can be obtained repeatedly from the same site of the nephron, and comparisons made of control and experimental values.

During micropuncture, a standard clearance technique with fifteen minutes periods were employed and heparinized blood samples were obtained at the midpoint of each period.

Chemical Methods

In all experiments, the glomerular filtration rate was measured by inulin clearance. The inulin was administered at a constant rate by a Harvard constant infusion pump, following the initial priming dose. Plasma inulin level was maintained at 100 mg% during the experiment. Inulin in plasma and urine was determined by a modification of the anthrone method of Fuhr, Kaczmarczk and Krüttgen (48). Microanalysis of tubular fluid inulin concentration was performed by a modification of the method of Vurek and Pegram (89).

A total of 28 macro inulin standards, with concentrations ranging from 100 to 400 mg% were analysed and the mean error was 3.7% (S.E. ± 0.51). A total of 25 micro inulin standards, with concentrations ranging from 100 to 400 mg% were analysed

with a mean error of 5.6% (S.E. ± 0.49). A comparison of TF/P inulin ratio in 28 pairs of recollected and control proximal tubular fluid during hydropenia yielded a mean (TF/P recollected/TF/P control) of 0.98 (S.E. ± 0.02).

Sodium and potassium concentration in plasma and urine were measured by flame photometry (Instrumentation Laboratory 143). Microanalysis of tubular fluid concentration of sodium and potassium was performed by a helium glow photometer after Vurek (88).

Experimental Protocol

Three phase recollection micropuncture experiments were performed in four group of experiments.

1) Group 1 experiments included 15 Stage II and 11 Stage III dogs in which tubule fluid samples were collected at the same micropuncture site during the control phase of hydropenia, a second phase of Ringer's solution (Na 145, K 3.5, Cl 128.5 and HCO_3 20 mEq/L) amounting to 3% body weight was infused at 12 ml/min over a period of 30 to 45 minutes. In the third phase, furosemide (10 mg/kg) was injected intravenously followed by a sustaining infusion of the same dosage per hour. In all phases, infusion of Ringer's solution was maintained at an appropriate rate to match the urine flow. The results of the first two phase in Group I experiments were compared with those of the

corresponding phases of the Stage I dogs in Group II experiments which were carried out in the same manner. The data for the third phase of furosemide in 8 Stage I dogs were taken from the previously published results of our laboratory for comparison.

2) Group II experiments included 10 Stage I, 10 Stage II and 8 Stage III dogs in which the effect of graded expansion of extracellular volume was studied. The first two phases of hydropenia and Ringer's infusion to 3% body weight were carried out as described in Group 1. In the third phase, additional expansion of extracellular volume to 7% body weight was made so the total expansion amounted to 10% body weight. This was followed by sustaining infusion with Ringer's solution to maintain fluid balance. Precautions were taken to maintain sodium balance in the Stage III dogs with the additional infusion of saline when necessary, prior to the experiments, to maintain normal hydration.

3) Group III experiments included 10 Stage I and 11 Stage II dogs in which two phase micropuncture study was performed in the remnant kidney before and after ligation of the main renal artery of the contralateral kidney. These experiments were performed to assess the effect of rapid reduction of renal mass on proximal reabsorption in the remnant kidney.

Similar experiments were carried out in an additional five Stage I dogs by obstructing the ureter of the opposite kidney

and sampling at 90-120 minutes after complete contralateral obstruction.

4) Group IV included four groups of normal dogs (Stage I) with nine animals in each group. The purpose of this study was to assess whether the effects of various factors on proximal sodium and water reabsorption are related to the effects on proximal reabsorption produced by acute clamping of the contralateral kidney or complete contralateral obstruction as demonstrated in Group III.

Three phase recollection micropuncture experiments were performed in all four groups.

In Groups IVa, a hydropenic control phase was followed by repunctures 0-15 and 45-60 minutes after contralateral clamping. Simultaneously, with the contralateral clamping, an aortic clamp was placed just above the left renal artery and was adjusted to keep the mean arterial pressure slightly below control.

In Group IVb, the protocol was identical to Group IVa except that the aortic clamp was adjusted so that the perfusion pressure was significantly lowered during the contralateral phase.

In Group IVc, animals received Ringer's infusion amounting to 3% of the body weight during the control phase. Then the contralateral clamping maneuver was carried out. Perfusion pressure was not controlled in this group by an aortic clamp.

Finally, Group IVd, assessed the role of renal nerves on the contralateral clamping response. The left kidney was denervated during the control period by stripping all the nerves along the renal vessels and painting the vessels with xylocaine. Again, two recollections were taken after contralateral clamping.

Statistical Analysis

Mean values for each experimental phase per animal were used for analysis of clearance and micropuncture data. Student's t test was performed for paired and unpaired comparison of various experimental data.

RESULTS

Group I Experiments (3% volume expansion and furosemide administration)

Recollection micropuncture studies were performed in 15 Stage II and 11 Stage III dogs during the control phase of hydropenia, the second phase of Ringer's infusion to 3% body weight and the third phase of furosemide administration at 10 mg/kg. The results of representative experiment in Stage II are shown in Table I.

A) Clearance Data

Summary of clearance data of Group 1 experiments in Stage I, II and III are listed in Table II. For comparison the data in Stage I dogs during hydropenia and Ringer's infusion were taken from the corresponding phases in Group II experiments, and those during furosemide phases were taken from previously published data by Seely and Dirks. In the control Stage I dogs, the glomerular filtration rate were comparable in all three phases (30-31 ml/min), but the fractional excretion of water, sodium and potassium increased from 1.3%, 1.2% and 27% to 5.0%, 3.0% and 36%, respectively, after Ringer's infusion, and to 40.5%, 34.9% and 118% after furosemide. In Stage II, the GFR of the contralateral control kidney in hydropenia had a mean of 36 ml/min which remained significantly unchanged at 33 ml/min after Ringer's but was reduced to 21 ml/min after furosemide. The GFR of the remnant kidney had a mean of 7 ml/min and remained unchanged throughout the experiment. These Stage II dogs were non-azotemic with a BUN of 16 mg% and plasma creatinine

TABLE I

Representative Micropuncture and Clearance Data in Stage II

Kidney	GFR (ml/min) H.	3% E.	10% E.	FE H_2O (%) H.	3% E.	10% E.	FE_{Na} (%) H.	3% E.	10% E.
CK	37.7	39.8	34.7	3.6	17.3	24.3	2.6	9.5	17.6
RK	7.3	8.2	8.0	5.7	22.0	35.4	5.2	12.9	27.2

Sample	Proximal Tubule TF/P_{IN} H.	3% E.	10% E.	TF/P_{Na} H.	3% E.	10% E.
1	2.12	1.84	1.83	0.96	0.93	0.92
2	1.85	1.66	1.64	0.97	0.96	1.01
	Distal Tubule					
3	3.55	2.53	2.93	0.27	0.31	0.64
4	3.34	3.22	2.30	0.33	0.32	0.32
5	2.62	2.98	2.13	0.16	0.22	0.21
6	3.39	2.42	2.30	0.15	0.36	0.50

Abbreviations: GFR = glomerular filtration rate; FEH_2O = fractional excretion of water; FE_{Na} = fractional excretion of sodium; H = hydropenia; 3% E = 3% extracellular volume expansion; 10% E = 10% extracellular volume expansion; CK = contralateral kidney; RK = remnant kidney; TF/P_{In} = tubule fluid-to-plasma inulin ratio; TF/PNa = tubule fluid-to-plasma sodium ratio.

TABLE II

Summary of Clearance Data in Remnant-Kidney Dog

Experimental Stage		Experimental Phase	GFR ml/min	FE_{H2O} %	UNaV µEq/min	FE_{Na} %	U_KV µEq/min	Fe_K %
I		Hydropenia	31±2.2	1.3±0.3	59±9	1.2±0.2	30±3	27±1.8
(10 dogs)		3% ECF volume expansion	30±1.9	5.0±0.7*	118±22*	3.0±0.5*	36±4*	36±2.0*
		Furosemide	30±2.1	40.5±3.0*	1633±249*	34.9±3.2*	806±8*	118±4.7*
	Control	Hydropenia	36±4.5	1.3±0.3	45±13	0.8±0.2	29±2	24±2.0
	Kidney	3% ECF volume expansion	33±4.6	3.8±0.6*	105±24*	1.9±0.3*	46±8*	37±4.9*
II		Furosemide	21±2.7*	29.6±2.4*	804±103*	25.2±2.0*	90±12*	118±8.6*
(15 dogs)	Remant	Hydropenia	7±1.3††	2.3±0.4††	12±3	1.3±0.3††	7±1	28±2.3
	Kidney	3% ECF volume expansion	7±1.6††	6.6±1.1*††	36±7*	3.4±0.6*††	12±3*	42±4.4*
		Furosemide	7±0.8††	49.5±4.0*††	487±71*	40.7±5.0*††	31±4*	121±7.8*
III		Hydropenia	10±0.8	2.8±0.6	30±9	2.0±0.6	14±2	41±3.5§§
(11 dogs)		3% ECF volume expansion	10±1.3	7.5±1.7*	96±28*	5.8±1.6*	22±6*	58±8.7*
		Furosemide	9±0.9	48±1.4*	576±63*	42.7±1.5*	41±7*	153±11.3*§

* Significantly different from preceding phase. § $p < 0.05$, §§ $p < 0.01$ in reference to the Stage II remnant kidney. †† $p < 0.01$ in reference to the corresponding contral kidney

at 0.9mg%. The mean fractional excretion of water, sodium and potassium of the remnant kidney in all three phases increased significantly after Ringer's infusion, and following furosemide administration as shown in Table II. The corresponding values in the contralateral kidney indicated a greater response of the remnant kidney to the diuretic maneuvers. This is illustrated in Fig. 1 in which the fractional excretion of water and sodium for the two kidneys in Stage II are compared. The difference in these values between the two kidneys increased with Ringer's infusion and was markedly exaggerated when the diuresis was greatly enhanced by furosemide administration.

In Stage III dogs seven days following nephrectomy of the contralateral kidney, the mean GFR in the remnant kidney was 10 ml/min and this remained unchanged during the three experimental phases. Mild azotemia developed with the mean BUN at 46 mg% and mean plasma creatinine at 2.5 mg%. Many animals appeared dehydrated but no attempt was made to correct for the apparently negative fluid balance prior to the experiments. Mean fractional excretion of water and sodium in the three phases are shown in Table II. Although the values of these parameters were slightly higher than the corresponding values of the remnant kidney in Stage II they were not significantly different. Mean fractional excretion of water and sodium during the furosemide phase were nearly identical in the remnant kidneys in Stage II and III. However, the mean fractional excretion of potassium in the three phases is significantly higher than the corresponding values.

B) Micropuncture Data

a) Proximal tubules

Mean micropuncture data for Group I experiments in Stage I, II and III are shown in Table III and IV. Table III summarizes the mean proximal micropuncture data. Mean proximal tubule fluid to plasma (TF/P) inulin ratios in Stage I were 1.67 in hydropenia, 1.49 after Ringer's infusion and 1.61 after furosemide. In Stage II, mean proximal TF/P inulin of the remnant kidney decreased from 1.73 in hydropenia to 1.37 after Ringer's infusion and remained unchanged at 1.40 after furosemide. The corresponding responses to these diuretic maneuvers in Stage III were similar with mean proximal TF/P inulin for the three phases at 1.51, 1.39 and 1.39 respectively. The mean hydropenic value of 1.51 in Stage III was significantly lower than the corresponding value in Stage II indicating that proximal reabsorption is reduced in Stage III. Proximal TF/P sodium and potassium remained constant close to unity in all experiments.

b) Distal tubules

The micropuncture data from distal tubules is shown in Table IV and Figures 2, 3, 4 and 5. There was no change in baseline distal water reabsorption in Stage I compared with Stage II. However, TF/P inulin values were reduced in Stage III. After the infusion of Ringer's, the TF/P inulin was significantly reduced to approximately the same level in all three stages. In the furosemide phase the TF/P inulin ratio was reduced in Stage I with further reductions in Stage II and Stage III.

TABLE III

Summary of Proximal Tubule Micropuncture Data in Remnant-Kidney Dogs

Experimental Stage	Experimental Phases	TF/P_{In}	TF/P_{Na}	TF/P_{K}
I	Hydropenia	1.67±0.1	1.00±0.02	1.07±0.06
	3% ECF volume expansion	1.49±0.1**	1.03±0.02	1.04±0.08
(10 dogs)	Furosemide	1.61±0.1	1.01±0.01	0.98±0.05
II	Hydropenia	1.73±0.02	1.00±0.2	1.01±0.04
	3% ECF volume expansion	1.37±0.04**	1.00±0.02	1.04±0.05
(15 dogs)	Furosemide	1.40±0.05	0.97±0.07	1.06±0.05
III	Hydropenia	1.51±0.05†	1.00±0.02	1.04±0.05
	3% ECF volume expansion	1.39±0.10*	0.98±0.02	1.07±0.04
(11 dogs)	Furosemide	1.39±0.04	0.98±0.02	1.07±0.05

* $p < 0.05$, ** $p < 0.01$; in reference to the preceding phase

† $p < 0.05$; in reference to corresponding Stage II value

TABLE IV

Summary of Distal Tubule Micropuncture Data in Remnant-Kidney Dogs

Experimental Stage	Experimental Phases	TF/P_{In}	TF/P_{Na}	TF/P_K
I	Hydropenia	3.86±0.29	0.19±0.02	0.92±0.19
	3% ECF volume expansion	2.96±0.76**	0.26±0.03	0.85±0.17
(10 dogs)	Furosemide	2.06±0.1**	0.90±0.02**	1.72±0.14**
II	Hydropenia	4.04±0.34	0.24±0.03	0.92±0.17
	3% ECF volume expansion	2.97±0.15*	0.30±0.03*	0.72±0.10
(15 dogs)	Furosemide	1.73±0.13**	0.91±0.02**	1.21±0.09**
III	Hydropenia	3.29±0.33	0.24±0.01	0.61±0.12
	3% ECF volume expansion	2.79±0.20**	0.34±0.03**	0.69±0.09
(11 dogs)	Furosemide	1.70±0.09**	0.88±0.02**	2.12±0.05††**

* $p < 0.05$, ** $p < 0.01$; in reference to the preceding phase

††$p < 0.01$; in reference to the preceding stage II.

The mean distal TF/P sodium and potassium values are also shown in Table IV and Figure 4 and 5. It is apparent that in Stage II, the TF/P sodium is slightly increased after Ringer's infusion and further increased dramatically following furosemide, as shown in Figure 4 where the points are above the identity line. The corresponding changes in Stage III are similar to those in Stage II.

Figure 6 illustrates the change in the fraction of the filtered sodium remaining in the distal tubules and urine for Stage II and III when Stage I values were substracted. The mean fraction of filtered sodium remaining in the tubules and the ureteral urine in all stages was calculated by the formula (TF/PNa)/(TF/PIn). Under most conditions, there is a progressive increase in the sodium remaining in both distal tubules and the urine in Stage I toward Stage III, except during furosemide administration when the sodium remaining in the distal tubules in Stage III did not exceed that remaining in Stage II. As the Stage III dogs may have been somewhat salt depleted, as judged by excessive loss of weight following nephrectomy, these differences may be an underestimate and may possibly be exaggerated with additional saline infusion prior to the experiment.

The mean fraction of filtered water, sodium and potassium remaining in the late proximal and distal tubules and ureteral urine in all three stages are illustrated in Figure 7, 8 and 9. In the proximal tubule, the reduced reabsorption during hydropenia in Stage III was shown by a higher remaining fraction as compared with that in Stage I and II in all figures ($p < 0.05$).

In the distal tubule, there appeared to be a trend to progressively higher remaining fractions of water and sodium from Stage I toward III during hydropenia and after Ringer's infusion, suggesting a greater reduction in sodium reabsorption in the loop of Henle from Stage I and III. Although the differences did not attain statistical significance except for those between Stage I and III ($p < 0.05$), the trend was also reflected in the corresponding values in the ureteral urine. After furosemide, there was a significantly greater inhibition of water and sodium reabsorption in loop of Henle in Stage II and III as compared with that in Stage I while no difference was observed in the markedly reduced loop reabsorption between the former two stages. This observations was also supported by the similar finding in the ureteral urine.

Group II Experiments (Graded Volume Expansion)

The purpose of this group was to explore further the proximal and distal tubular function in the dog kidney during progressive reduction in renal mass in response to graded volume expansion. The experimental protocol consisted of a hydropenia control phase, followed by isotonic Ringer's infusion of 3% body weight, with a third phase of isotonic Ringer's infusion to 10% body weight. Urinary losses were balanced with equal volume of Ringer's infusion. An example of such experiments in Stage II is shown in Table I. Micropuncture data were obtained from the remnant

kidney while clearance data were from both the remnant and contralateral control kidneys. In this experiment, GFR was reduced in the remnant kidney to about 1/5 of the contralateral kidney. There was a progressive increase in fractional sodium and water excretion following graded extracellular volume expansion with the values in the remnant kidney consistently higher than those in the contralateral kidney. The graded diuretic response in the final urine was generally reflected in progressive reduction in distal TF/P inulin ratios and a progressive rise in distal TF/P sodium. However, the effect on proximal tubule reabsorption was observed only following 3% extracellular volume expansion and no further reduction in its reabsorption occurred following additional expansion to 10% body weight.

A) Clearance Data

The mean clearance data from Group II are tabulated in Table V. In 10 Stage I dogs, having comparable GFRs in all three phases there was a greater diuretic response to 10% than to 3% expansion with the mean fractional excretion of water, sodium and potassium increasing progressively with increased expansion. In 10 Stage II dogs, the mean GFR of the contralateral kidney in hydropenia was 38.9 ml/min and this remained significantly unchanged after 3% loading but decreased slightly after 10% loading. The GFR of the remnant kidney had a mean of 7 ml/min

TABLE V

Summary of Clearance Data in Graded ECF Volume Expansion in Remnant Kidney Dog

Experimental Stage		Experimental Phase	GFR ml/min	FE_{H2O} %	UNaV µEq/min	FE_{Na} %	U_KV µEq/min	Fe_K %
I		Hydropenia	31.4±1.9	1.3±0.3	59±10	1.2±0.2	30±3	27. ±1.8
		3%E	29.8±1.9	5.0±0.7*	118±22*	3.0±0.5*	36±2	35.5±2.2*
(10 dogs)		10%E	28.8±1.8	11.8±1.1*	369±53*	8.6±1.0*	52±4*	51.3±3.0*
	Control	Hydropenia	38.9±3.2	2.6±0.7	97±23	1.8±0.4	36±4	25.0±2.7
II	Kidney	3%E	37.5±2.8	7.2±2.1*	257±74*	4.0±1.1*	58±11*	40.6±6.2
(10 dogs)		10%E	33.6±1.1	13.9±2.2*	205±136*	8.7±1.9*	73±12*	64.3±8.3
	Remnant	Hydropenia	7.1±0.6	2.8±0.6	18±4	1.9±0.3	8±0.9	32.9±2.1††
		3%E	7.1±0.8	12.2±2.4*††	78±21*	6.7±1.4†	12±2*	49.3±6.2*†
	Kidney	10%E	7.9±0.8	22.2±3.7*††	202±46*	16.2±2.4††	18±2*	66.9±6.7*
III		Hydropenia	7.2±1.3	8.5±1.1§§	71±24	5.0±0.9§§	14±3	51.6±12.0§§
(8 dogs)		3%E	8.3±1.9	17.7±2.7*	116±34*	8.8±1.9*	20±6	62.6±16.7§
		10%E	9.1±2.0	29.0±4.2*	233±66*	18.1±4.4*	29±10*	75.1±20.5*

* Significantly different from preceding phase

† $p < 0.05$, †† $p < 0.01$ in reference to the corresponding control kidney

§ $p < 0.05$, §§ $p < 0.01$ in reference to the Stage II remnant kidney

Abbreviations: 3% E = 3% extracellular volume expansion; 10% E = 10% extracellular volume expansion.

which remained almost the same throughout the experiment. Serum BUN and creatinine were normal, with a mean BUN of 16 mg% and plasma creatinine of 0.8 mg%. The remnant kidney in Stage II had a greater response during volume expansion than its contralateral kidney. The mean fractional excretion of water, sodium and potassium increased with 3% expansion and increased to a greater extent with 10% expansion. This is illustrated in Figure 10. This difference is augmented by 3% volume expansion and even further by 10% expansion. This supports the observation in Group I experiments that the remnant kidney showed greater response to the diuretic maneuveurs.

In Stage III dogs, precautions were taken to maintain sodium balance by a single intravenous infusion of 500 ml of Ringer's lactate over a period of 2 to 3 hours, 3 to 4 days prior to the micropuncture study. These Stage III dogs were mildly azotemic, with a BUN of 47 mg% and a plasma creatinine of 2.3 mg%. The remnant kidney of azotemic dogs had a significantly higher baseline fractional water, sodium and potassium excretion and responded more briskly with 3% and 10% expansion.

The mean fractional excretion of sodium in Stage I, II and III during graded volume expansion is plotted in Figure 11. Stage I kidneys had a progressive increase in fractional sodium excretion from 1.2% to 3% and 8.6%, respectively, with 3% and 10% loading. The normal kidney of Stage II responded

similarly. Stage II remnant kidney which had a greater response with volume expansion, 1.9 in contrast to 6.7 with 3% expansion, and to 16% with 10% expansion. The remnant kidney of Stage III azotemic dogs had a significantly higher baseline fractional sodium excretion and responded more briskly to 9% and 18% with 3% and 10% expansion.

B) Micropuncture Data

a) Proximal tubules

Table VI summarizes the mean proximal tubule micropuncture data in the normal kidney of Stage I and the remnant kidney of Stage II and III. The mean TF/P inulin in hydropenia was 1.67 in Stage I, which is not significantly different from 1.89 obtained in Stage II. However, in Stage III the mean TF/P inulin was highly significant, reduced to 1.47, which reflects a reduction in proximal fluid reabsorption of 10% when nephron mass is reduced to the point of azotemia. The lower mean control TF/P inulin in Stage III further confirmed the similar observation in Group I experiments that proximal tubule reabsorption is reduced in azotemic animals. In each group, TF/P inulin ratios and, hence, fluid reabsorption were significantly depressed by 3% body weight Ringer's infusion to 1.49 in Stage I, 1.41 in Stage II, and somewhat lower to 1.24 in Stage III. With additional volume expansion to 10% body weight a further small but insignificant

TABLE VI

Summary of Proximal Tubule Micropuncture Data in Remnant-Kidney Dogs

Experimental Stage	Experimental Phases	TF/P_{In}	TF/P_{Na}	TF/P_{K}
I	Hydropenia	1.67±0.09	1.00±0.02	1.07±0.06
	3% ECF Volume Expansion	1.49±0.09**	1.03±0.02	1.04±0.08
(10 dogs)	10% ECF Volume Expansion	1.40±0.10	1.03±0.04	0.99±0.05
II	Hydropenia	1.89±0.15	.97±0.005	1.00±0.06
	3% ECF Volume Expansion	1.41±0.08*	.99±0.17	1.06±0.07
(9 dogs)	10% ECF Volume Expansion	1.36±0.10	1.00±0.11	1.08±0.09
III	Hydropenia	1.47±0.10††	.98±0.02	1.04±0.05
	3% ECF Volume Expansion	1.24±0.05**	.97±0.02	1.06±0.07
(8 dogs)	10% ECF Volume Expansion	1.20±0.07	.96±0.02	1.11±0.06

* $0.01 < p < 0.05$, ** $p < 0.01$ in reference to the preceding phase

†† $p < 0.01$ in reference to corresponding Stage II value.

decrease in TF/P inulin ratios occurred in all stages; 1.40 in I, 1.36 in II and 1.20 in III. These data indicate that proximal tubular reabsorption is readily reduced by volume expansion but the response is not graded. Proximal TF/P sodium and potassium tended to remain at unity in all experiments.

b) Distal tubules

The mean distal TF/P inulin, sodium and potassium data are illustrated in Table VII and Figures 12, 13, 14 and 15. There is again no change in baseline distal water reabsorption in hydropenia in Stage I and II, with TF/P inulin ratios at 3.86 and 4.37 respectively. However, a significant reduction to 3.05 occurred in the azotemic Stage III dogs, complementing the reduced fluid reabsorption observed in the proximal tubule. Distal TF/P inulin ratios then decreased to 2.96 in Stage I and to 2.74 in Stage II with 3% Ringer's infusion. A lower value of 2.22 occurred in the Stage III dogs. In Stage I there is a progressive increase in TF/P sodium values with progressive volume expansion. The mean distal TF/P sodium ratios in Stage I in these three phases were 0.19, 0.26 and 0.34 respectively. In both Stage II and III a slightly higher TF/P sodium value of 0.26 occurs in hydropenia, with significantly greater elevations to 0.34 and 0.30 with 3% expansion and values of 0.52 and 0.48, respectively, with 10% expansion. The development of higher

TABLE VII

Summary of Distal Tubule Micropuncture Data in Remnant-Kidney Dogs

Experimental Stage	Experimental Phases	TF/P_{In}	TF/P_{Na}	TF/P_{K}
I	Hydropenia	3.86±0.29	.19±0.02	.92±0.19
	3% ECF Volume Expansion	2.96±0.16**	.26±0.03	.85±0.17
(10 dogs)	10% ECF Volume Expansion	2.11±0.14**	.34±0.03	1.07±0.14
II	Hydropenia	4.37±0.55	.26±0.04	.77±0.12
	3% ECF Volume Expansion	2.74±0.21**	.34±0.05**	1.03±0.17
(9 dogs)	10% ECF Volume Expansion	2.28±0.22*	.52±0.06	1.24±0.21
III	Hydropenia	3.05±0.21	.26±0.03	.93±0.18
	3% ECF Volume Expansion	2.22±0.20**	.30±0.03	.81±0.22
(8 dogs)	10% ECF Volume Expansion	1.99±0.15	.48±0.05*	1.40±0.36*

* <0.05, ** <0.01 in reference to the preceding phase.

TF/P sodium values during volume expansion in Stage II, suggests that distal tubular sodium reabsorption is reduced in the remnant kidney prior to the development of azotemia, and that this defect is maintained when azotemia ensues.

The mean fraction of filtered sodium remaining in the distal tubules and ureteral urine in Stage II and III when Stage I values were substracted are compared in Figure 16. In both distal tubules and the urine there is a progressive increase in sodium remaining in the three phases and the values for Stage III exceed Stage II. This supports our observation in Group I experiments that the remnant kidney showed greater reduction in loop reabsorption in responses to extracellular volume expansion.

Group III Experiments (Acute Clamping and Ureteral Obstruction of the Contralateral Kidney)

To assess the effect of rapid reduction in renal mass on proximal reabsorption, additional experiments were performed in 10 Stage II dogs and 11 Stage I dogs. Proximal tubules were studied before and after ligation of the main renal artery of the contralateral kidney. Similar experiments were carried out in 5 Stage I dogs by obstructing the ureter in the opposite kidney.

The results of the representative experiment in Stage II are shown in Table VIII. Micropuncture data were obtained from the remnant kidney while clearance data were from both the remnant and contralateral control kidneys. In this experiment, GFR was reduced in the remnant kidney to about 20% of the contralateral kidney. There was a decrease in PAH clearances following contralateral clamping. However, there was an increase in fractional water, sodium and potassium excretion following contralateral clamping. Proximal TF/P inulin ratios were depressed by acute clamping of the right kidney.

A) Clearance Data

A summary of the clearance data in the 11 Stage I dogs is presented in Table IX. The mean GFR of the experimental kidney did not change significantly at 27 ml/min and 30 ml/min in the two phases, while PAH clearance fell from 79 ml/min to 65 ml/min. Contralateral clamping increased the fractional excretion of water sodium and potassium.

The mean clearance data of Stage II dogs are given in Table X. In 10 Stage II dogs, the mean GFR remained unchanged at 10 ml/min after clamping the contralateral control kidney, but PAH clearance decreased significantly from 36 ml/min to 25 ml/min. After clamping the contralateral kidney the fractional excretion of water, sodium and potassium significantly increased.

TABLE VIII

Representative Micropuncture and Clearance Data in Stage II Following Contralateral Clamping

Kidney	GFR (ml/min) H	GFR (ml/min) C.C.	C_{PAH} (ml/min) H	C_{PAH} (ml/min) C.C.	FE_{H2O} (%) H	FE_{H2O} (%) C.C.	FE_{Na} (%) H	FE_{Na} (%) C.C.	FE_{K} (%) H	FE_{K} (%) C.C.
CK	29.9		120.7		1.0		0.8		24.1	
RK	6.6	4.9	24.4	15.4	2.3	3.6	1.4	1.9	31.4	40.0

Sample	Proximal Tubule (TF/P_{IN}) H	Proximal Tubule (TF/P_{IN}) C.C.
1	1.92	1.43
2	1.86	1.26
3	1.86	1.62
4	1.78	1.35
5	1.71	1.45
6	1.36	1.40
7	1.50	1.31

Abbreviations: H = Hydropenia RK = Remnant kidney

C.C. = Contralateral clamping CK = Control kidney

TABLE IX

Summary of Clearance Data in Acute Clamping of Contralateral Kidney in Normal Dogs (11 Dogs)

Experimental Phases		GFR ml/min	CPAH ml/min	FE_{H2O} %	FE_{Na} %	FE_{K} %
Control Period	Right Kidney	31±3.3	86±7.7	2.0±0.69	1.0±0.21	22.8±3.0
	Left Kidney	27±2.8	79±7.8	1.6±0.46	1.0±0.21	25.0±3.5
Acute Clamping Right Kidney	Left Kidney	30±3.1	65±7.7*	2.0±0.52	1.1±0.19	32.3±3.6*

* p <0.05 in reference to the preceding phase

TABLE X

Summary of Clearance Data in Acute Clamping of Contralateral Kidney

in Stage II Dogs (10 Dogs)

Experimental Phases		GFR ml/min	CPAH ml/min	FE_{H2O} %	FE_{Na} %	FE_{K} %
Control Period	Right Kidney	36±3.2	129±10.9	1.8±0.6	1.1±0.4	25.4±3.1
	Left Kidney	10±1.7	36± 4.3	1.5±0.3	.8±0.2	27.6±3.1
Acute Clamping Right Kidney	Left	10±1.7	25± 4.0**	2.8±0.7*	1.3±0.2*	37.2±4.5*

* $0.01 < P < 0.05$ in reference to the preceding phase

** $P < 0.01$ in reference to the preceding phase

Table XI summarizes the clearance data in the ureteral obstruction studies. Contralateral obstruction did not change mean GFR, but mean PAH clearances decreased significantly from 101 to 67 ml/min for the micropuncture kidney. A small but insignificant decrease in fractional water (1.4 vs 1.0) and sodium excretion (1.5 vs 1.1) occurred. A significant increase in potassium excretion also occurred.

B) Micropuncture Data

Figure 17 illustrates a plot of TF/P inulin ratios obtained during the control phase, compared to values after contralateral clamping in Stage I dogs. The majority of TF/P inulin ratios are below the identity line, indicating reduced proximal reabsorption. Mean proximal TF/P inulin ratios decreased from 1.85 to 1.45 a highly significant reduction.

Micropuncture data during acute clamping of the right kidney in Stage II are shown in Table XII. Mean proximal TF/P inulin ratios were significantly depressed by contralateral clamping from 1.90 to 1.51. Distal TF/P inulin ratios fell from 5.63 to 3.98, reflecting a small diuretic response observed following contralateral clamping. Mean single nephron glomerular filtration rates in Stage II in the two phases were 70.9 nl/min and 69.2 nl/min, respectively, and the corresponding TF/P sodium and potassium ratios were 0.24 and 0.30 and 0.64 and 1.34, respectively.

TABLE XI

Summary of Clearance Data in Contralateral Ureteral Obstruction

in Normal Dogs (5 dogs)

Experimental Phases		GFR ml/min	CPAH ml/min	FE_{H2O} %	FE_{Na} %	FE_{K} %
Contralateral	Right Kidney	34±2.0	105±12	1.7±1.0	1.9±1.1	19.3±6.0
	Left Kidney	31±2.4	101±10	1.4±0.7	1.5±0.9	20.5±5.6
Ureteral Obstruction of Right Kidney		27±2.8	67±10**	1.0±0.2	1.1±0.3	28.9±3.6*

* $p < 0.05$ in reference to the preceding phase

** $p < 0.01$ in reference to the preceding phase

TABLE XII

Summary of Micropuncture Data in Acute Clamping of Contralateral Kidney in Stage II Dogs

Experimental Phase	SNGFR nl/min	TF/P_{IN} Proximal	TF/P_{IN} Distal	TF/P_{Na} Distal	TF/P_{K} Distal
Control Period	70.9±7.2	1.90±0.15	5.63±0.93	0.24±0.04	0.64±0.15
Exptl. Period	69.2±10.4	1.51±0.07**	3.98±0.39*	0.30±0.03	1.34±0.21*

* $p < 0.05$ in reference to the preceding phase

** $p < 0.01$ in reference to the preceding phase

The plots of proximal and distal TF/P inulin ratios during the control phase as compared to values after clamping the right kidney are shown in Figure 18 and 19. The majority of the proximal and distal TF/P inulin ratios are below the identity line, indicating inhibition of both proximal and distal reabsorption as a result of acute reduction in renal mass.

Figure 20 shows a plot of control proximal TF/P inulin from 5 dogs on the abscissa, compared to values after 90-120 minutes of complete contralateral ureteral obstruction. The majority of TF/P inulins are below the identity line, indicating reduced proximal reabsorption. Mean TF/P inulin declined from 1.78 to 1.49, representing a 11% decrease in fractional water reabsorption.

Group IV Experiments (Role of perfusion pressure, volume expansion and renal nerves in contralateral kidney clamping response)

The previous group of experiments have shown that sodium and water reabsorption in the late proximal tubule is depressed following acute clamping of the contralateral kidney or complete contralateral obstruction. The aim of this group of experiments was to enable us to assess the effect of various factors known to affect proximal tubule sodium reabsorption and its relation to the contralateral clamping maneuver. The representative experiment for this group of experiments is shown in Table XIII.

TABLE XIII

Representative Micropuncture and Clearance Data in a Normal Dog Following Contralateral Clamping and Reduction in Mean Arterial Pressure

Kidney	GFR ml/min			C_{PAH} ml/min			FE_{H2O} %			FE_{Na} %			FE_K %			MAP mmHg		
	H	0-15 min	45-60 mins	H	0-15 min	45-60 mins	H	0-15 min	45-60 mins	H	0-15 min	45-60 mins	H	0-15 min	45-60 mins	H	0-15 min	45-60 mins
Right	29.3			63.2			0.27			0.23			9.9					
Left	30.2	26.7	32.3	71.3	62.0	66.7	0.29	0.44	0.48	0.34	0.31	0.41	13.6	19.8	28.0	118	90	95

Sample	Proximal Tubule TF/P_{IN} H	0-15 min	45-60 mins
1	1.42	1.62	1.64
2	1.71	1.62	1.72
3	2.08	1.38	1.44
4	1.66	1.29	1.54
5	1.78	1.19	1.50

Abbreviations: H = hydropenia
45-60 mins after contralateral kidney
0-15 mins after contralateral clamping
MAP = mean arterial pressure

A) Clearance Data

The mean arterial pressure measured at the level of the renal artery is shown in Table XIV. In Group IVa, simultaneously with the contralateral clamping, an aortic clamp was adjusted so as to keep the control mean arterial pressure slightly below control at 114 and 111 mm Hg. In Group IVb, the aortic clamp was adjusted so that the perfusion pressure was significantly lowered during the contralateral clamping phase to 95 and 93 mm Hg respectively. In Group IVc, perfusion pressure was not controlled in this group by an aortic clamp, but remained unchanged at 118 and 122 mm Hg. Finally, in Group IVd, blood pressure tended to fall with continued clamping.

The mean clearance data in Group IV are tabulated in Table XIV and graphically illustrated in Figure 21 and 22. Figure 21 summarized the mean changes in GFR and PAH clearances in all four groups. The mean GFR remained unchanged in each group, however, PAH clearances tended to decrease in each group. Using these measurements for calculating filtration fraction as shown in Table XIV, a small but insignificant increase was observed in each group. Figure 22 summarizes the fractional excretion of sodium and potassium in the four group. Fractional excretion of sodium remained unchanged from control in each instances. However, a highly significant increase in fractional potassium excretion was observed in each protocol.

TABLE XIV

Summary of Clearance Data in Group IV Experiments

Experimental Protocol		Experimental Phases	GFR ml/min	C_{PAH} ml/min	FF (%)	FE_{H2O} (%)	FE_{Na} (%)	FE_{K} (%)	MAP (mmHg)
Group IVa	I	Control	30±2	109±20	34±4	0.4±0.1	0.7±0.2	22.8±3.2	119±6
Control	II	0-15 mins	25±2	73±10	40±5	0.4±0.1	0.8±0.2	27.4±3.0	114±5
MAP	III	45-60 mins	25±3	69±9	39±4	0.5±0.1	0.5±0.1	32.8±3.6	111±7
Group IVb	I	Control	33±3	95±15	41±3	0.7±0.2	0.8±0.2	17.6±2.0	113±4
Lowered	II	0-15 mins	28±3	83±12	35±6	0.7±0.1	0.6±0.1	23.8±2.5	94±5**
MAP	III	45-60 mins	30±3	78±13	46±4	1.0±0.3	0.7±0.1	30.9±4.1**	93±3**
Group IVc	I	Control	37±3	121±19	34±3	5.6±1.5	3.9±0.9	38.4±4.0	116±4
3% Volume	II	0-15 mins	36±4	94±10	40±2	6.0±1.8	4.2±0.8	41.7±3.8	118±5
Expansion	III	45-60 mins	39±3	98±13	40±2	6.2±1.9	3.5±0.8	49.1±6.3**	122±6
Group IVd	I	Control	35±3	144±14	24±2	1.1±0.2	1.2±0.3	24.8±1.8	120±3
Denervation	II	0-15 mins	32±4	118±16	30±3	1.9±0.4	1.6±0.2	30.5±1.5*	124±6
	III	45-60 mins	33±5	117±21	29±3	1.6±0.3*	1.0±0.1	31.4±2.2*	110±8

* $p < 0.05$, ** $p < 0.01$ in reference to the corresponding control phase

Abbreviations: FF = filtration fraction
MAP = mean arterial pressure.

B) Micropuncture Data

Figure 23 summarizes the proximal TF/P inulin ratios for each of the four groups. In group IVa, following contralateral clamping there was a reduction in mean proximal TF/P inulin from 1.71 to 1.59 and significantly to 1.40 in late collection. A similar decrease occurred in Group IVb, with a reduction from 1.82 to 1.53 after clamping, and remained unchanged at 1.51. It can be seen that TF/P inulin fell significantly whether the perfusion pressure remained constant (Group IVa) or even when deliberately reduced (Group IVb), suggesting that the response to contralateral clamping occurs despite reduced perfusion pressure. In the volume expansion animals (Group IVc) a significant reduction in TF/P inulin from 1.61 to 1.44 and 1.41 was obtained in early and late collections respectively. Finally, in Group IVd, following renal denervation, a smaller but significant reduction in proximal TF/P inulin ratio from 1.67 to 1.49 was still observed.

Figure 24 illustrates the individual TF/P inulin ratios in the second group of dogs (Group IVb) in which the perfusion pressure was maintained 30 mm Hg below control. On the left hand side the control inulin ratios on the horizontal line are plotted against the early recollections on the vertical line. The majority of the points are below the identity line with a mean reduction in inulin ratio from 1.82 to 1.53. Similarly, on the right hand side the control inulin ratios are plotted against

the recollections obtained 45-60 minutes after contralateral clamping. Again, the majority of points are below the identity line with a mean decrease to 1.51, highly significant at both time periods. There was no difference between the TF/P inulin ratios collected immediately after contralateral clamping and in about one hour.

DISCUSSION

The functional characteristics of the remnant kidneys and individual nephrons of dogs and rats have been examined using clearance and micropuncture techniques. The present thesis presents a more detailed study of renal function in the remnant kidney of dogs.

I. Importance of Clearance Data

As the nephron population was reduced by the ligation of secondary branches of the renal artery and subsequent unilateral nephrectomy, the fractional excretion of water, sodium and potassium progressively increased, as shown by the clearance data in Table I and V. The clearance data shown are of importance for the following reasons. First, they provide an assessment of the severity of the lesion, which in functional terms, may be obtained by comparing the GFR of the remnant organ with that of its contralateral control organ or its corresponding stages. In most dogs, the decrease in total function was marked, as indicated by depressed GFR in Group I and II experiments. Second, by relating the values for fractional water excretion, fractional sodium excretion, and fractional potassium excretion from the diseased kidneys to those of the contralateral control kidney of the same animals in Stage II, certain aspects of electrolytes and water excretion

can be compared in diseased and normal kidneys. Both similarities and differences between the paired kidneys emerged. The fractional excretion of water and sodium for the remnant organs exceeded those of the contralateral control kidneys, as illustrated in Figure 1 and 6 where the difference between the two organs is plotted. Third, by comparing the clearance ratios in the kidneys of these animals with previously published data in dogs with unilateral and bilateral renal disease, it is possible to assess the appropriateness of this experimental model. The patterns observed in the Stage II animals, in which the clearance ratios for the diseased kidneys exceeded that of its contralateral control, closely resemble those recently reported by Rieselbach (51, 52).

II. Adaptive Increase in Glomerular Filtration Rate

Increase in fractional excretion of water, sodium and potassium have been attributed in the past to the increase in filtration rate of individual nephrons which occurs as renal mass is reduced. Such an increase would result in a greater delivery of solutes to the renal tubule, and part of the increased solute load would presumably escape reabsorption. For example, Bricker, Klahr and Rieselbach (27) found that the GFR increased by 60% in a single deseased kidney of dogs when the contralateral control kidney was removed. Bank and Aynedjian (9, 10) found

that the single nephron filtration rate rose about 60% above normal in rats with bilateral pyelonephritis that had produced a 43% reduction in total filtration rate. This is confirmed by Hayslett, Kashgarian and Epstein (56) who found that the inulin clearance of individual surface nephrons increased by 65% to 75% after partial nephrectomy. It seems unlikely, however, that changes in filtration rate are primarily responsible for the modulation of electrolyte excretion. Hayslett et al. found that as renal mass was progressively reduced by nephrectomy, the proportion of filtered sodium excrete in the urine simultaneously increased, but nephron glomerular filtration rate apparently attains a maximum of approximately 75 nl/min after 50% of the nephrons are removed. Further ablation of renal tissue did not change the filtration rate despite considerable increase in sodium excretion.

In the present experiments, mean inulin clearance had increased from 7 ml/min to 10 ml/min in the remnant kidney one week after contralateral nephrectomy. The single nephron glomerular filtration rate was also measured in the present experiments in some animals, and is tabulated in Table XV. It can be seen that SNGFR increased from 64 nl/min in Stage I to 73 nl/min in Stage II. This was further significantly increased to 100 nl/min following nephrectomy of the right kidney (Stage III). The increase in inulin clearance and SNGFR after nephrectomy is in general agreement with other reports.

TABLE XV

SNGFR Measurements at Different Stages of Renal Failure

	Stage I	Stage II	Stage III
SNGFR (nl/min)	63.6±4.1	73.2±6.8	99.5±9.4†*
% of change compared to Stage I		15%	56%
n	64	54	23

* p <0.01 compared to Stage I

† p <0.05 compared to Stage II

III. Enhanced Responsiveness of the Remnant Kidney

The present data indicate that the remnant kidney in Stage II shows a greater response to furosemide administration and graded volume expansion, than its contralateral control kidney. Earley et al. (41, 42, 43), and Brenner (17, 18) have shown that alteration in Starling forces is one factor governing the natriuretic response to saline infusion in dogs. Furosemide increases cortical blood flow (14). The present studies demonstrated that diuretic maneuvers which alter physical forces, have different effects upon the remnant kidney. This suggests that the remnant kidney is more susceptible to changes in Starling forces than its contralateral control. This might be due to an altered characteristic of the remnant kidney or a greater increase in cortical blood flow (14) following intravenous furosemide. The altered functional characteristics of the remnant kidney shown by furosemide and graded volume expansion indicated that the remnant kidney possesses altered functional behaviour in the absence of azotemia.

IV. Behaviour of Superficial Cortical Nephrons

The micropuncture data are limited to the behaviour of superficial cortical nephrons. Thus, the conclusion relating to the data obtained must be interpreted as the behaviour of

these nephrons. Furthermore, in common with all micropuncture studies, only a very small percentage of the total nephron population could be sampled, and there is a possibility that by chance the tubules sampled were not a true representation of the overall nephron population. However, sufficient micropuncture data were obtained from the surface tubules during hydropenia, volume expansion and furosemide infusion to offset such a possibility. Punctures were made in persisting nephrons which demonstrated no apparent anatomical abnormalities. The infarcted area was well delineated and involved the upper and lower pole of the kidney. Each of the tubules sampled had continuous flow of tubular fluid and therefore may be presumed to have contributed their fluid to the final urine.

a) Proximal Tubule Behaviour in Remnant Kidney

Bank and Aynedjian (9) showed no significant difference in proximal tubule reabsorption between the diseased kidney of uremic pyelonephritic rats and the kidney of normal control rats, whereas fractional fluid reabsorption in the distal tubule was significantly reduced in the former. Hayslett, Kashgarian and Epstein (56) using the shrinking drop technique in partially nephrectomized rats also found no change in the proximal tubule transport. On the other hand, Lubowitz et al. (66) observed a reduction in proximal tubule bicarbonate reabsorption in uremic,

pyelonephritic rats but failed to demonstrate changes in proximal tubule fluid reabsorption. However, reduction in the latter parameter was shown in the remnant kidney of saline infused uremic rats on a high salt diet (83). The animals with the three stages of renal failure were challenged with graded volume expansion and furosemide administration in order to create greater differences for comparison. Our results demonstrate a significant reduction in proximal tubule reabsorption in hydropenic Stage III dogs as compared to that in Stage II in both Group I and II experiments. In the Group III experiments we have demonstrated a reduction of similar order of magnitude in proximal tubule reabsorption in the remnant kidney of Stage II dogs immediately following clamping of the contralateral renal artery. Since our Stage III dogs also underwent nephrectomy of the contralateral kidney, the reduced proximal tubule reabsorption observed in Stage III is consistent with the findings following contralateral renal artery clamping. All these observations support the theory extended by Bricker (19) that proximal tubule sodium reabsorption is reduced when the functioning nephron population is greatly diminished.

The mechanism by which reduction in proximal tubule reabsorption may occur in the kidney with reduced renal mass is entirely unknown. Bricker (19) has shown recently that a natriuretic humoral factor exists in uremic plasma and acts directly in

the proximal tubule of uremic rats to inhibit sodium reabsorption. The development of lower TF/P inulin values during graded volume expansion and furosemide administration in Stage II suggested that proximal water and sodium reabsorption is reduced in the remnant kidney prior to azotemia, and this defect is maintained when azotemia ensues. The greater natriuresis observed in the remnant kidney following furosemide and volume expansion in Stage II could be due to a greater inhibitory effect of diuretics on sodium transport in nephrons of the remnant kidney, as shown by lower TF/P inulin ratios, or greater changes in Starling forces in response to diuretic maneuvers. Thus, the presence of a humoral factor during azotemia may not provide the sole explanation for the altered characteristics of the remaining nephrons in Stage III. The finding of diminished proximal tubule reabsorption in the Stage II remnant kidney immediatley following clamping of the contralateral control kidney may be explained by alternative mechanisms. Since these dogs were not uremic immediatley following contralateral clamping, the observed reduction in proximal tubule reabsorption could not be related to uremia per se but rather to a certain as yet unidentified intrarenal mechanism. However, it remains possible that the reduction of proximal sodium reabsorption observed in acute and chronic reduction in nephron mass is not mediated by the same factors. The role of elevated plasma urea in our Stage III dogs must

then be evaluated. Kauker, Lassiter and Gottschalk (60) observed no reduction in proximal water reabsorption in the rat when plasma urea was elevated to 39 mM and care was taken not to expand extracellular volume. Nevertheless, recent studies in our laboratory (3, 8, 45) have shown that acute infusion of urea into normal dogs, so as to increase the plasma BUN to 40-50 mg%, inhibited proximal reabsorption. In our Stage III animals, mean plasma BUN was 46 mg% which is comparable to the above study, indicating that urea also played a vital role in the inhibition of proximal sodium reabsorption. Additional Stage III experiments may be necessary to assess the effect on proximal reabsorption of restoring plasma BUN to normal.

It is of interest to note that graded volume expansion (Group II) in both normal and remnant kidneys did not result in progressive reduction in proximal tubule reabsorption, and the greater natriuresis observed following 10% volume expansion was mainly due to its additional effect in the distal nephron since no further reduction in proximal TF/P inulin ratios was observed. It was demonstrated by Davis et al. (35) that the magnitude of reduction in proximal tubule reabsorption remained unchanged at different levels of volume expansion. This is consistent with our findings. On the other hand, micropuncture studies in the rat by Brenner and Berliner (16) showed a progressive

change in proximal tubule reabsorption with graded volume expansion. Therefore, the higher degree of natriuresis observed in the final urine following 10% volume expansion and furosemide administration could not be accounted for by proximal inhibition alone, suggesting the involvement of a more distal site of inhibition of sodium and water transport.

b) Impairment of the Distal Site

The distal tubule in the dog is poorly permeable to water under most conditions (12), therefore changes in distal TF/P inulin ratio following graded expansion and furosemide reflect changes in fluid reabsorption that have occurred mainly in the loop of Henle. There is evidence in the literature which supports the concept that sodium transport at a distal site, especially in the loop of Henle, is important in determining the degree of natriuresis that occurs in the final urine. Thus, intravenous infusion of hyperoncotic albumin and dextran to inhibit proximal tubule reabsorption did not result in significant natriuresis (57). Micropuncture studies (32, 38, 39, 84) have shown that the main site of action of most diuretics is in the loop of Henle. This again emphasizes the important role of the distal nephron in producing diuresis. The further depression of distal TF/P inulin ratios in response to 10% volume expansion and furosemide administration supported the clearance study of Eknoyan et al. (46) who demonstrated

that massive volume expansion inhibits sodium reabsorption in the ascending limb of Henle as indicated by changes in free water clearance. A similar degree of volume expansion depressed sodium reabsorption to a greater degree in the remnant kidney as compared to the normal kidney. This reduction in fractional loop reabsorption of sodium could be due to a direct effect of volume expansion and furosemide or to an increase in sodium delivery to the loops, thereby exceeding its reabsorptive capacity. The fact that fractional loop reabsorption was further reduced with 10% volume expansion and furosemide, while no additional effect was observed in the proximal tubule speaks for its direct effect on the loop of Henle.

The mechanism by which the remnant kidney responds to the diuretic maneuvers with a greater fall in fractional loop reabsorption is not clear since little is known about factors which modulate sodium reabsorption in this segment. However, evidence from both clearance and micropuncture studies (11,30,85) indicates that sodium reabsorption in the loop is reduced when perfusion pressure to the kidney is increased by systemic hypertension. These observations suggest that, as in the proximal tubule (44,63), physical factors in the peritubular capillaries also affect loop reabsorption. The changes in physical factors in the peritubular capillaries of the Henle's loop cannot be evaluated without direct measurement. Although the blood pressure in both Stage II

and III dogs was not elevated in the present studies, peritubular capillary pressure of the loop may be increased in the remnant kidney. Other abnormalities such as increased medullary blood flow could result in a decrease in loop reabsorption. Renal blood flow studies in Stage II and III dogs using a radioactive Xenon washout technique were made in a separate study (31), but we failed to detect any change in intrarenal blood distribution in Stage II. However, in Stage III remnant kidneys showed an increase in medullary flow, which should tend to wash out medullary interstitial solutes. Other considerations included architecture of the remaining medullary nephrons. Presumably, the spatial arrangement of the loops of Henle, vasa recta and collecting ducts in the medulla plays a vital role in the complex interplay between these during the countercurrent operation. Disruption of medullary architecture by a disease process might reduce concentrating ability, not only because some of the individual units were destroyed but also because the spatial arrangement of the remaining intact units would be disturbed and the efficiency of these units in the countercurrent mechanism would be impaired. However, the surgical procedure of ligating secondary renal arteries left the medullary architecture of the remaining nephrons undisturbed.

V. Renal Function Following Acute Reduction of Renal Mass

Group III experiments were designed to assess the effect of rapid reduction in renal mass on proximal reabsorption in the experimental kidney by clamping the normal contralateral kidney in Stage I and II. Following acute ligation of the contralateral renal artery in Stage I and II, fractional reabsorption in the proximal tubule of the experimental kidney was reduced to a similar degree in azotemic Stage III dogs. This was associated with a significant increase in the fractional excretion of sodium, potassium and water in the Stage II dogs. However, the fractional excretion of sodium and water tends to be higher after ligation of the contralateral kidney in normal dogs, but the increase was not significant statistically. The present observation indicates that the greater load delivered to the more distal sites following clamping was adequately handled in normal dogs, but in Stage II animals part of the increased load was excreted into the final urine. This revealed a distal defect in the remnant kidney. A similar observation was reported by Allison et al. (5) in uninephrectomized rats following ligation of branches of the renal artery of the remaining kidney. Since the reduction in renal mass was induced acutely and the animals were non-azotemic, the observed changes in proximal tubule reabsorption should be caused at least in part by factors other than azotemia. To rule out the possibility of the role of urea, plasma BUN

was measured before and after clamping. BUN remained the same in control and experimental phase, and thus urea does not play a role in depressing proximal reabsorption. Since the depression of proximal TF/P inulin ratios occurs very rapidly after the reduction in renal mass, a change in renal hemodynamics should bring about an abrupt change in proximal reabsorption. Separate studies with Carriere (31) in which PAH extraction ratios and Xenon washout curves were studied following acute contralateral clamping, total blood flow and its distribution remained unchanged. In the present studies, mean arterial pressure did not increase significantly following clamping or obstruction, although it did tend to increase. Contralateral clamping or obstruction did not alter GFR, but PAH clearance fell by more than 20%. The hemodynamic changes observed, such as increased filtration fraction should favor an increase in proximal reabsorption. The mechanism of rapid reduction in proximal reabsorption with reduced nephron mass may then depend on subtle hemodynamic changes, such as an increased peritubular capillary pressure or the rapid release of a humoral agent. More direct measurements of peritubular protein concentrations and hydrostatic pressure will be necessary to evaluate physical forces further.

The mechanism involving reduction in proximal tubule reabsorption in the Stage II remnant kidney following acute clamping of the

contralateral control kidney may be involved in the azotemic Stage III kidneys. However, it should be noted that the magnitude of natriuresis induced following uninephrectomy was less than that observed in azotemic animals, suggesting that azotemia per se may have an additional effect of inhibiting water and electrolyte reabsorption.

VI. Role of Perfusion Pressure, Volume Expansion and Denervation in Acute Reduction of Renal Mass

Experimental evidence from Group III indicated that contralateral clamping or obstruction was followed by a significant depression of the proximal sodium and water reabsorption, accompanied by an increase in excretion of water sodium and potassium. The present series of experiments (Group IV) was undertaken to investigate the effect of various factors known to affect proximal tubule sodium reabsorption and its relation to the contralateral clamping maneuver.

As a group (Group IV), on the whole, GFR remained unchanged, however, the PAH clearances tended to decrease in each protocol. Using this measurement for calculation of filtration fraction, a small but insignificant increase was observed in each instance, which would be expected to increase proximal reabsorption since filtration fraction is a reflection of the peritublar protein concentration. However, more direct measurements of peritubular

protein concentrations and hydrostatic pressure will be necessary to evaluate the physical forces. The urinary excretion of sodium remained unchanged from control in each instance, whereas the potassium excretion rate was significantly augmented with contralateral clamping in each protocol. Increased delivery of proximal tubular fluid into the loop of Henle and the distal tubule segments are accompanied by the typical response of this nephron segment to such an event; more sodium is reabsorbed and more potassium is secreted. Increase in the excretion of potassium with the increased delivery of tubular fluid to the distal tuble which is the main site of potassium excretion (13, 70).

The rapidity of the response would at first glance suggest hemodyanmic factors are involved. To investigate the possible role of perfusion pressure (Group IVa and IVb) as a cause of reduced reabsorption, an aortic clamp was employed to maintain or lower mean arterial pressure. This study demonstrated that clamping the control right kidney, rapidly and significantly reduced proximal tubule sodium and water reabsorption whether the perfusion pressure remained constant (Group IVa) or even when deliberately reduced (Group IVb), suggesting that the response to contralateral clamping occurred despite reduced perfusion pressure. It appears that the clamping response is mediated by factors other than the perfusion pressure.

Extracellular fluid volume expansion in animals leads to depressed reabsorption of sodium in the proximal tubule and also to increased sodium excretion (37, 41). This depressed sodium reabsorption is believed to be due to an undefined natriuretic hormone (64) or to be hemodynamic changes which occur during volume expansion. In Group IVc, reduction in proximal reabsorption following contralateral clamping can still be demonstrated in the presence of modest volume expansion, indicating that contralateral clamping may affect the site of reabsorption within the proximal tubules not responsive to saline infusion or augmenting the effect of volume expansion. Finally, in the fourth group following renal denervation, a smaller but significant reduction in proximal TF/P inulin ratio was still observed, indicating that the response could not be negated by denervation. It should also be mentioned that the mean arterial pressure measured at the level of the renal artery did not increase in all protocol. Thus, changes in physical factors do not appear at first glance to influence the contralateral response, even though this question will need to be examined further with detailed studies of surface peritubular hydrostatic and colloid oncotic pressures. As the response occurred despite decreased perfusion pressure, volume expansion and denervation, it suggested that other chemical or humoral mediation may be important in rapidly changing proximal reabsorption when nephron mass is reduced.

ORIGINAL CONTRIBUTION

This thesis represents the first investigation of water and electrolyte transport in the proximal and distal tubule of the remnant kidney of a dog using micropucnture technqiue.

This thesis is therefore the first to show the following:

Group I and II Experiments (Furosemide administration and graded volume expansion)

In these two groups of experiments a proximal defect was revealed in Stage II animals following the diuretic maneuvers. These experiments also presented data to suggest impairment of the loop of Henle as a result of the reduction in renal mass. These defects are present prior to the development of azotemia (Stage II) and can still be seen following unilateral nephrectomy in which the animals become azotemic (Stage III).

Group III Experiments (Acute contralateral clamping)

This group of studies demonstrated that acute reduction of renal mass by clamping the contralateral control kidney in both normal and Stage II dogs led to a significant depression in proximal sodium and water reabsorption. Depression in proximal reabsorption can also be brought about by obstructing the ureter of the contralateral control kidney.

Group IV Experiments (Role of perfusion pressure, volume expansion, and denervation in contralateral clamping)

Results from this series of experiments showed that the depressed proximal reabsorption observed in Group III experiments were independent of the followng: perfusion pressure, volume expansion and renal denervation. This suggests the possible role of a chemical or humoral agent mediating the contralateral clamping response.

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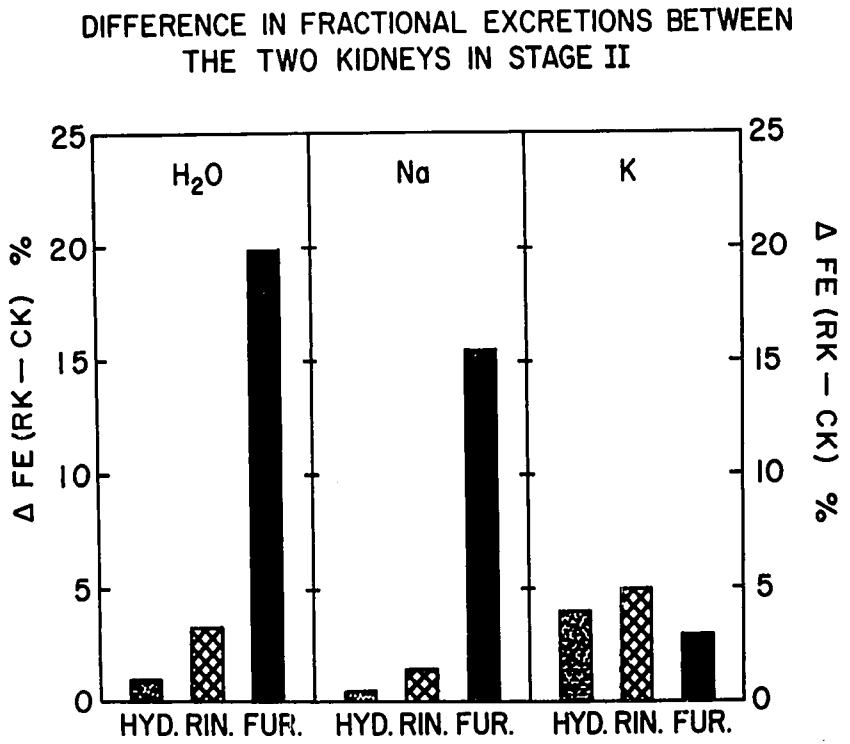


HYD = Hydropenia; RIN = 3% Ringers; FUR = Furosemide.

Figure 1

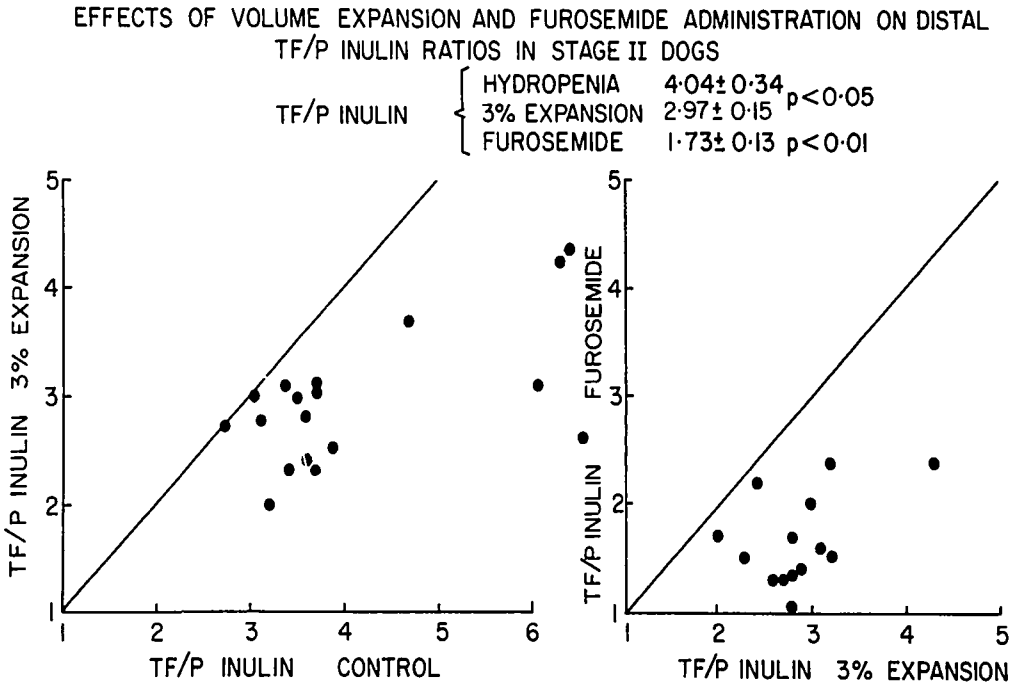

EFFECTS OF VOLUME EXPANSION AND FUROSEMIDE ADMINISTRATION ON DISTAL TF/P INULIN RATIOS IN STAGE II DOGS
TF/P INULIN
HYDROPENIA 4·04± 0·34
3% EXPANSION 2·97± 0·15 p<0·05
FUROSEMIDE 1·73± 0·13 p<0·01
TF/P INULIN 3% EXPANSION
TF/P INULIN CONTROL
TF/P INULIN FUROSEMIDE
TF/P INULIN 3% EXPANSION

Figure 2

EFFECT OF VOLUME EXPANSION AND FUROSEMIDE ADMINISTRATION ON DISTAL TF/P INULIN RATIOS IN STAGE III DOGS

TF/P INULIN	HYDROPENIA	3·29± 0·33	
	3% EXPANSION	2·79± 0·20	$p < 0·01$
	FUROSEMIDE	1·70± 0·09	$p < 0·01$

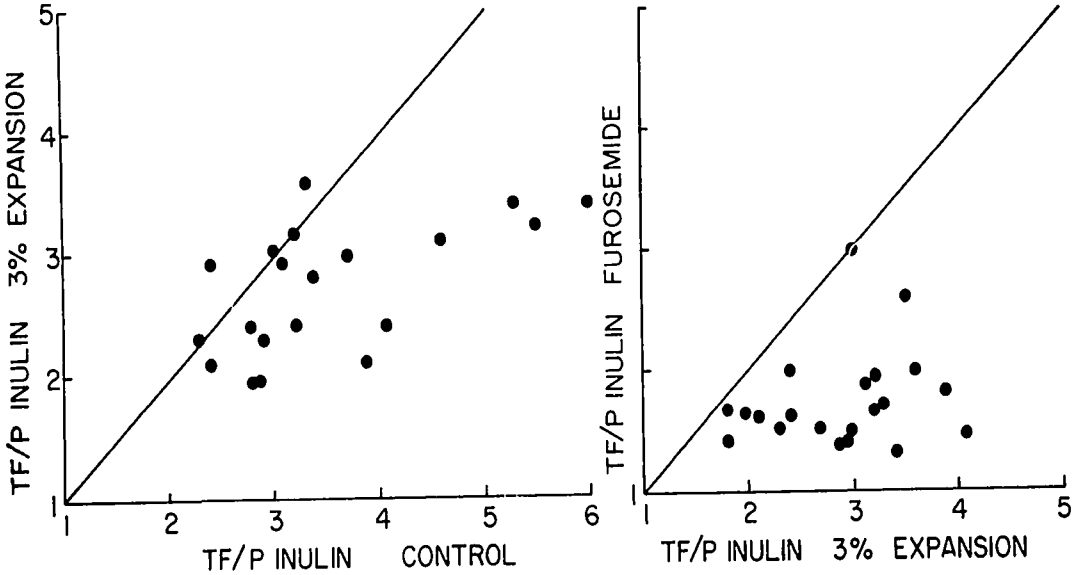


Figure 3

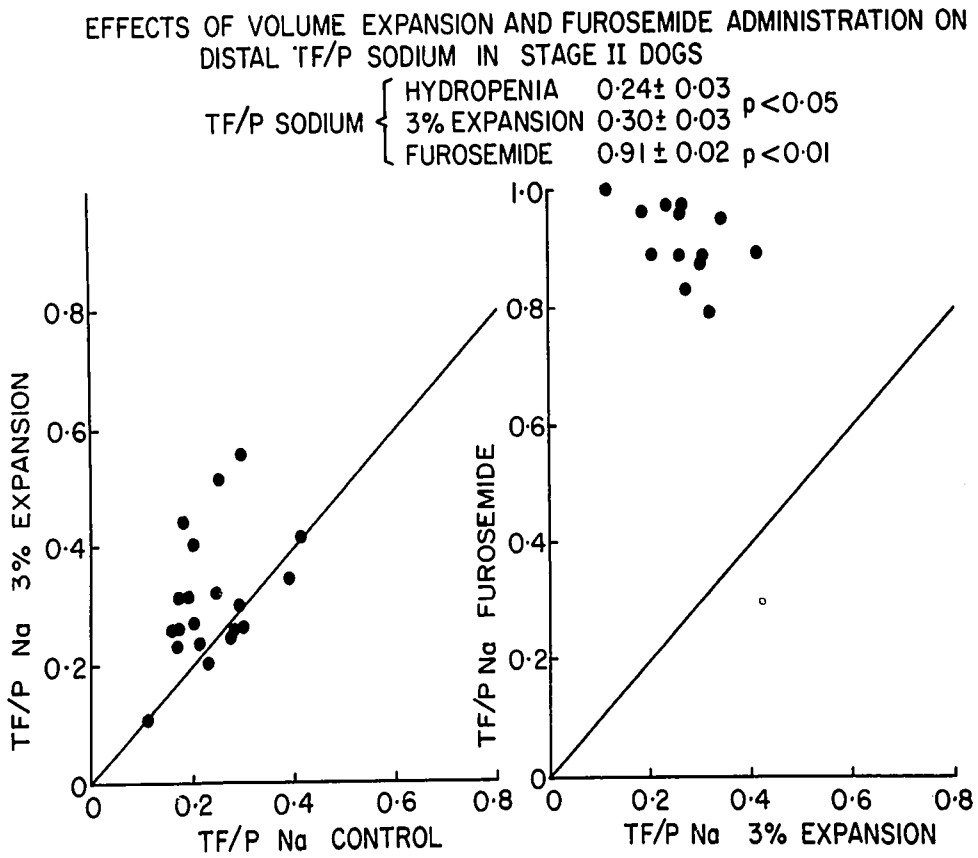

EFFECTS OF VOLUME EXPANSION AND FUROSEMIDE ADMINISTRATION ON DISTAL TF/P SODIUM IN STAGE II DOGS
TF/P SODIUM
HYDROPENIA 0·24± 0·03
3% EXPANSION 0·30± 0·03 p<0·05
FUROSEMIDE 0·91 ± 0·02 p<0·01
TF/P Na 3% EXPANSION
TF/P Na CONTROL
TF/P Na FUROSEMIDE
TF/P Na 3% EXPANSION
0
0·2
0·4
0·6
0·8
1·0

Figure 4

EFFECT OF VOLUME EXPANSION AND FUROSEMIDE ADMINISTRATION ON DISTAL TF/P SODIUM IN STAGE III DOGS

TF/P SODIUM		
HYDROPENIA	0·24± 0·01	
3% EXPANSION	0·34± 0·03	$p < 0·01$
FUROSEMIDE	0·88± 0·02	$p < 0·01$

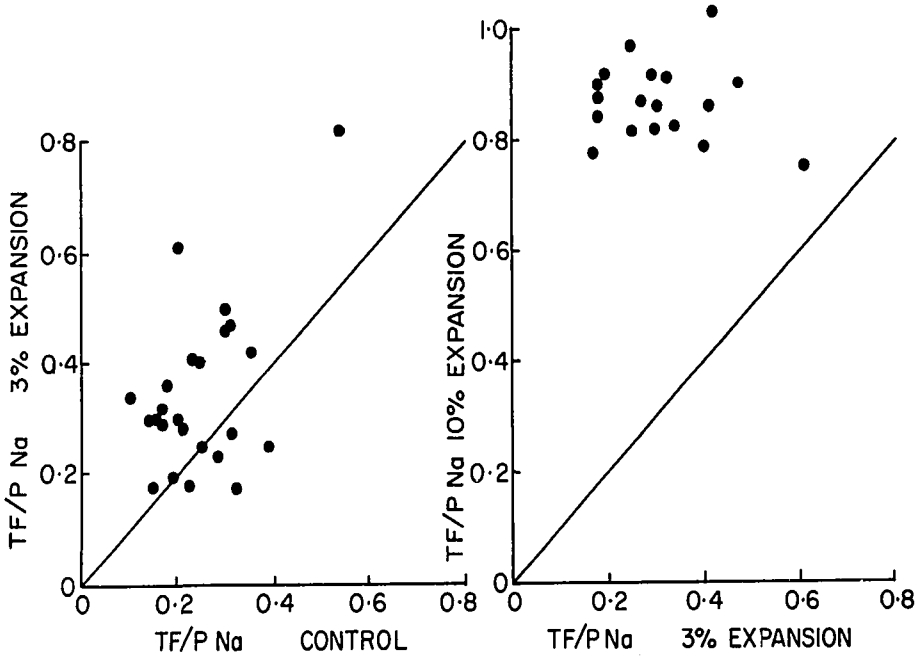
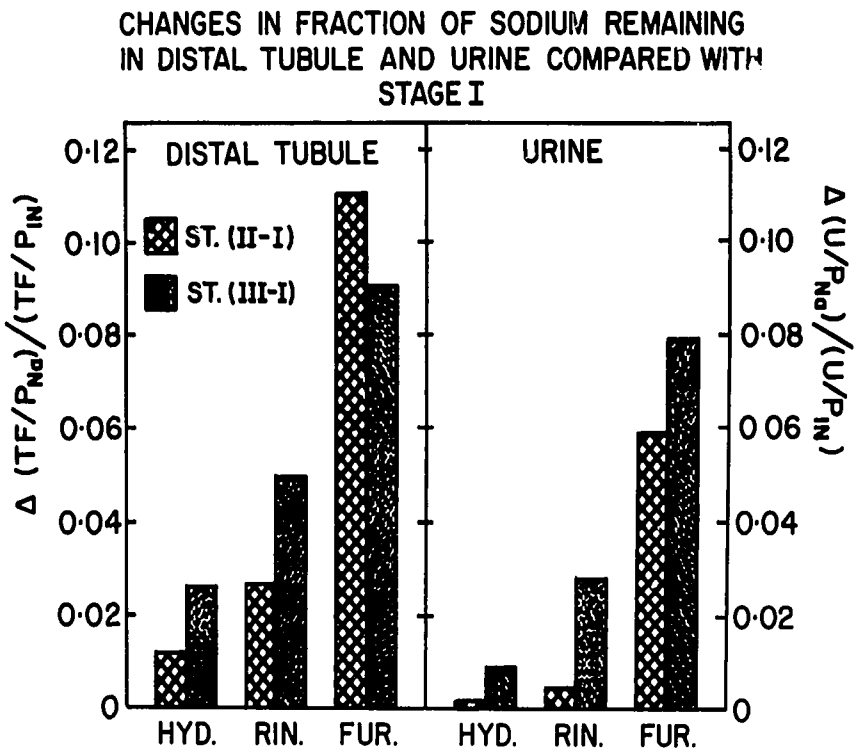
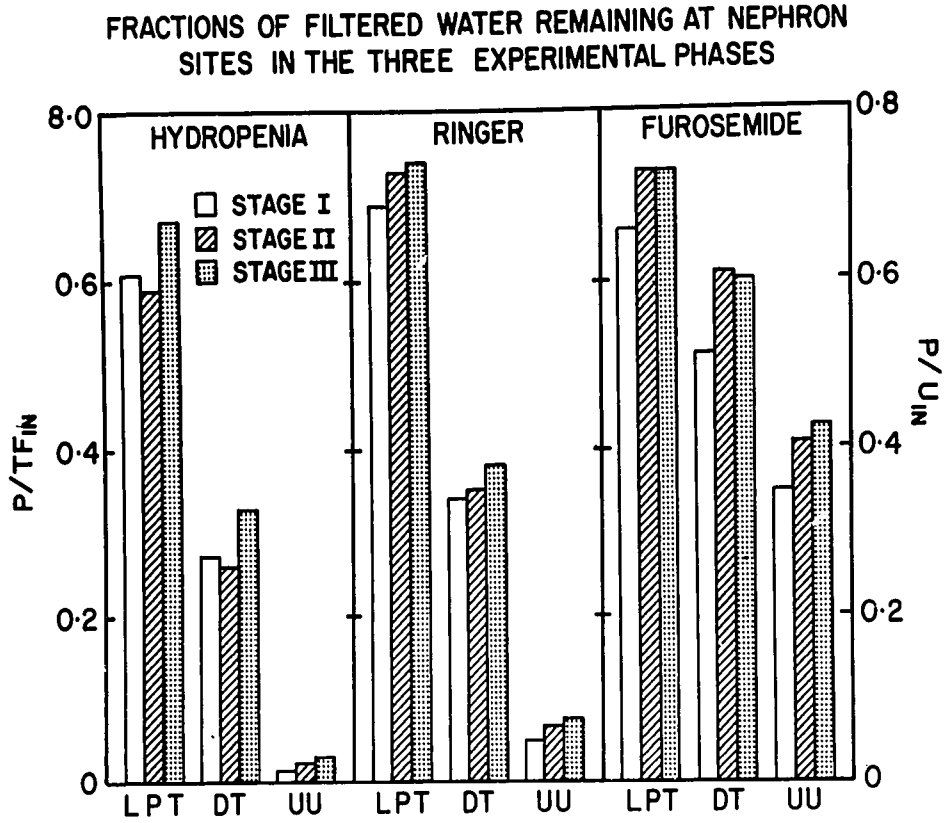


Figure 5



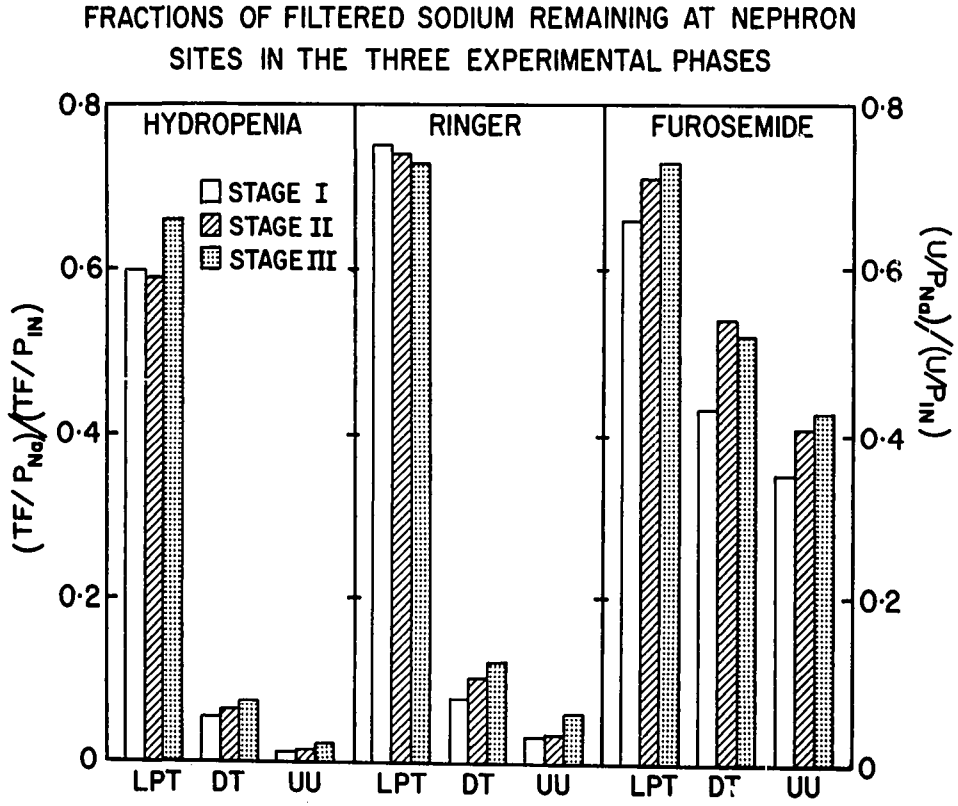
HYD = Hydropenia; RIN = 3% Ringers; FUR = Furosemide.

Figure 6



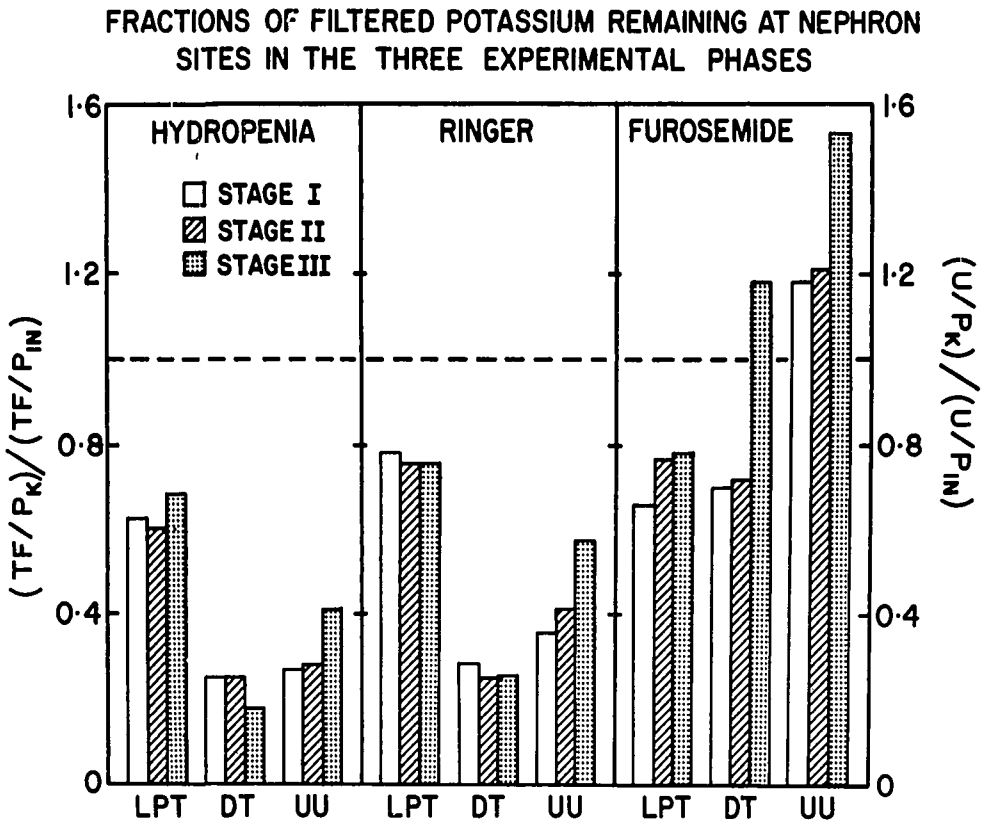
LPT = Late proximal tubule; DT = Distal tubule; UU = Ureteral urine.

Figure 7



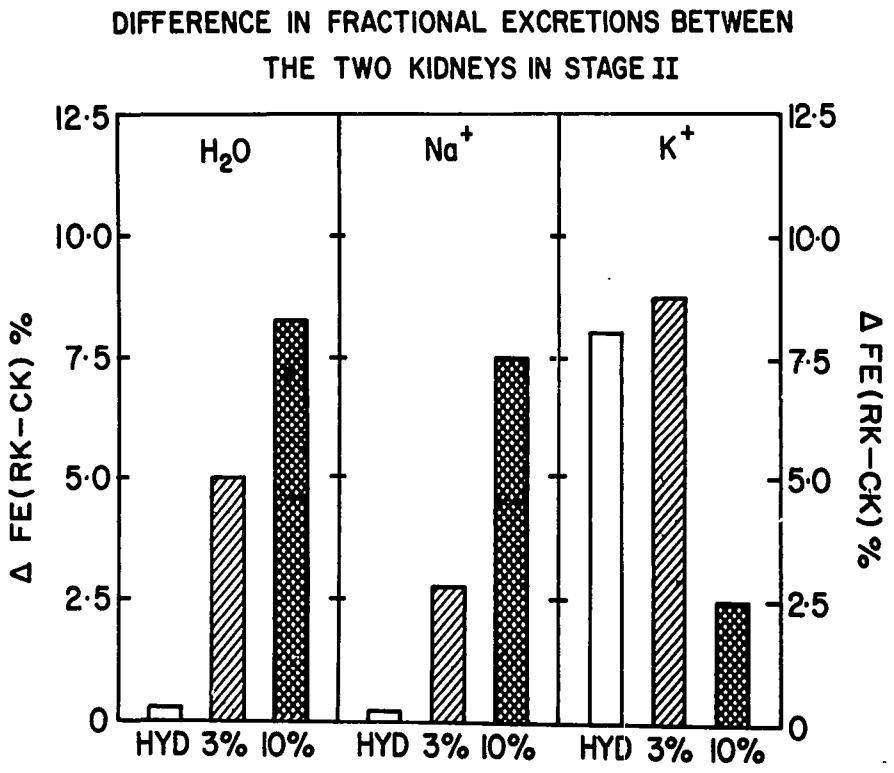
LPT = Late proximal tubule; DT = Distal tubule; UU = Ureteral urine.

Figure 8



LPT = Late proximal tubule; DT = Distal tubule; UU = Ureteral urine.

Figure 9



HYD = Hydropenia; 3% = 3% expansion; 10% = 10% expansion.

Figure 10

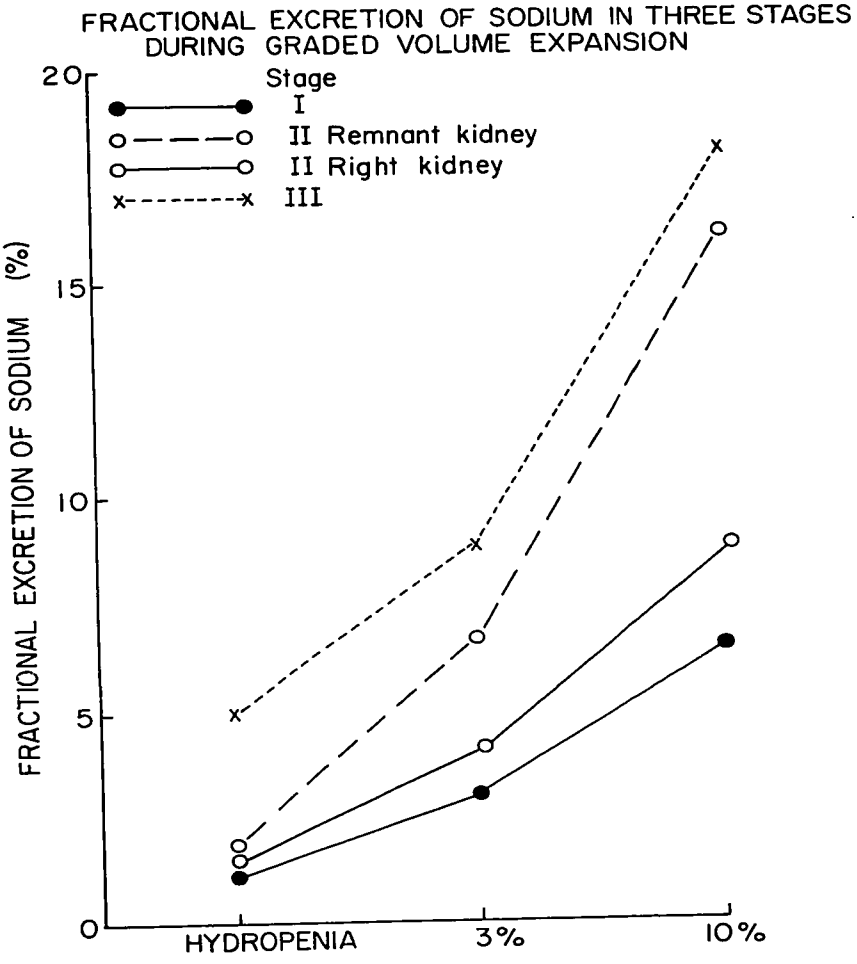

FRACTIONAL EXCRETION OF SODIUM IN THREE STAGES
DURING GRADED VOLUME EXPANSION
Stage
I
II Remnant kidney
II Right kidney
III
FRACTIONAL EXCRETION OF SODIUM (%)
20
15
10
5
0
HYDROPENIA
3%
10%

Figure 11

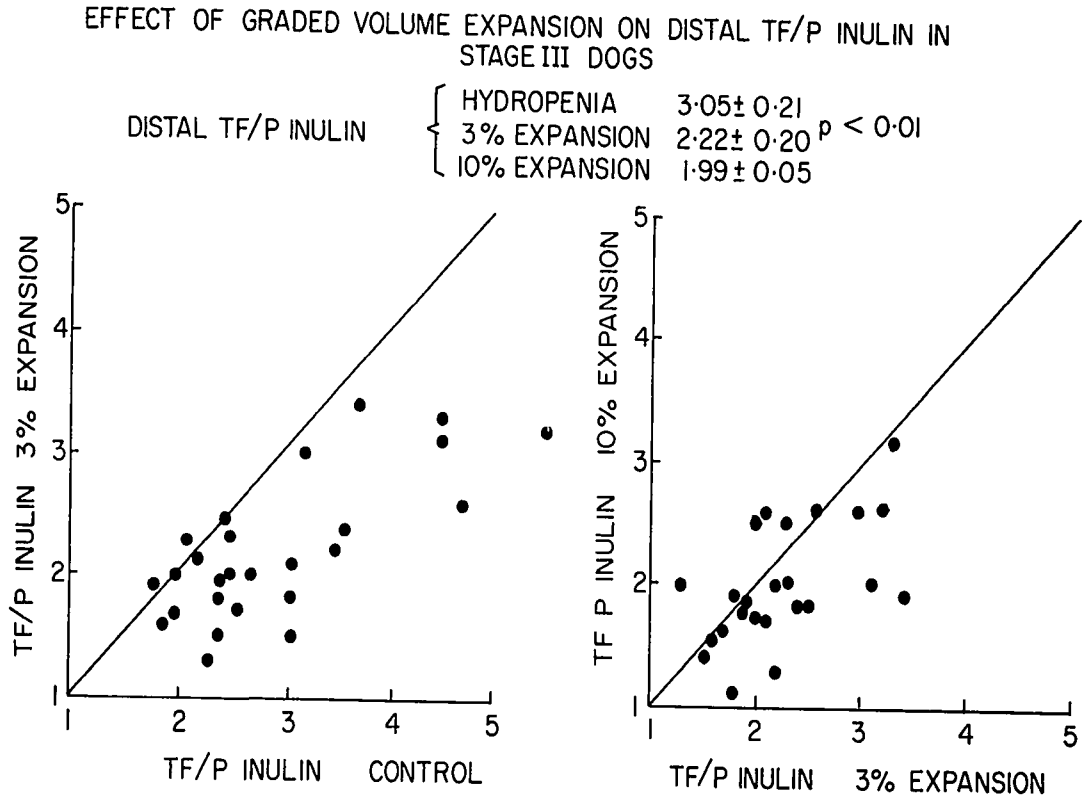

EFFECT OF GRADED VOLUME EXPANSION ON DISTAL TF/P INULIN IN STAGE III DOGS
DISTAL TF/P INULIN
HYDROPENIA 3·05± 0·21
3% EXPANSION 2·22± 0·20
10% EXPANSION 1·99 ± 0·05
p < 0·01
TF/P INULIN 3% EXPANSION
TF/P INULIN CONTROL
TF P INULIN 10% EXPANSION
TF/P INULIN 3% EXPANSION

Figure 12

EFFECT OF GRADED VOLUME EXPANSION ON DISTAL TF/P INULIN IN STAGE II DOGS.

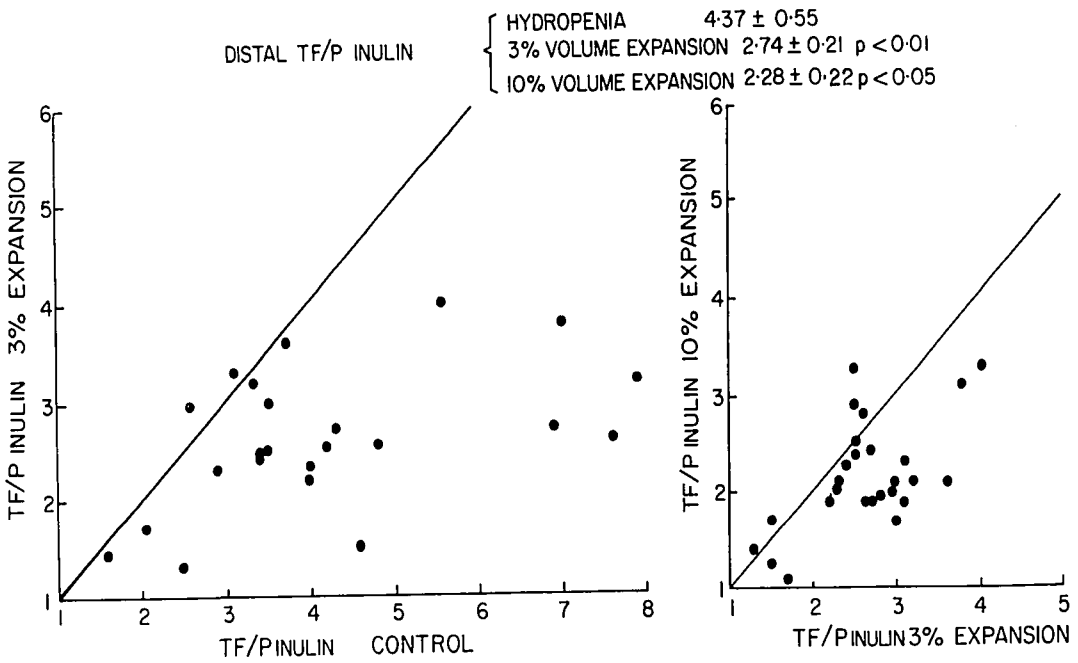


Figure 13

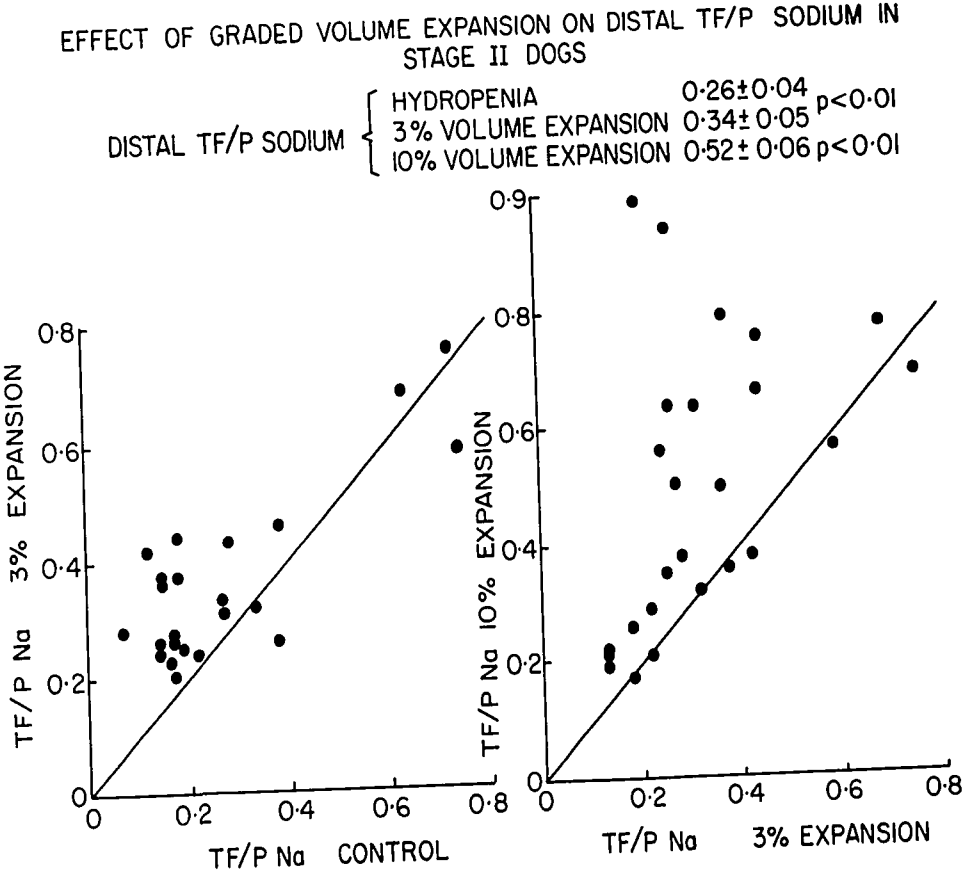

EFFECT OF GRADED VOLUME EXPANSION ON DISTAL TF/P SODIUM IN STAGE II DOGS
DISTAL TF/P SODIUM
HYDROPENIA 0·26±0·04
3% VOLUME EXPANSION 0·34±0·05 p<0·01
10% VOLUME EXPANSION 0·52±0·06 p<0·01
TF/P Na 3% EXPANSION
TF/P Na CONTROL
TF/P Na 10% EXPANSION
TF/P Na 3% EXPANSION
0
0·2
0·4
0·6
0·8
0·9

Figure 14

EFFECT OF GRADED VOLUME EXPANSION ON DISTAL TF/P SODIUM IN STAGE III DOGS

MEAN TF/P Na	HYDROPENIA	0·26±0·03
	3% VOLUME EXPANSION	0·30±0·03
	10% VOLUME EXPANSION	0·48±0·05

$p<0·05$

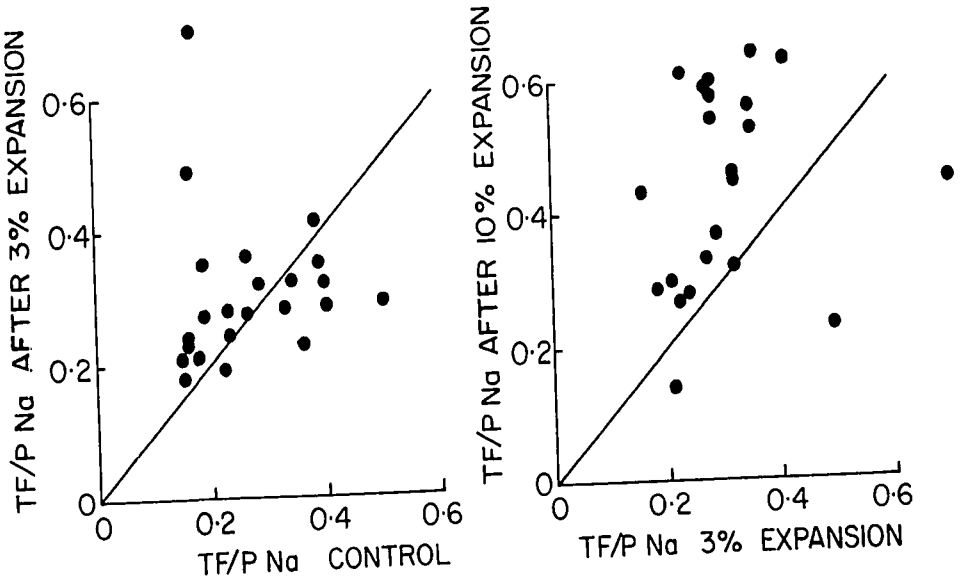
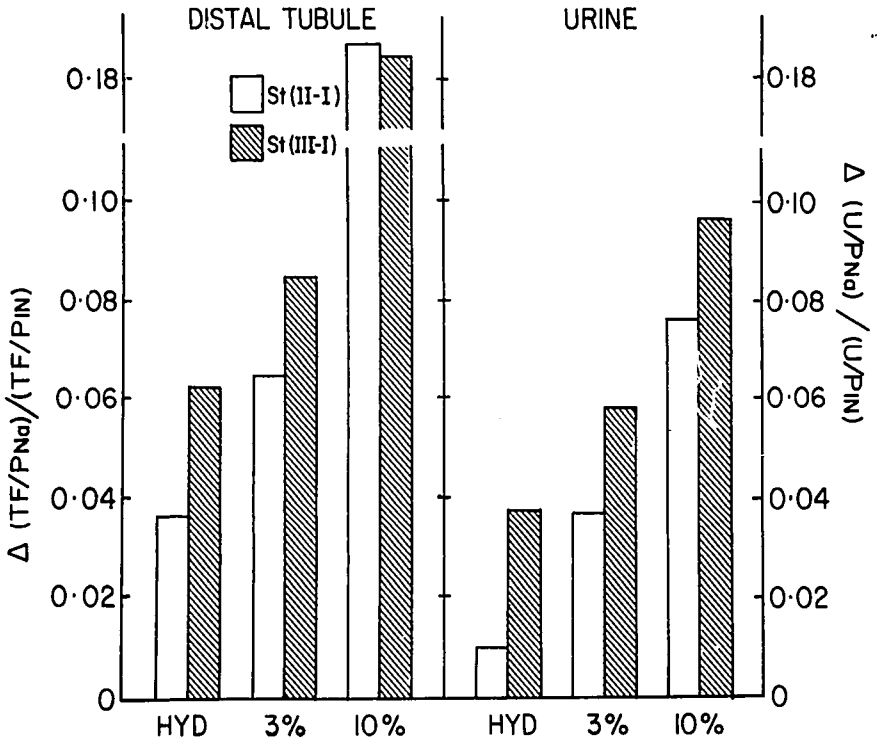


Figure 15

CHANGES IN FRACTION OF SODIUM REMAINING IN DISTAL TUBULE AND URINE COMPARED WITH STAGE I.



HYD = Hydropenia; 3% = 3% expansion; 10% = 10% expansion.

Figure 16

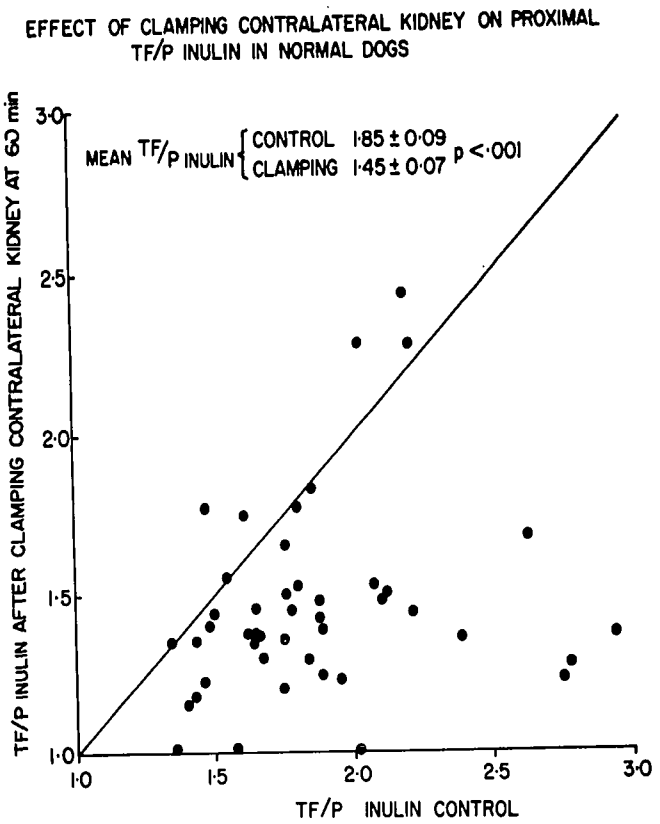

EFFECT OF CLAMPING CONTRALATERAL KIDNEY ON PROXIMAL TF/P INULIN IN NORMAL DOGS
MEAN TF/P INULIN { CONTROL 1·85 ± 0·09 / CLAMPING 1·45 ± 0·07 p < ·001
TF/P INULIN AFTER CLAMPING CONTRALATERAL KIDNEY AT 60 min
TF/P INULIN CONTROL
1·0
1·5
2·0
2·5
3·0

Figure 17

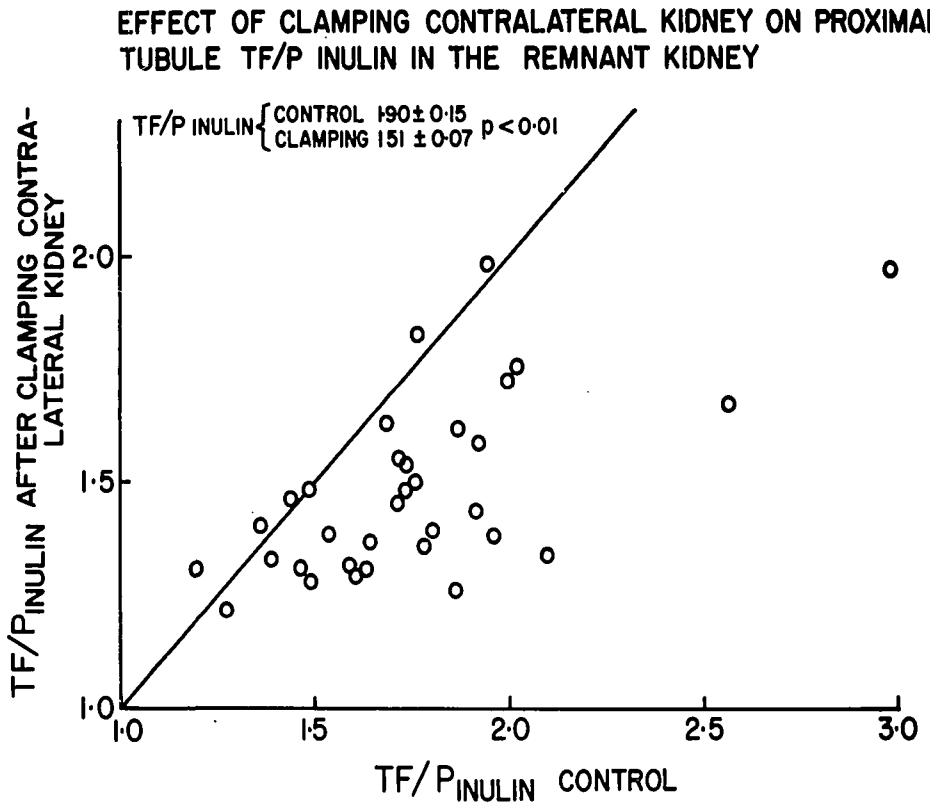

EFFECT OF CLAMPING CONTRALATERAL KIDNEY ON PROXIMAL TUBULE TF/P INULIN IN THE REMNANT KIDNEY
TF/P INULIN { CONTROL 1·90 ± 0·15
CLAMPING 1·51 ± 0·07 p < 0·01
TF/P INULIN AFTER CLAMPING CONTRA-LATERAL KIDNEY
2·0
1·5
1·0
1·0
1·5
2·0
2·5
3·0
TF/P INULIN CONTROL

Figure 18

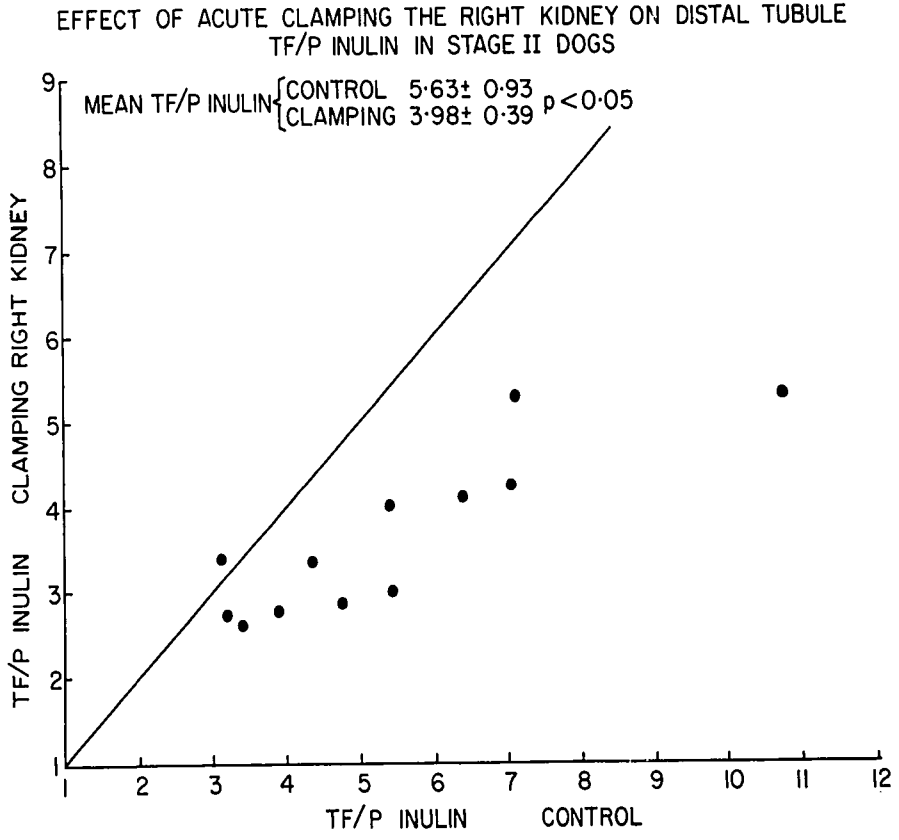

EFFECT OF ACUTE CLAMPING THE RIGHT KIDNEY ON DISTAL TUBULE
TF/P INULIN IN STAGE II DOGS
MEAN TF/P INULIN
CONTROL 5·63± 0·93
CLAMPING 3·98± 0·39
p<0·05
TF/P INULIN CLAMPING RIGHT KIDNEY
TF/P INULIN CONTROL
1
2
3
4
5
6
7
8
9
10
11
12

Figure 19

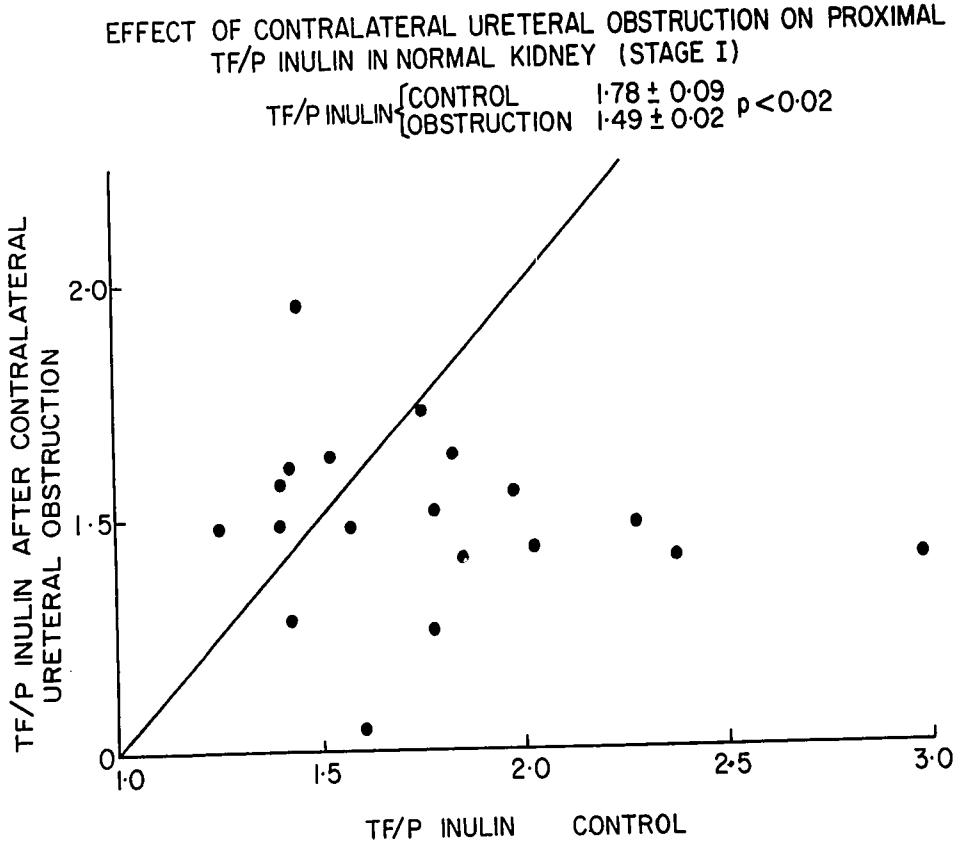

EFFECT OF CONTRALATERAL URETERAL OBSTRUCTION ON PROXIMAL TF/P INULIN IN NORMAL KIDNEY (STAGE I)
TF/P INULIN {CONTROL 1·78 ± 0·09
OBSTRUCTION 1·49 ± 0·02 p<0·02
TF/P INULIN AFTER CONTRALATERAL URETERAL OBSTRUCTION
2·0
1·5
0
1·0
1·5
2·0
2·5
3·0
TF/P INULIN CONTROL

Figure 20

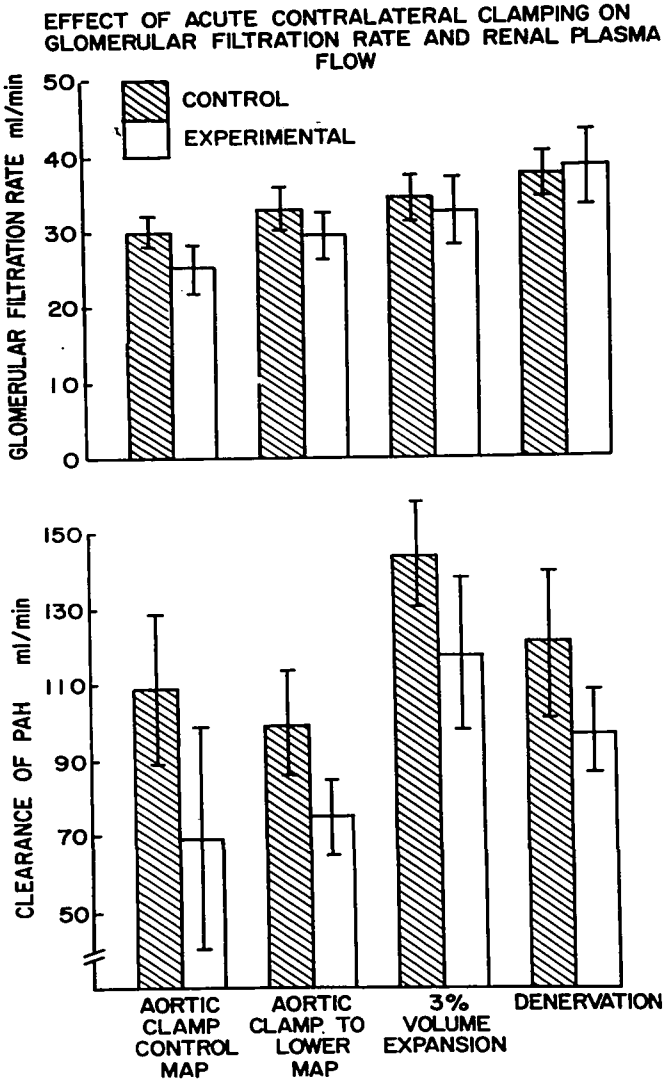

EFFECT OF ACUTE CONTRALATERAL CLAMPING ON GLOMERULAR FILTRATION RATE AND RENAL PLASMA FLOW
GLOMERULAR FILTRATION RATE ml/min
50
40
30
20
10
0
CONTROL
EXPERIMENTAL
CLEARANCE OF PAH ml/min
150
130
110
90
70
50
AORTIC CLAMP CONTROL MAP
AORTIC CLAMP TO LOWER MAP
3% VOLUME EXPANSION
DENERVATION

Figure 21

EFFECT OF CLAMPING ON THE FRACTIONAL EXCRETION OF SODIUM AND POTASSIUM

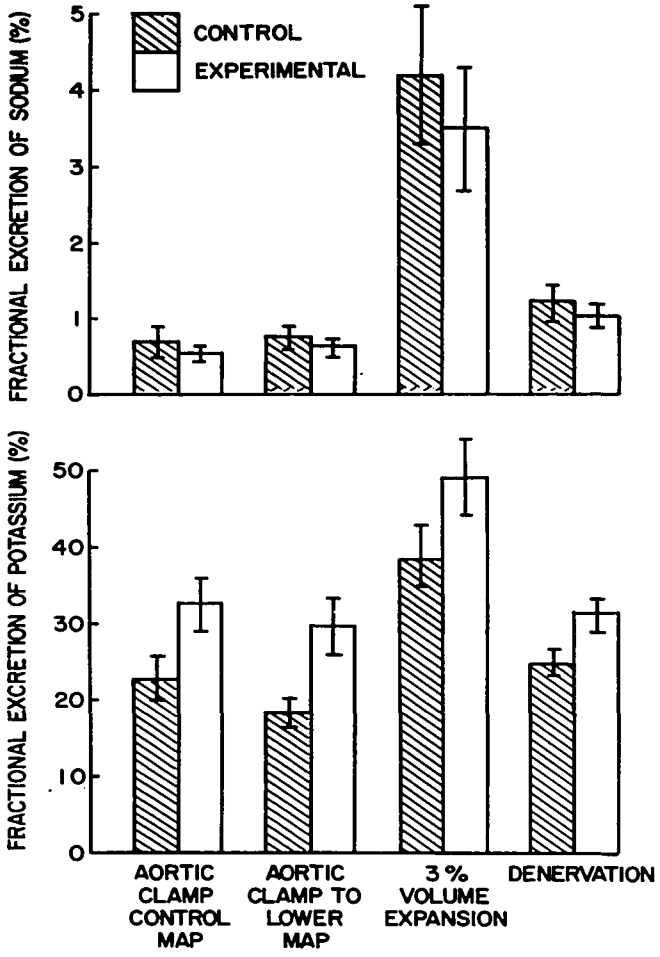


Figure 22

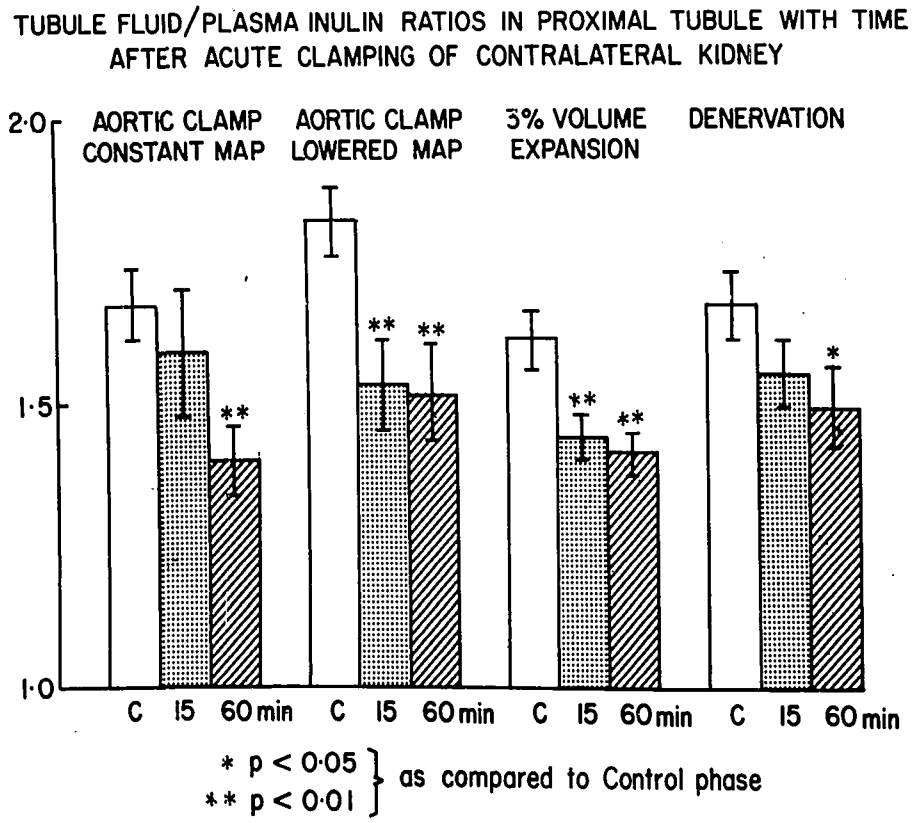

TUBULE FLUID/PLASMA INULIN RATIOS IN PROXIMAL TUBULE WITH TIME AFTER ACUTE CLAMPING OF CONTRALATERAL KIDNEY
AORTIC CLAMP CONSTANT MAP
AORTIC CLAMP LOWERED MAP
3% VOLUME EXPANSION
DENERVATION
2·0
1·5
1·0
C 15 60 min C 15 60 min C 15 60 min C 15 60 min
* p < 0·05
** p < 0·01
as compared to Control phase

Figure 23

COMPARISON OF TUBULE FLUID/PLASMA INULIN RATIOS BEFORE AND AFTER ACUTE CONTRALATERAL CLAMPING

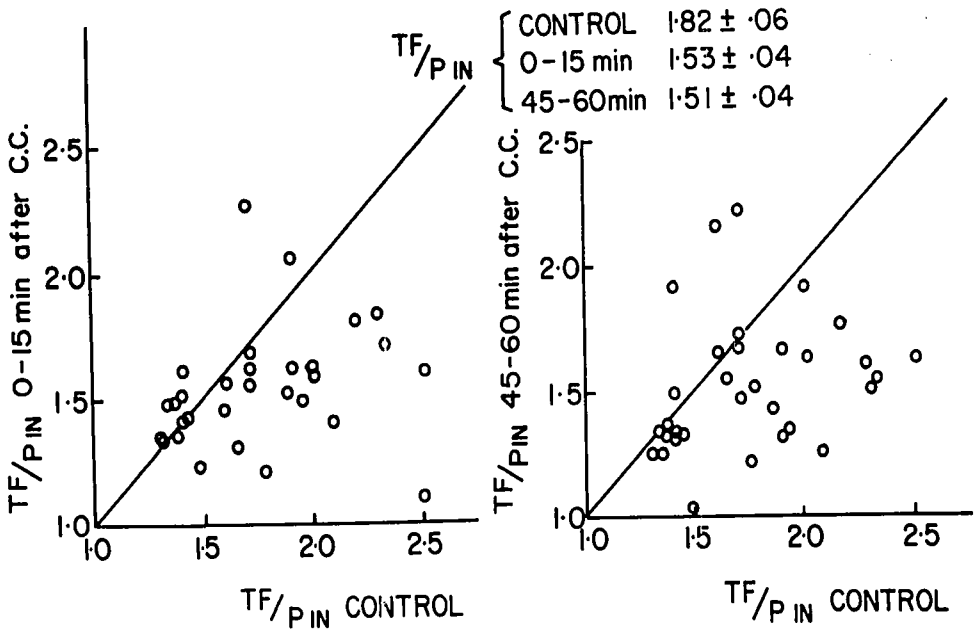


Figure 24