# ABSTRACT

"The effects of total renal ischemia on canine renal function"

by

Mohammad Sharif, M. B. , B. S.

Forty-two healthy mongrel dogs were subjected to complete renal arterial occlusion of varying duration ranging from 1 to 3 hours. A contralateral nephrectomy was performed simultaneously. The post-operative effect of ischemia was followed with serial serum creatinine determinations and serial $^{131}I$ hippuran renograms. Each animal was followed for four weeks from the date of the operation, or until death.

An attempt was made to define the ischemic tolerance of the canine kidney. At the same time, correlation between serum creatinine and renogram was attempted, to determine the validity of the latter in evaluating renal function. No detectable renal damage was noticed in control and 1/2 hour ischemia groups. A variable amount of reversible renal insufficiency was evident in ischemia groups of 1 - 2 hours duration. The 50% lethal dose of complete renal ischemia was demonstrated to be two hours.

A qualitative classification of renograms was developed, and this was shown to correlate well with other parameters of renal function.

The renogram was found to be particularly reliable in the acute phase of renal damage.

Clinical application of the information obtained was discussed specifically with respect to human cadaveric renal homo transplantation.

M. Sharif M.Sc. Experimental Surgery

"Effects of total renal ischemia on canine renal function"

# THE EFFECTS OF TOTAL RENAL ISCHEMIA ON CANINE RENAL FUNCTION

by

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A thesis submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfilment of the requirements for the degree of Master of Science.

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## INTRODUCTION

The rate of failure of human cadaver renal homo transplantation has been high despite recent advances in tissue matching. Approximately 20% of cadaver transplants never function. Some of these failures may be due to hyperacute rejection, but others occur because of irreversible ischemic damage. The conditions leading to this damage have been our prime interest.

Donor kidneys are obtained from cadavers, who have suffered a lingering death. There have been varying degrees of terminal shock. Donor blood pressure and flow has been maintained by large doses of vasoactive drugs. In some situations, phyotension is prolonged despite all therapeutic efforts. Moreover, the donor kidney also goes through a period of obligatory ischemia during nephrectomy, and a varying length of perfusion and storage under unphysiologic circumstances. Undoubtedly, such a pre-transplant condition affect the future function of the kidney. How much renal sensitivity to ischemia is altered by the action of these factors, has not been fully investigated. It has been shown that certain nephrotoxins (methemoglobin, cell pigments and bacterial toxins) potentiate the effect of ischemia. Whether such a synergistic association between the above mentioned circumstances and ischemia also exists, has interested us. To observe any such relationship, the renal threshold to ischemia must first be ascertained. The effect of such ischemia combined with other factors, can then be objectively assessed. The aim of this project is to establish quantitative parancliss of tolerance of the canine kidney to total renal ischemia.

# PART I

# HISTORICAL REVIEW

## Acute Renal Failure

The history of acute renal failure is relatively recent. Bywaters in 1941, treating air raid casualties, noticed that a wide variety of crush injuries developed renal insufficiency, after recovery from shock. The syndrome of "shock kidney" had not come under observation until then, because, in absence of blood transfusion, patients seldom survived the acute effects of shock itself. An immediate interest was aroused to study the pathophysiology of this lesion.

Van Slyke and colleagues in 1944 showed, in dogs, that severe trauma or hemorrhagic shock resulted in a sharp decrease of renal blood flow. This decrease in blood flow persisted after blood pressure was restored to above 110 mm/Hg. He explained that the decreased blood flow was due to renal vasoconstriction, and was the cause of anuria. Lauson et al. reported similar observation in human patients.

Lucke in 1946 introduced the term "lower nephron nephrosis". Studying 538 army casualties, he found that many different injuries resulted in similar renal lesions. Glomeruli and proximal tubules were intact, but distal tubules and loop of Henle showed acute necrosis. With Richard's production of acute tubular necrosis, in frogs kidneys using mercury bichloride, and by his own experience from patients with

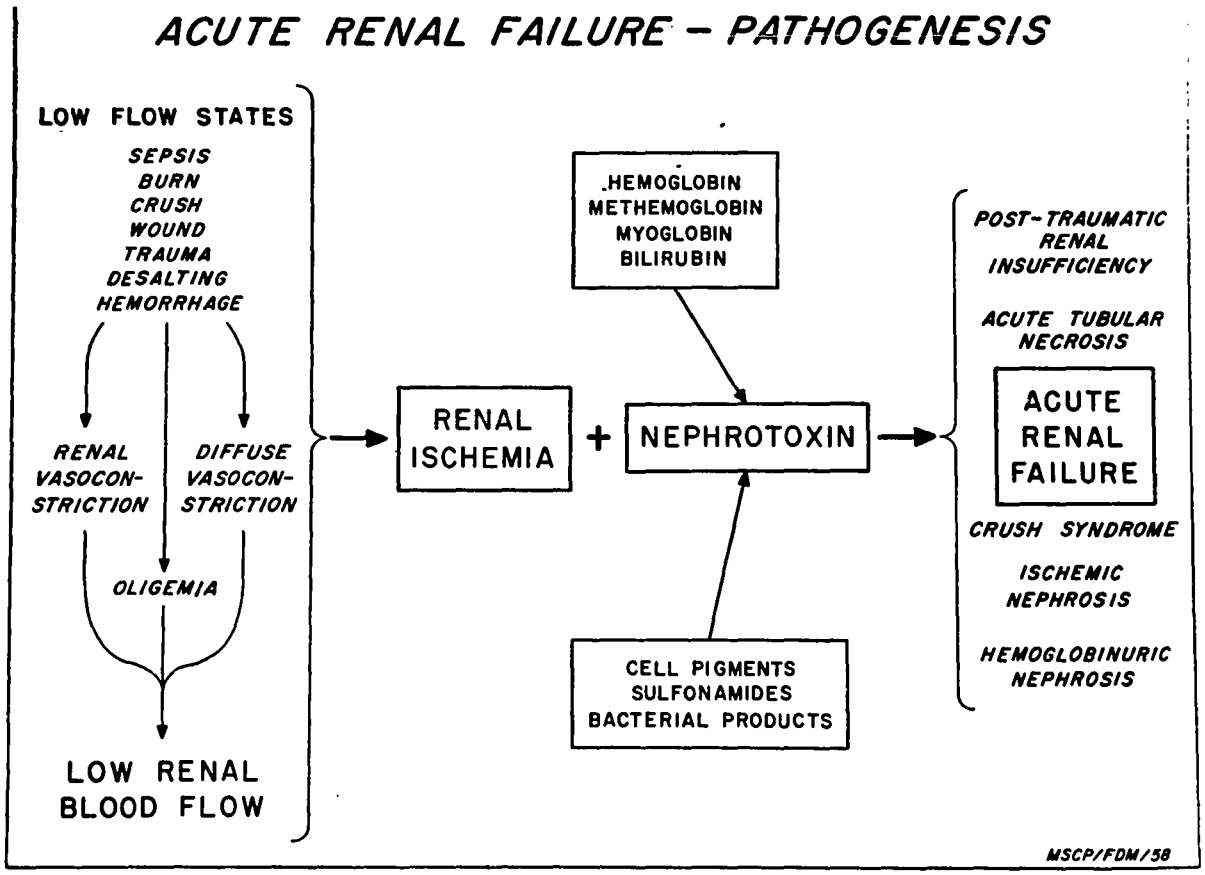



Fig. 1.

Aetielogy and pathogenesis of acute renal failure. From Metabolic care of surgical patient, Moore, F. D., W. B. Saunders Co., Philadelphia.

battle injury, Lucke suggested that cause of post shock uremia was not failure of glomerular filtration, but that filtrate was completely re-absorbed across necrotic tubular epithelium. His terminology of "lower nephron nephrosis" was challenged, because lesions produced by mercuric chloride poisoning were located in proximal tubules, rather than distal tubules. Oliver (1951) showed by microdissection of the nephron, that the entire tubular portion of the nephron may be involved by discontinuous foci of necrosis, or actual tubular disruption (tubulorrhexis). He also distinguished ischemic nephrosis from toxic nephrosis, using a term "ischemuric nephrosis". Allen has been the chief opponent of tubular damage theory. He argues that, a) excessive tubular reabsorption has never been demonstrated. b) He objected that the number of complete nephrons studied by Oliver was too few (14) to make a conclusive judgement. The technique of microdissection is so tedious that some of the tubules in the mass of edema and inflammation might be traumatized. Thus, the tubular disruption may actually be an artefact than real. c) Even if the tubular epithelium was disrupted, there is no real evidence that it would necessarily result in reabsorption of filtrate. d) It is also difficult to explain how recovery in these patients is marked by sudden diuresis, and is so complete.

Allen suggested that a decrease, or stoppage of glomerular filtration may be the possible mechanism in acute anuria. Evidence in

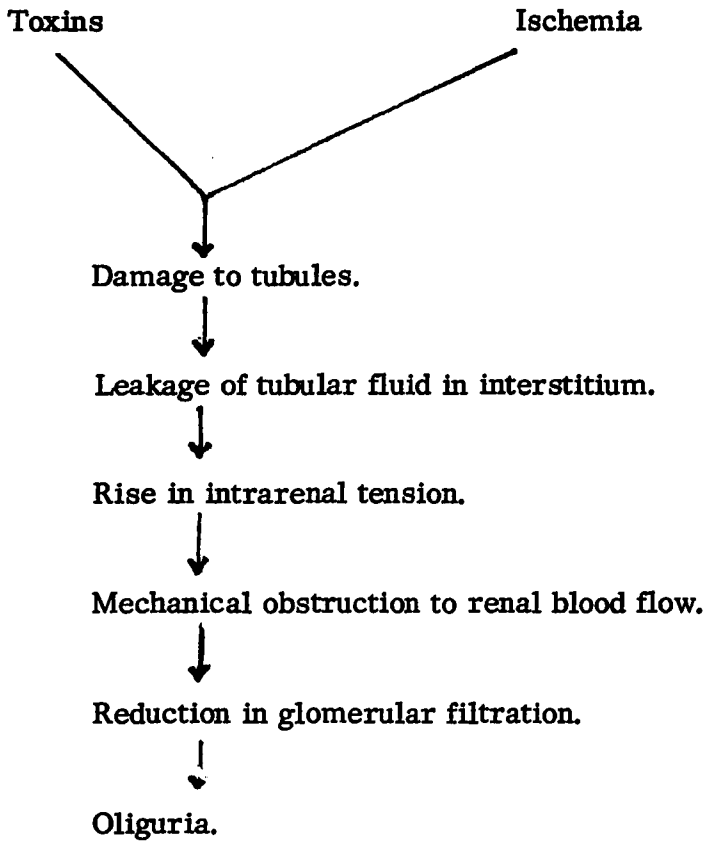



Fig. 2.

Pathogenesis of oliguria in acute renal failure. From Pathology of the kidney, Hepinstall, R. H., Little, Brown and Co., Boston.

favour of this hypothesis, again is tenuous. Decrease of blood flow has been shown in PAH clearance studies in shock, but there is no decrease of blood flow noticed in pathologically identical lesion of hemoglobinuric nephrosis. Histological changes in glomeruli are also less prominent features of acute uremic nephrosis.

Auxilliary pathogenetic factors; e. g. mechanical blockage of the tubular lumen by hemoglobin casts has been suggested. This explanation is difficult to support, because upper tubular dilatation that would result from such an obstruction, is not seen.

EXPERIMENTAL STUDIES ON EFFECT OF RENAL ISHCEMIA

Soon after Bywaters drew attention to the syndrome of "shock kidney", several laboratories studied the lesion under controlled conditions.

Van Slyke showed, in hemorrhagic shock in dogs, that there was an almost complete cessation of renal blood. Hamilton and associates, excised the right kidney and clamped the left renal artery for one, two, three, four, and six hours respectively. However, some of the capsular blood vessels and small anastomosing vessels in the pedicle may not have been divided. Five out of six dogs survived three hours of ischemia, and three out of six survived four hours. After the clamp was removed, urea clearance studies dropped to 10% of normal level. In dogs which recovered, renal function gradually improved to normal after three weeks.

Phillips et al. with a similar model, clamped the left renal artery for

20, 60, and 120 minutes, to define the cause of functional deficit in acute uremia. A marked drop in PAH and creatinine clearance levels occured after two hours of renal ischemia. They concluded that, lowered clearances were due to tubular damage with increased reabsorption of creatinine and decreased extraction of PAH. Roof et al. proposed that decrease in clearance after renal ischemia was mainly due to factors other than reduction of renal blood.

Porch et al. (1959) found that 3 hour ischemia caused maximum tubular damage and death, but if nephrectomy was postponed for three weeks, enough recovery in the ischemic kidney occured to sustain life. No protection was offered by leaving capsular vessels intact.

Morris et al. (1956) compared renal function after two hours of ischemia caused by different modes of vascular occlusion, aortic occlusion, renal arterial occlusion, and combined aortic and renal arterial occlusion. The most severe depression of glomerular filtration rate was noticed after combined aortic and renal arterial occlusion, showing that renal artery was not the only source of blood supply to the kidney and renal damage was less, unless renal artery and aorta were both occluded.

Creatinine

Creatinine is an aminoacid derived from creatine phosphate as a breakdown product after it has given up high energy phosphate for production of ATP. Striated muscle is the biggest reservoir of creatine phosphate. Production of serum creatinine is strikingly constant and directly proportional to lean muscle mass. Hoverman et al. (1948), (19), found that daily turnover of this store is less readily influenced by muscle catabolic factors; e.g. hyperpyrexia, muscle injury or steroid therapy. The serum level of creatinine depends on balance between production from muscle creatine, versus, excretion in urine. Schaffers analysis proves that daily excretion in a healthy man remains remarkably constant, and on successive days, in a single individual, differs less than 10% of the mean.

Significance of the endogenous creatinine level in plasma compared to the urea level lies in the fact that the former is relatively independent of Nitrogen intake in food. Bloch and Schoenheimer, (1939) (4), showed that $^{15}N$ labelled creatine fed to rats was deposited in the tissies. A single dose of creatine causes no increase in urinary creatinine, though extra creatinine is found in urine on prolonged feeding. Another outcome of the study was a confirmatory proof that in a creatine free diet, body creatine was the only precursor of creatinine.

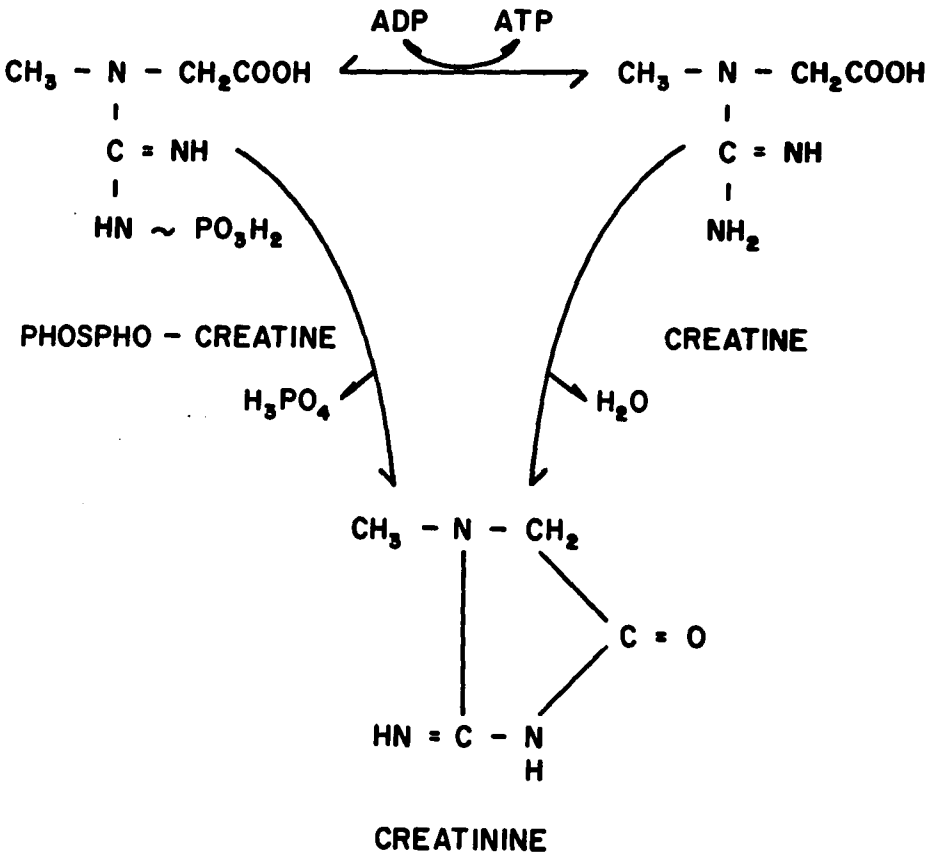



Fig. 3.

Production of serum creatinine.

The ratio of body weight to daily creatinine excretion is, and is expressed as the creatinine coefficient. Creatinine coefficient normally averages 20 - 26 for man, and 14 - 22 for woman.

Creatinine excretion

It has been known for a long time that of all the substances the normal kidney normally has to secrete, creatinine is concentrated to the greatest extent; i.e. it has the highest U/P ratio. Rehberg in 1926, (not knowing the concept of tubular excretion) suggested that creatinine must be maximally filtered through glomeruli. He tried to apply this concept to measure glomerular filtration rate. Observers doing comparative work on creatinine excretion got different excretion values in dog and man, suggesting non identicle mechanism of excretion. Shannon in 1935, (45), demonstrated that in dogs the exogenous creatinine clearance was equivalent to insulin clearance. However, in man, the rate of exogenous creatinine clearance changed from 1.4 to 1.1 as serum creatinine concentration was increased from 10 to 100 mg. %. He regarded this as evidence for existence of a second method of excretion through the tubules, and that the mechanism had a maximal rate of excretion ($T_m$). Similar conclusions were reached by Smith and Hare et al. They found that patients with renal failure showed an increase in the ratio $\frac{\text{Creatinine clearance}}{\text{Insulin clearance}}$ which may rise over 2.

Crawford, (8) demonstrated that there was a common mechanism

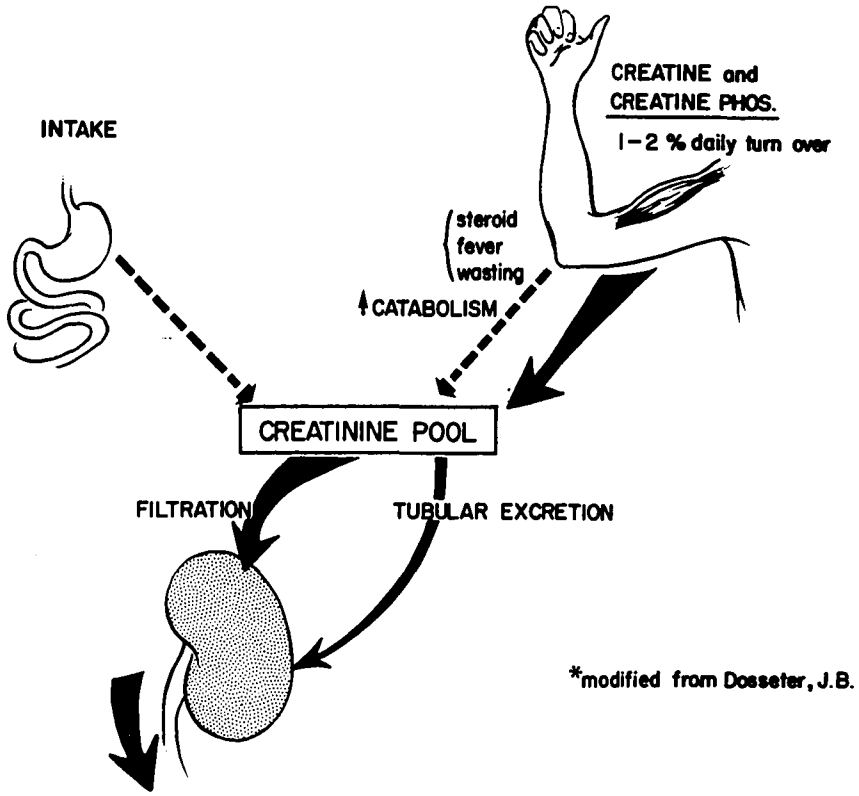



Fig. 4.

Metabolic turnover and renal clearance of serum creatinine.

for the tubular excretion of creatinine diodrast and PAH; therefore , when she administered large doses of PAH or diodrast, the excretion of creatinine was depressed.

The evidence of tubular excretion of creatinine in dog came much later than that for man. Even Smith in 1951, did not believe in the existence of such a mechanism. However, since then, several authors, namely, O'Connel, J.M., Romeo, J.A., Mudge, F.H.,(39) have come up with evidence of presence of this mechanism.

Prognostic value of serum creatinine in relation to renal disease was first remarked upon by Folin and Denis in 1913. This was substantiated by Myers and Killian, (32) who reported that, cases in which serum creatinine had risen above 5 mgm% rarely showed marked improvement and almost invariably died within a comparatively limited time. All these considerations have given blood creatinine measurement a very high reputation in many centres. Endogenous creatinine concentration, when elevated, is a valid index of renal insufficiency. However, when normal, creatinine clearance is needed to accurately measure the functional reserve of the kidneys. It is almost unanimously agreed that there estimations are far more reliable than blood urea and urea clearance, in estimating renal function. In an aduly, daily production and secretion of creatinine fluctuates less than 10% from the mean, and is less readily influenced by catabolic processes, whereas urea production is markedly affected by these. Serum creatinine level in contrast to urea, is also independent of dietry protein intake. Urea is liable to be reabsorbed in tubular damage, whereas creatinine is not.

P.-Aminohippuric Acid and Hippuran $^{131}I$

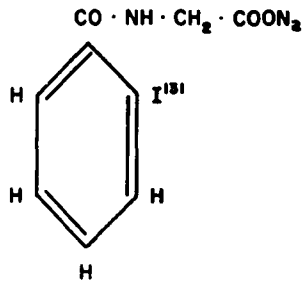
The quantitative study of renal plasma flow and tubular function by saturation methods evolved historically in the sequence: phenol red, diodrast, and p-aminhippuric acid. The fact that hippuran and diodrast are excreted by the tubules in rabbits, dog, and man was first established by Elsom et al. in 1936. Some of the substances eg. probenecid and phenylbuazone, also use the same mechanism of excretion but because of the high lipid solubility of their undissociated form are able to escape from the tubular lumen by diffusion in more distal portion of the nephron. Because of this cycle these substances act as competitive inhibitor though they themselves appear in urine in very low concentration. PAH is also inhibited by dinitrophenol which is considered to be due to uncoupling of phosphorylation from oxidation. This suggests that the process of excretion is active and required high energy phosphate expenditure.

Average normal extraction ratio of diodrast ( $E_D$ ) or PAH ( $E_{PAH}$ ) in man is 92% but taking into account part of the renal arterial blood flow passing through inert renal excretory tissue and blood that passes to the renal vein from perirenal fat which amounts to about 8% therefore extraction of PAH which perfuses through excretory tissue must be close to 100%. The tubular extraction of PAH is limited by a constant and reproducible maximal rate $Tm_{PAH}$ in both dog and man. Because of high extraction ratio of diodrast its clearance at low plasma levels, was used to measure renal blood flow, while saturation of the tubules and measurement of diodrast Tm at high plasma levels was utilized

as a means of determining the quantity of functional (tubular) tissue in the kidney. However analytical difficulties of determining organic iodine led to popularity of other substances having essentially complete renal clearance, and aminable to easier analysis than diodrast.

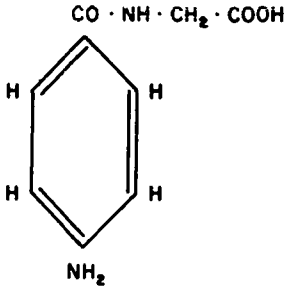
In view of the facts that at low plasma level the PAH clearance is identical with that of diodrast and hence equally valid as an estimate of the renal plasma flow, that the chemical determinaltion is simple, that it does not penetrate the red cells, that it is non toxic and can be used for the evaluation of total tubular excretory tissue, and that it is less extensively bound by plasma proteins than is diodrast, p-aminohippuric acid has virtually replaced diodrast as a measure of measure of renal plasma flow and tubular function, both in the laboratory and clinical investigations. The added advantages of use of hippuran $^{131}I$ in renography are discussed in the next chapter.

HIPPURAN




SODIUM ORTHO – IODO – HIPPURATE

| | |
|---|---|
| AMT. USED | 20 μ CURIES |
| PHYSICAL HALF LIFE | 8·08 DAYS |
| BIOLOGICAL " " | 15 MINUTES |
| RADIATION | 2 MILLI RADS |
| SPECTRUM | 5 γ EMISSIONS 80% 364 MEV. |
| | 4 β EMISSIONS 87% 608 MEV. β |




PARA – AMINO HIPPURIC ACID

Fig. 5.

$^{131}$I hippuran and para-amino hippuric acid. From Renal function using hippuran $^{131}$I and neohydrin $HG^{203}$, Goodwin, D. A., McGill thesis, 1963.

## RENOGRAM

The concept of renogram evolved from a prior development of a similar technique used for measuring excretory function of the liver. Taplin had used $^{131}I$ rose bengal to test biliary patency by external monitoring of Gamma radiation. Naturally, the idea of testing ananogous renal function by external counting was next. In 1956 Taplin and Winter (51) described the method of renography in rabbits using $^{131}I$ Urokon. After reassuring reproducibility, and human safety, the test was applied to screen hypertensive patients. INitial results were most discouraging, because of the lack of differentiation between curves of a good and a poorly functioning kidney. Urokon was soon discarded in favour of $^{131}I$ idiopryacet (diodrast). The diodrast renogram, although an improvement in discrimination of renal function, had the disadvantage of partial accumulation in the liver. This distorted the right renal tracing.

The investigators (Johnson, Winter, (53), Nordyke(32) ) encountering similar problems with diodrast tried Miokon (sodium diprotrizoate), Renografin (diatrizoate methyl glucamine), and hypaque sodium. These substances, although more kidney specific, were much more slowly excreted, thus masking differences in tracing.

The breakthrough came when Tubis et al. (1960) introduced radioiodinated O-iodohuppurate, and Nordyke (32) proved its clinical application.

Hippuran $^{131}I$ had all the advantages and none of the disadvantages. The substance was selectively and promptly removed by the kidneys (U/P ratio 92%). It did not appreciably accumulate in the liver.

## COMPOSITION OF RENOGRAM

When renogram was first described by Taplin and Winter, the tracing was divided into three phases. 1. VASCULAR PHASE: The initial spike rising, almost vertically, attributed to renal and background vascularity. 2. FUNCTIONAL PHASE: The slower ascending slope after vascular phase representing tubular activity and intraluminal accumulation of the isotope. 3. EXCRETORY PHASE: The final descending part of the curve interpreted as discharge of radioactivity into the urinary bladder. Since the first description, a heated debate has built up on physiological significance of these three phases of renogram. Several attempts, both clinical and experimental, have been made to analyse the renographic changes and correlate it with renal pathology; e. g. unilateral nephrectomy, renal artery ligation, renal vein occlusion, tubular necrosis produced with mercuric chloride, and use of vaso active drugs. The other methods of analysis include; substitution of hippuran with albumin, prior loading with para-aminohippuric acid, blocking tubular excretion with probenecid.

Taplin who coined the original terminology of vascular, functional, and excretory phase, renamed them as tracer appearance, blood flow, and drainage respectively. Lack of conformity of nomenculature reflects the doubt in the minds of the authors about the pathophysiological significance of these

segments. To avoid any furthur confusion of terminology in this paper, they will be referred to, henceforth, as; First, Second, and Third segment.

The only area of general agreement seems to be the third, or excretory segment. Most investigators believe that it reflects elimination of radioactive material from the kidney. The knowledge has been used beneficially in differentiating urinary outflow obstruction. The current concept of the second segment is the most controversial. Taplin presently believes it to represent renal blood flow. He argues that; 1. The tracer dose (micrograms / Kg) of hippuran used for renography uses about 1/10,000th. of maximum tubular cell transport capacity. 2. A dose of hippuran which produces plasma concentration below 10 mg. has a very high plasma clearance. In one passage through the kidney more than 90% is excreted out of plasma. 3. Nearly all of the extracted hippuran remains in the field of the collimator vision during the entire second phase. 4. In experimental constriction of the renal artery, the decrease in renal blood flow is matched by proportional flattening of the second segment slope. 5. Acute mercuric chloride poisoning depresses the second segment proportional to severity of cellular damage, but also in proportion to associated reduction in effective plasma flow and extraction efficiency.

## EVALUATION OF RENOGRAMS

Interpretation of a renographic tracing has been the most difficult subject in the history of the procedure. There is no uniform agreement on the basic criteria of normality, and significance of an abnormal curve is even

disputed. Various authors have suggested from simple qualitative visual inspection to, all the way to computer assisted analysis. Winter, the pioneer of the test, still believes that qualitative interpretation of the renogram is not only the most logical, butalso the most dependable method for general use. Koplowitz, (22) compared accuracy of his qualitative inspection against semiquantitative "$T_{max}$" of Stewart and Haynie and the renogram index method of Hirakawa and Corcoran. He noticed that diagnostic accuracy was significantly decreased by application of quantitative methods. In an important control study, Spencer, investigating normal human renograms for statistical analysis of reproducibility, concluded that despite carefully controlled test conditions, a statistical analysis indicated that reproducibility was insufficient. The slopes and ratios of amplitudes showed the greatest reproducibility.

Stewart and Haynie, (50) had measured several parameters and concluded that most diagnostic accuracy was achieved by measuring "$T_{max}$" and $T_{\frac{1}{2}}$. They reported normal range of figure for the right kidney as 2.5 - 7.2 min. and 4.4 - 21.3 min. for the left kidney. The value for the left kidney was slightly higher than the right.

Hirakawa (18) had suggested that combined function of both kidneys was best expressed by measuring radioactivity at the end of 20 minutes, expressed as percentage of that injected. He also presented a formula based on ratio of the squares of segments of the radioisotope renogram.

Meade, (28) while investigating normal renograms, measured six points along the time axis, and calculated mean and standard deviation for each point. This was then plotted to show standard deviation of the whole tracing. He used a transparent photocopy of this figure as an overlay in evaluating renograms. He found that 30 minutes excretion was $67.7 \pm 5\%$ and individual values could be duplicated quite accurately. However, excretory slopes of the renogram tracing did not correlate well with the 30 minutes excretion.

More sophisticated analysts have tried to use a mathematical approach. One of the most thorough studies was done by Pircher, who measured 14 parameters derived from linear and angular measurement of the renograms of nine normotensive subjects without evidence of kidney disease. In calculating the difference between the two kidneys of these 14 parameters, 14 additional parameters were derived. A probability and statistical approach was used to determine the limits within which a single observation on a normal person would be expected to fall. Three tolerance ranges were obtained with respective limits of 75 - 90 and 95% . The normal range was wider on the left side than on the right.

Rosler, (43) investigated 721 patients with radionephrography and simultaneous blood sampling for residual hippuran $^{131}I$ radioactivity after 7 minutes and 17 minutes. He plotted the figures on a semilog paper, and with the aid of graphic analysis, was able to define separate curves for most important partial functions of the kidney; namely, the renal vascular pool,

filtration and secretion segments, excretion via renal dead space and additional loss into extrarenal dead space; etc.

Luck, (23) tried to correlate the slope of the first 45 seconds of second segment with clinical progress, laboratory data and histology of the kidney in renal transplants. He contended an exact correlation between "T" ( a mathematical expression obtained for slope of the second segment obtained by use of a computer ) and BUN,serum creatinine and creatinine clearance was obtained. However, he was unable to evolve any statistical significance from the data.

Knudsen, (21) described a mathematical model for simulation of radiohippuran renogram test on an analogue computer. The model incorporated one part for simulation of the kidney dynamics, and another part for the simulation of the hippuran transport in the human body from the place of tracer injection to the kidney.

Meade, (28) tested the validity of digital computer simulation model for renograms. He was able to effectively simulate various disease states; e. g. reduction of renal blood flow, mechanical obstruction of the drainage, effect of dehydration, nephritis and heminephrectomy. The model gave an estimate within $\pm$ 5% of the mean normal renogram and the 30 minutes excretion. However, simultaneous good fit of different parameters did not seem possible.

# PART II

# MATERIAL AND METHODS

## Animal Selection

Healthy male and female adult mongrel dogs were selected, because of the ease of management, greater anesthetic tolerance, and similarity in anatomy and physiology to human beings. The dogs were properly immunized, ranged in weight between 10-20 kg. They were fed Dr. Ballards dog food, and were given supplemental vitamins. Dietry habits and fluid intake were closely observed. They were placed in individual metabolic cages in immediate post-operative periods, and were transferred to large pens after 3-4 days.

## Pre-operative preparation

Pre-operative renal function was screened. Any dog with serum creatinine above 1 mgm%, or abnormal renogram was excluded.

## Alimentation

All dogs were fasted overnight. Intravenous infusion of 5% dextrose in water, at 50 cc per hour was given through the cephalic vein during each experiment.

## Position

The animals were placed supine, on the operating table. The abdomen was shaved and scrubbed with tincture of iodine, and draped for midline sterile exposure.

## STUDY GROUPS

| Group No. | Period of ischemia | No. of experiments |
|---|---|---|
| 1 | 3 hr. arterial occlusion | 6 |
| 2 | 2 1/2 hr. " " | 6 |
| 3 | 2 hr. " " | 6 |
| 4 | 1 1/2 hr. " " | 6 |
| 5 | 1 hr. " " | 6 |
| 6 | 1/2 hr. " " | 6 |
| 7 | Control | 6 |

Total number of experiments 42

Anesthesia

Intravenous pento barbital sodium 25 mgm/kg. per body weight was used for anesthetic induction. Additional amount of the same was used as required to keep the animal in the second plane of the third stage of anesthesia, continuously. Endotracheal intubation was done to assure free air passage. Continuous blood pressure monitoring was carried out for the duration of the operation using femoral arterial catheterization.

## OPERATIVE TECHNIQUE

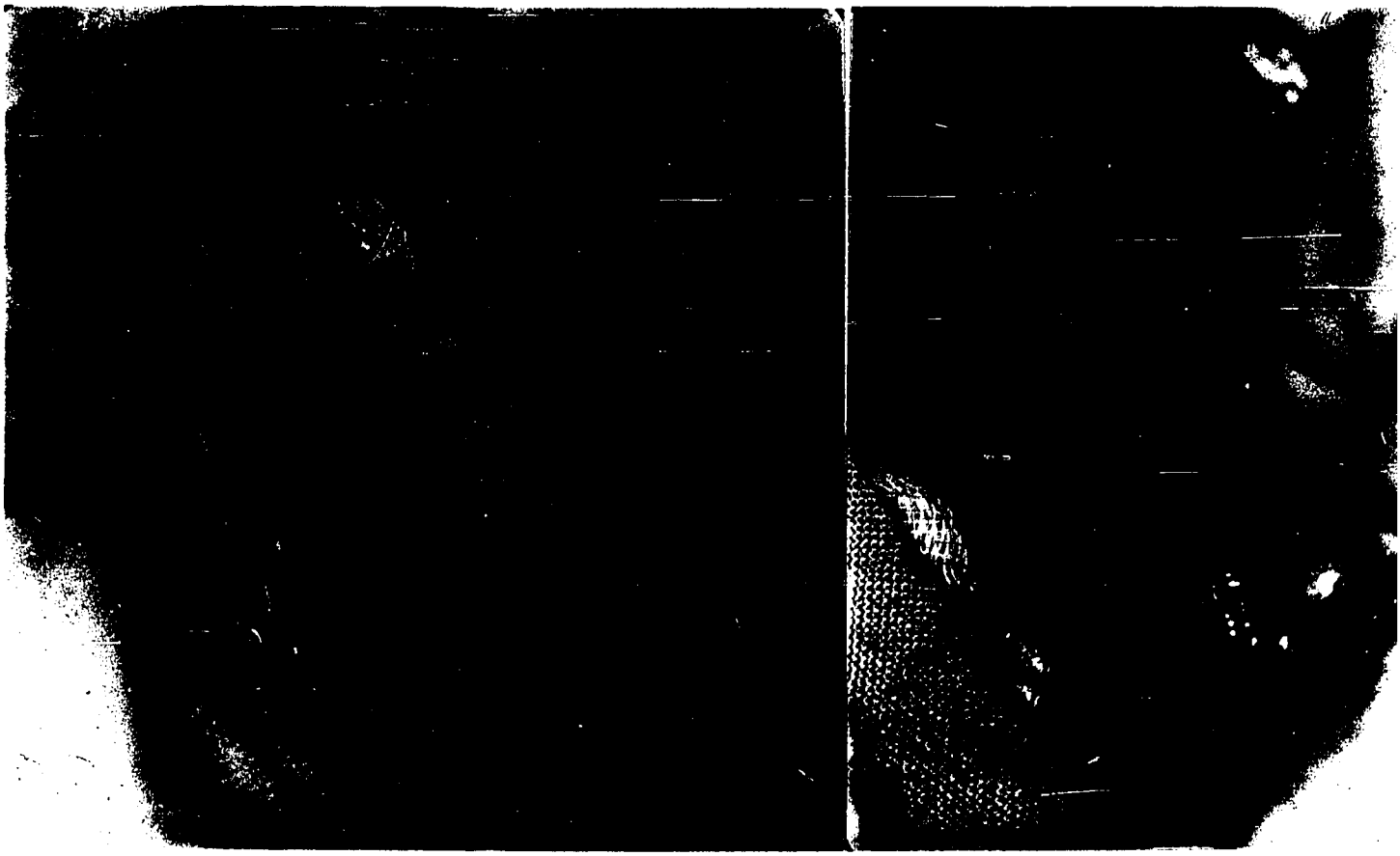
A midline upper abdominal incision was used for surgical exposure. An exploratory laporotomy was carried out to rule out any intraperitoneal or renal disease.

a) Right nephrectomy

Peritoneal coverings over the right renal pedicle were dissected exposing renal artery, vein and ureter. Pericapsular vessels were ligated and divided, separating the kidney from its bed. The kidney was mobilized ventro-medially identifying the renal artery from the dorsal side. The renal artery was doubly ligated with 00 black silk and divided. Renal vein was dissected next from the ventral side. It was ligated and divided similarly. Last of all, the ureter was tied and bissected about one inch below the pelvis. The kidney was removed and saved for use as a control of morphological studies.

b) Dissection of the left kidney

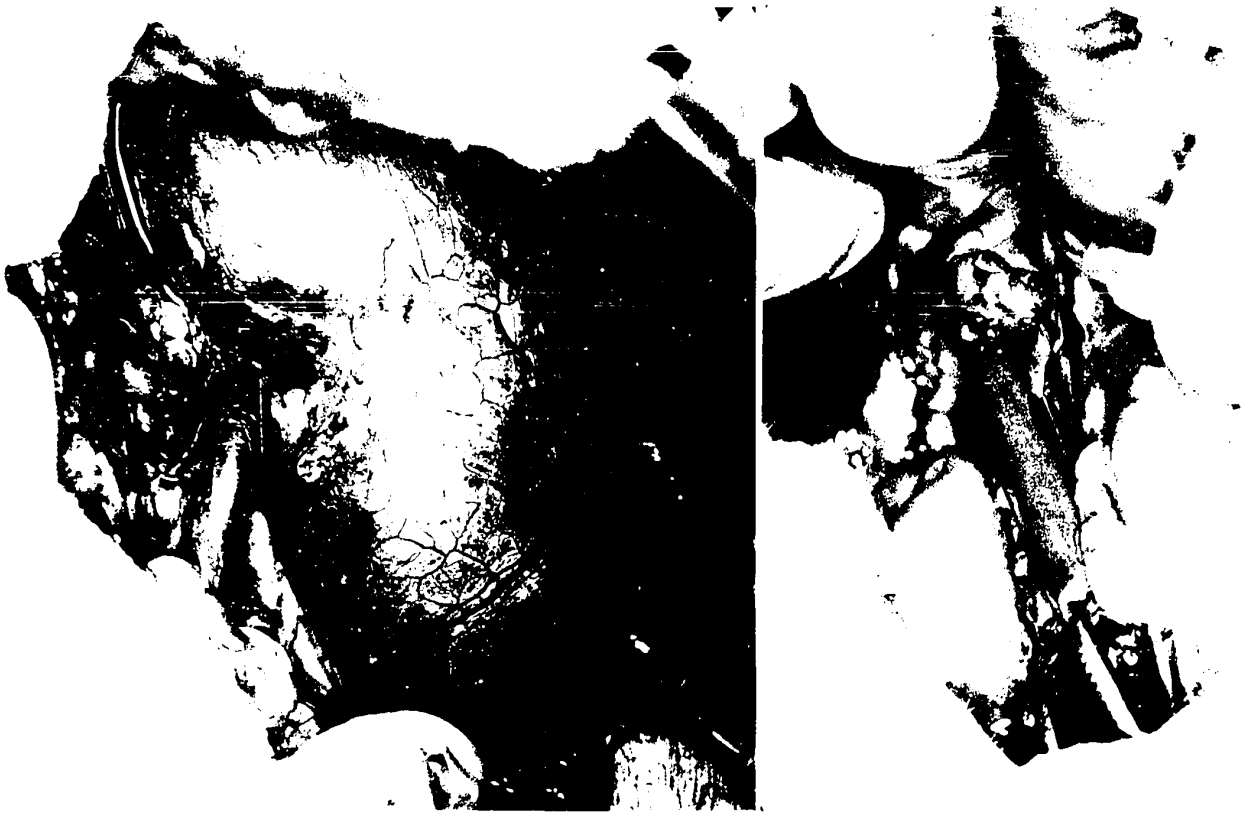
In all experiments it was the left kidney that was studied. The left



A B

Fig. 6.

Operative technique.
A. Dissection of left kidney.
B. Occlusion of the left renal artery.



kidney was exposed similar to the right kidney. Special precautions were taken to allow only the minimal handling of the organ. A meticulous peri-capsular dissection was carried out to completely isolate the organ. Special attention was paid to a thorough dissection around the pedicle with an aim to ligate any vascular source, except the renal artery. A complete denervation of the kidney was carried out to remove any neuro-humoral influence on the renal blood flow.

c) Occlusion of the left renal artery.

Renal artery was clamped for predetermined duration, to occlude the blood flow completely. Ischemic period for the experiment of the day was chosen at random to avoid any statistical error. The abdomen was temporarily closed for the duration of ischemic period. After completion of the desired length of arterial occlusion, the clamps were removed and free flow of blood was established. The abdomen was closed using a continuous braided steel suture on peritoneum and linea alba. Superficial fascia was approximated with 000 chromic catgut. A continuous subcuticular stitching was done with 000 chromic catgut to approximate the skin. A thin layer of plastic spray was applied to close the wound.

## EVALUATION PROCEDURES

1) Post-revascularization renal consistency

Renal consistency was checked fifteen minutes after re-establishment of arterial flow. It was graded into one of the following categories for future comparison: a) flabby, b) soft, c) medium, e) firm (equivalent to normal pre-operative consistency) f) tense.

2) Endogenous serum creatinine estimation

Daily serum creatinine estimation was done for the first 3 days. This was followed by the determination on alternate days for the next 7 days, and 2 to 3 times weekly thereafter up to the 28th post-operative day when the experiment was terminated. Estimation of creatinine clearance was attempted in the beginning, but because of difficulty in collecting exact, and unadulterated samples, effort was mostly wasted. Most of the samples thus collected were considered unreliable and we had to suffice with serum creatinine values.

3) Serial hippuran $^{131}I$ renography

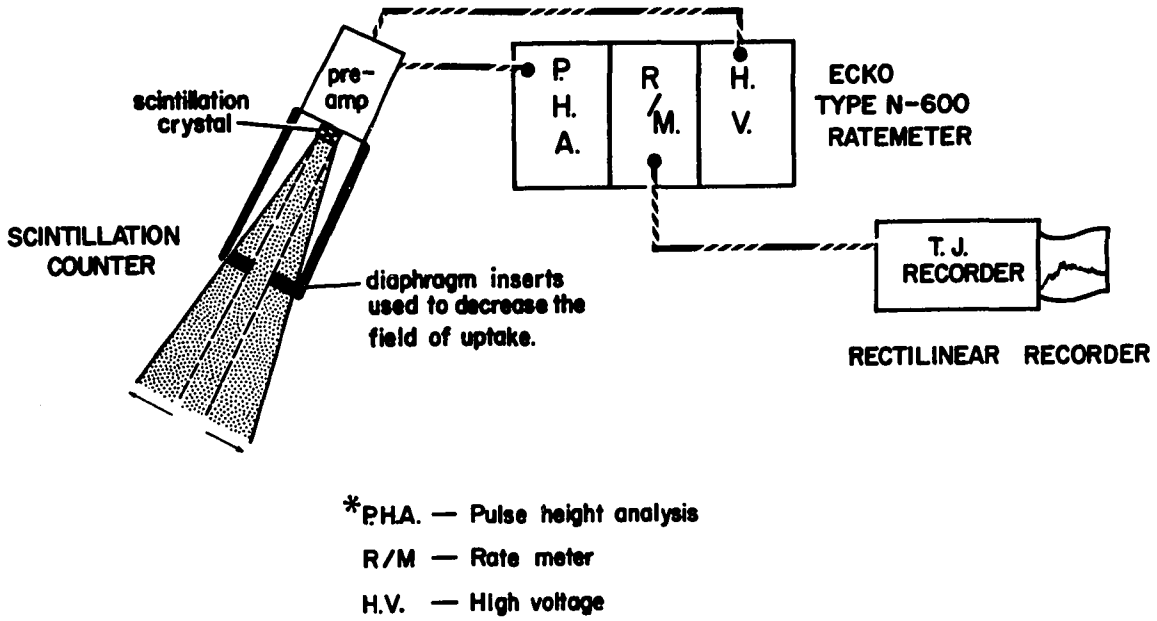
A pre-operative renogram followed by at least, 2 renograms in the first week and one renogram every succeeding week. At times a scheduled renogram had to be cancelled, because of excessive accumulation of residual radioactivity.

4) Morphology

The right kidney, removed at the time of the operation, was used as control for morphological changes in ischemic (left) kidney.

Morphological studies were carried out under following heading:

1. gross examination
2. microscopic examination
   a) H & E staining
   b) PAS staining




*P.H.A. — Pulse height analysis

R/M — Rate meter

H.V. — High voltage

Fig. 7.

Block diagram of scintillation recorder.

## THE EXPERIMENTS EXCLUDED FROM SERIES

1. Dogs with abnormal pre-operative renal function; i. e. serum creatinine more than 1 mgm%,or abnormal renogram.

2. A rise or fall of blood pressure more than 30 mm/Hg during the operation, specially after revascularization of the kidney. This was carried out to assure that period of ischemia lasted only for determined lengths of time and did not carry on as relative ischemia even after re-vascularization.

3) Death from extrarenal causes; e. g. distemper, intussception, pneumonia, or other surgical complications.

## RENOGRAMS

A) Instrumentation

A locally assembled single probe renographic apparatus was used. An Ekco Type N 559 scintillation detector crystal in a collimator of design, similar to the standard thyroid uptake collimator, operated an Ekco Type N 600 (tube model) ratemeter and a rectilinear recorder (Model Rectiwriter by Texas Instruments). The collimator was shaped like an inverted cone, and the crystal was recessed 7 inches from the face of the collimator to minimize background count from surrounding tissue. The instruments were installed in a Picker X-Ray porter stand and case.

All renograms were recorded under standard tuning at the following specifications. Scale range 100 K (100. 000 CPM) time constant 3 seconds, mean probability of error 5% tracing was made at a speed of 12" /hour (approx.

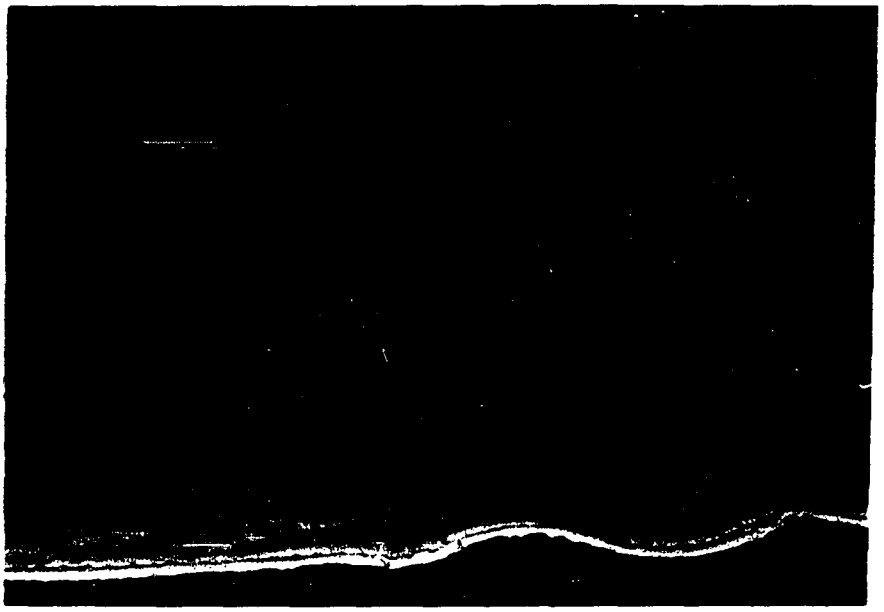


Fig. 8.

Position of scintillation tube.

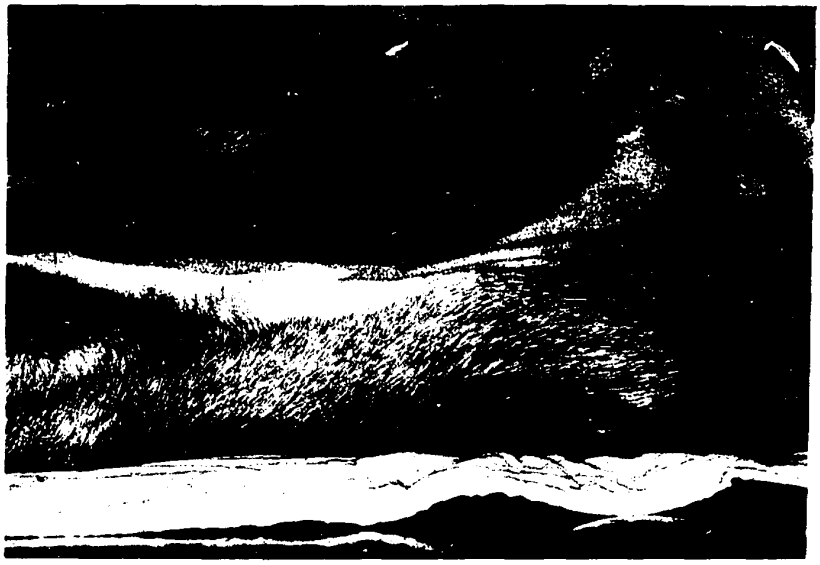


Fig. 8.

Position of scintillation tube.

0.5 cm/m). Calibration of the machine was carried out every two weeks by a radio-physicist.

B) Technique and standardization

Anesthetization and positioning of animal was done as for operative procedure. An intravenous infusion with 5% dextrose and water was started to keep the vein open.

Position of left kidney was marked on the skin. Localization in - variably was easy as the kidney could be palpated bi-manually.

Collimator tube was centered over the kidney with slight tilt looking cephaled. In this position it was possible to avoid most of the radiation coming from the spleen and urinary bladder, and the renographic tracing showed greatest amplitude. The tube of the collimator was always touching the skin and every attempt was made to keep it at the same distance with each successive experiment. The machine was always switched on well in advance of the tracing to avoid any error of equilibrium and lack of warm up. A brief strip of tracing was taken before injection of isotope to establish the baseline. The rectilinear recorder was set at a speed of 0.5 cm/minute. A standard dose; i.e. 20 mic. of hipputan 1-131 was used for every renogram. Time of injection was marked on the baseline. The tracing was recorded for more than 20 minutes. Grading of the renogram was attempted before the serum creatinine level for the day was known.

# PART III

# RESULTS

## Mortality Compared to Duration of Renal Ischemia

Table 2 shows the number of fatalities in each group according to duration. All the dogs of 2 1/2 hour and 3 hour renal ischemia group died. The three hour group dogs died on the 4th post-operative day on the average. For 2 1/2 hour ischemia group the mean number of days of survival was 7. Three out of six dogs died in the 2 hour ischemic group. The average survival period in these three dogs was 8 days. There was only one mortality in the 1 1/2 hour ischemia group. This dog died on the 8th post-operative day. There was no mortality in the 1 1/2 hour ischemia and control groups.

## ENDOGENOUS CREATININE LEVEL COURSE IN DIFFERENT GROUPS

### GROUP I

### Duration of Renal Ischemia - 3 hours

The 3 hour graph in figure 9 depicts mean levels of serum creatinine in 6 experiments on each day. All dogs in this group rapidly died. One animal died on the 3rd day, another on the 5th day, and the rest died on the 4th post-operative day. The course in all dogs was quite similar, with unrelenting rise in serum creatinine ending in death. Urine output remained poor. The dogs looked acutely ill, vomited repeatedly, and reduced their fluid and food intake drastically.

## MORTALITY

| No. of dogs | Duration of ischemia | No. of dogs died | Average day of death |
|---|---|---|---|
| 6 | 3 hr. | 6 | 4 |
| 6 | 2 1/2 hr. | 6 | 7 |
| 6 | 2 hr. | 3 | 8 |
| 6 | 1 1/2 hr. | 1 | 8 |
| 6 | 1 hr. | 0 | - |
| 6 | 1/2 hr. | 0 | - |
| 6 | control | 0 | - |

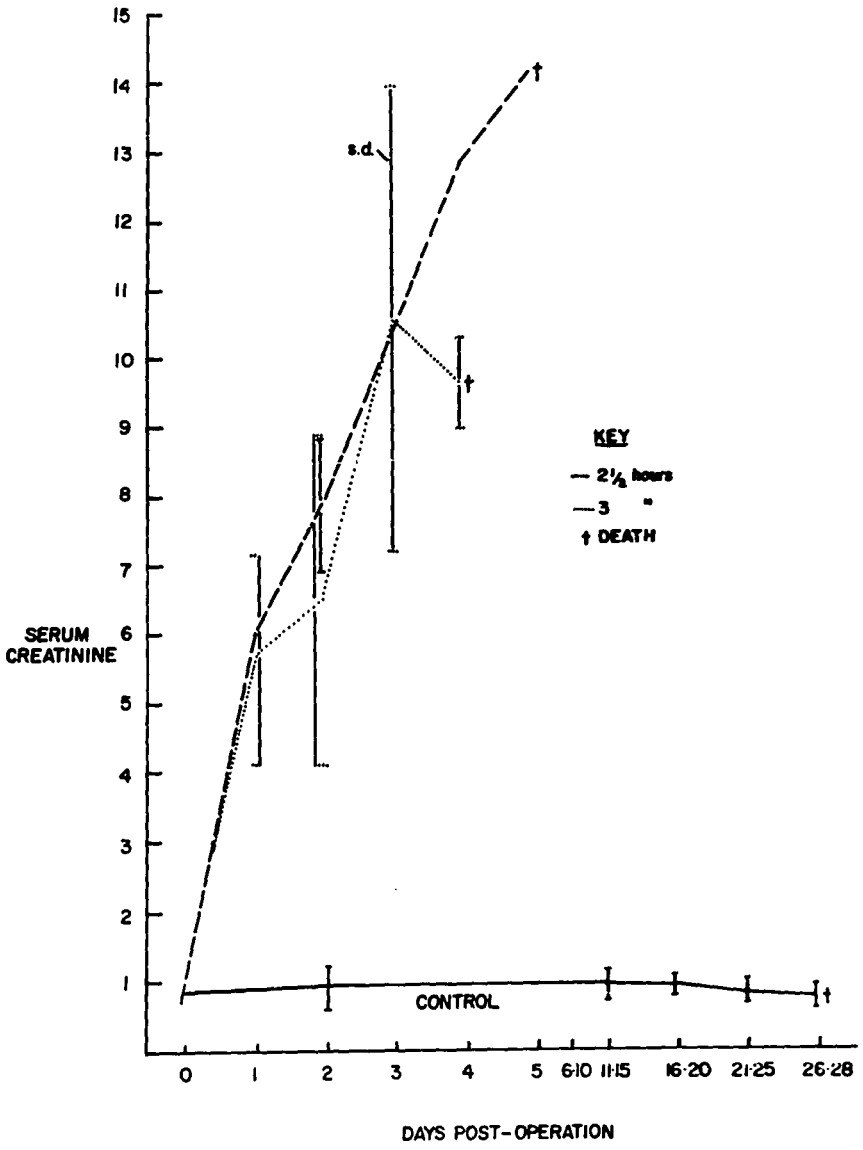



Fig. 9.

Mean creatinine levels of
2 1/2 and 3 hours groups.

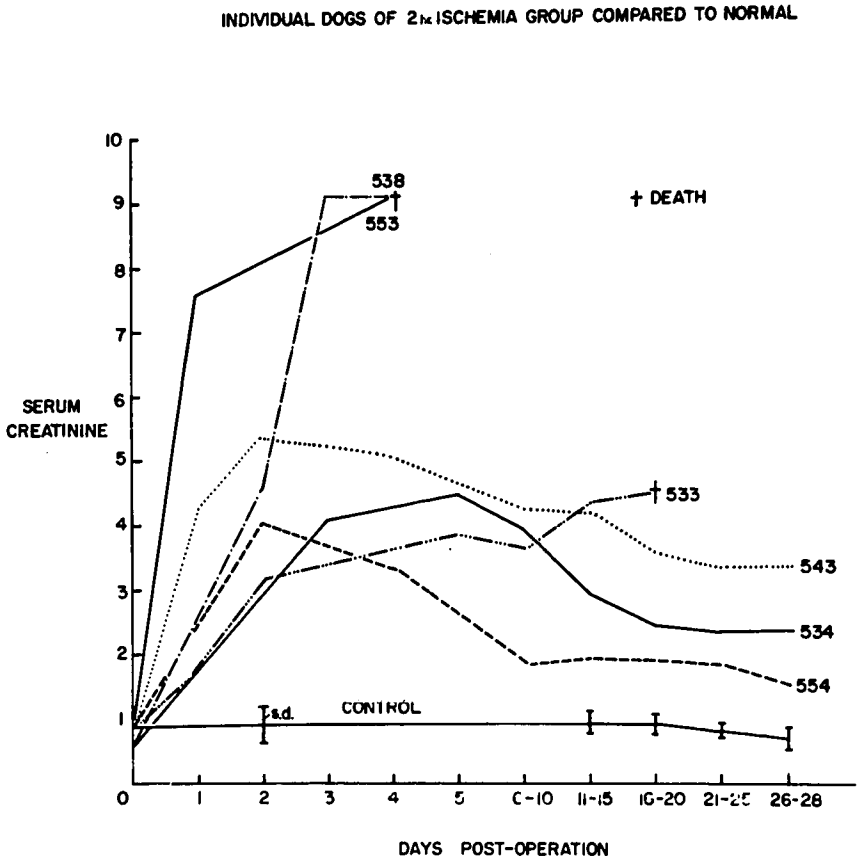



Fig. 10.

Creatinine levels of individual dogs of 2 hours group.

## GROUP II

### Duration of Renal Ischemia - 2 1/2 hours

The creatinine level course of this group is plotted in figure 9 comparing it with the 3 hour and control groups. The creatinine levels and the clinical picture of this group closely resembled the 3 hour group. All six animals died. The average period of survival was 7 days. The range of survival was 3 to 12 days. The clinical appearance showed no change, except longer average survival as compared to the 3 hour group.

## GROUP III

### Duration of Ischemia - 2 hours

Figure 10 shows the serum creatinine level course of the 2 hour ischemia group. There is a marked variation of functional response, by the animals, to the same duration of ischemia. Out of the six animals, two follow a pattern similar to that of the 2 1/2 hour and 3 hour ischemia groups. The serum creatinine levels rose steeply to 9 mg% in each dog. The third dog which died showed a much slower but gradual rise of serum creatinine. It died on the 16th day, with serum creatinine level of 4.95 mg% . On autopsy, however, no other significant abnormality could be observed other than renal infarction.

The three survivors in this group followed a separate pattern. Peak level of serum creatinine was over by the 5th post-operative day, after which they showed a gradual improvement of renal function. Their serum

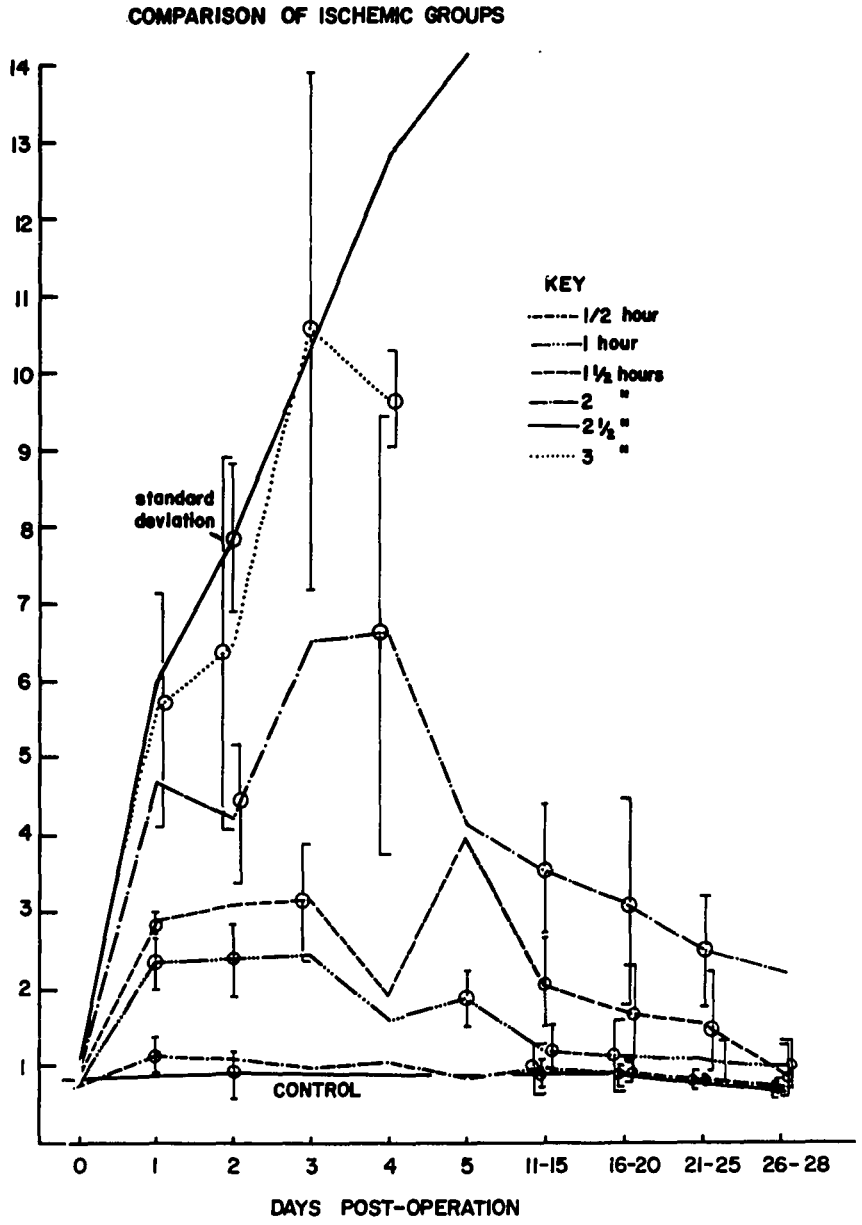



Fig. 11.

Summary of mean creatinine levels of all groups.

creatinine levels never reached the pre-operative level. Variable amount of renal insufficiency was evident throughout the course. These three dogs had gained 2.3 Kg. in average weight. A wide difference of response to ischemia is seen in this group, because a critical threshold of reversibility of damage may have been reached.

## GROUP IV

### Duration of Ischemia 1 1/2 hours

There was only one mortality in this group. Animal, 571, died with serum creatinine level 10.5 mg% on the 8th post-operative day. The course of changes in this animal was similar to the 2 1/2 hour and 3 hour group. In figure 11 mean creatinine levels of the 5 survivors of this group have been compared with similar plotting of the other groups. (The three deaths in the 2 hour group have not been included in this figure for the sake of simplicity of comparison) The mean creatinine level for the whole group on each post-operative day is entered upto day 5. From day 11, mean level for each 5 days is computed for better statistical correlation. The figure shows that the peak of serum creatinine in this group occurred earlier than that in the 2 hour ischemia group. Recovery was progressive and followed a distinctly lower level of insufficiency to the proceeding groups.

## GROUP V

### Duration of Ischemia - 1 hour

There was no death in this group. Serum creatinine peak was lower than

proceeding groups. Final serum creatinine level reached almost the pre-operative levels.

## GROUP VI

### Duration of Ischemia - 1/2 hour

There was no mortality in this group. Pre-operative serum creatinine peak was vague and rudimentary. Mean serum creatinine values reached lower than 1 mg% by the 5th post-operative day.

## GROUP VII

### Control, Right Nephrectomy with no Renal Ischemia

Figures 10 and 11, and table 3 compare mean serum creatinine values of this group against other groups. Maximum post-operative level of serum creatinine reached was, .92 mg%. It slowly declined with time, reaching .72 $\pm$ .1 mg% before the end of the experiment. All animals were sacrificed after a period of 28 days.

## COMPARISON BETWEEN VARIOUS GROUPS

Table 3 and figure 11 summarize the results of the whole project. Table 3 is a summary of the course of serum creatinine level at different times after operation for different groups. From day 0 to day 5, the figure shows an average of creatinine levels for all dogs for that day in the group. After day 11 up to day 28, the creatinine levels are pooled for 5 day periods and an average figure is entered. The control, 1/2 hour and 1 hour ischemia groups had no mortality; so data comprises all dogs in these groups. In

2 1/2 and 3 hour ischemia groups all dogs died, and data is entered only up to the 5th day. In the case of the 1 1/2 hour and 2 hour ischemia groups, data has been separated into two, survivors and deaths.

Figure 11 depicts the results of table 3 in graphical form, showing course of changes in serum creatinine levels of various groups. Please note that for the sake of comparison, the dogs which died in the 2 hour and 1 1/2 hour groups have not been included in the figure. The mean features of figure 1 1 are, that serum creatinine levels in all ischemic groups rose above control. In survivors after a peak, serum creatinine gradually decreased towards normal. Mean peak values reached were: 1/2 hour ischemia group, 1.13 mg%; 1 hour ischemia group, 2.4 mg%; 1 1/2 hour ischemia group, 3.12 mg%; 2 hour ischemia group, 6.58 mg%. Occurrence of peak tended to be later with longer duration of ischemia. Peak level of creatinine was over by the 5th day in all groups. Time taken for recovery was also proportionately longer with longer ischemia duration of ischemia.

In fatal cases, mean time of survival for the 3 hour group was 4 days, as compared to 7 days in the 2 1/2 hour group. The difference could not be proven to be due to severity of renal failure, as other factors, like longer duration of operative exposure and longer duration of anesthesia might have been contributing factors. Another sub experiment could be designed to show it if necessary.

## SUMMARY OF COURSE OF SERUM CREATININE LEVELS IN VARIOUS GROUPS

| Group | Day 0 Crt. ** S. D. | Day 1 Crt. S. D. | Day 2 Crt. S. D. | Day 3 Crt. S. D. | Day 4 Crt. S. D. | Day 5 Crt. S. D. |
|---|---|---|---|---|---|---|
| Control | .85 ± .27 | | .90 ± .29 | .9 * | | .9 * |
| 1/2 hr. | .77 ± .23 | 1.13 ± .22 | 1.1 * | 1.0 | 1.07 * | .87 * |
| 1 hr. | .72 ± .23 | 2.34 ± .29 | 2.36 ± .45 | 2.40 * | 1.60 * | 1.87 ± .33 |
| 1 1/2 hr. | .78 ± .09 | 2.88 ± .90 | 3.06 ± * | 3.12 ± .78 | 1.9 * | |
| | .7 | | 5.3 | | | 9.5 |
| 2 hr. | .78 ± .17 | 4.66 * | 4.23 ± .92 | 6.50 * | 6.58 ± 2.8 | 4.1 * |
| | .81 | 4.9 | 3.8 | 7 | 9 | |
| 2 1/2 hr. | .75 ± .14 | 5.4 * | 7.82 ± .94 | | 12.75 * | 14.06 * |
| 3 hr. | .75 ± .14 | 5.1 ± 1.5 | 6.45 ± 2.4 | 10.5 ±3.3 | 9.6 ± .6 | |

** S. D. Standard deviations

* Not enough readings for standard deviations

SEE REVERSE SIDE FOR TABLE

## SUMMARY OF COURSE OF SERUM CREATININE LEVELS IN VARIOUS GROUPS

| Group | Day 0 Crt. ** S. D. | Day 1 Crt. S. D. | Day 2 Crt. S. D. | Day 3 Crt. S. D. | Day 4 Crt. S. D. | Day 5 Crt. S. D. |
|---|---|---|---|---|---|---|
| Control | .85 ± .27 | | .90 ± .29 | .9 * | | .9 * |
| 1/2 hr. | .77 ± .23 | 1.13 ± .22 | 1.1 * | 1.0 | 1.07 * | .87 * |
| 1 hr. | .72 ± .23 | 2.34 ± .29 | 2.36 ± .45 | 2.40 * | 1.60 * | 1.87 ± .33 |
| 1 1/2 hr. | .78 ± .09 | 2.88 ± .90 | 3.06 ± * | 3.12 ± .78 | 1.9 * | |
| | .7 | | 5.3 | | | 9.5 |
| 2 hr. | .78 ± .17 | 4.66 * | 4.23 ± .92 | 6.50 * | 6.58 ± 2.8 | 4.1 * |
| | .81 | 4.9 | 3.8 | 7 | 9 | |
| 2 1/2 hr. | .75 ± .14 | 5.4 * | 7.82 ± .94 | | 12.75 * | 14.06 * |
| 3 hr. | .75 ± .14 | 5.1 ± 1.5 | 6.45 ± 2.4 | 10.5 ±3.3 | 9.6 ± .6 | |

** S. D. Standard deviations

* Not enough readings for standard deviations

| Days 11 - 15 Crt. S. D. | Days 16 - 20 Crt. S. D. | Days 21 - 25 Crt. S. D. | Days 26 - 28 Crt. S. D. | Remarks |
|---|---|---|---|---|
| .92 ± .15 | .92 ± .12 | .83 ± .06 | .72 ± .10 | All survived |
| .96 ± .30 | .91 ± .15 | .85 ± .11 | .78 ± .09 | All survived |
| 1.21 ± .30 | 1.21 ± .46 | 1.08 ± .27 | 1.04 ± .29 | All survived |
| 2.05 ± .49 | 1.72 ± .59 | 1.6 ± .62 | .92 ± .36 | Survivors only (five) |
| 10 .9 | | | | Deaths only (one) |
| 3.5 ± .9 | 3.13 ± 1.3 | 2.48 ± .71 | 2.26 ± .84 | Survivors only (three) |
| | | | | Deaths only (three) |
| | | | | All died |
| | | | | All died |

PROBABILITY OF NON SIGNIFICANCE OF THE DIFFERENCE BETWEEN CREATININE LEVELS OF CONTROL AND ISCHEMIA GROUPS AT DIFFERENT TIMES OF RECOVERY

| Group | days 11 - 15 | 16 - 20 | 21 - 25 | 26 - 28 | Estimated Recovery Time |
|---|---|---|---|---|---|
| Control + 1/2 hr. | * N. S. | N. S. | N. S. | N. S. | < 11 days |
| - 1 hr. | < .01 | N. S. | < .02 ? | < .01 ? | 16 days |
| - 1 1/2 hr. | < .001 | < .001 | < .01 | N. S. | 26 days |
| - 2 hr. | < .001 | < .001 | < .001 | < .001 | > 28 days |

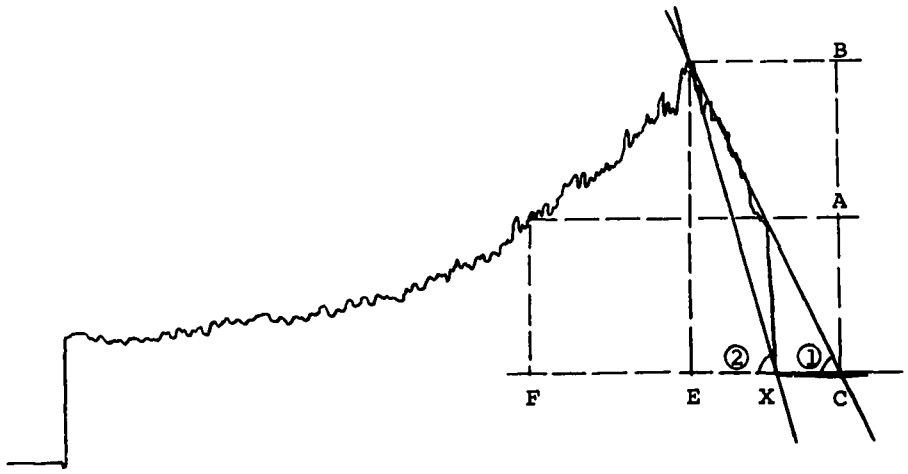
* N. S. = Non - significant difference

## RENOGRAM RESULTS

Renograms were analysed with regard to measurement of multiple parameters. Figure 12 shows various parameters measured along the time axis, amplitude axis, slope of first and second seconds and their combinations and ratios. The results were compared with other indices of renal function, in order to find the parameter which would reflect best the changes consistently. The attempts, however, were futile and no consistently significant relationship between these parameters and renal function was noticed. The only two parameters which showed a reasonable accuracy were: 1. Slope of the second segment. 2. $T_{max}$ or transit time; i.e., time taken by the tracer to reach maximum amplitude. Eventually a semiqualitative method of grading was adopted, keeping the most significant changes in mind. The method has previously been described by Dossetor et al. (Transplantation, vol. 5, 851, 1967) and is in use in renographic follow up of transplanted patients. The method depends, more or less, on over all configuration, rather than mathematical measurement of any individual parameter. Later on when correlation was noticed, with slope of the second segment and transit time, the criteria were also included in the classification.

## CLASSIFICATION

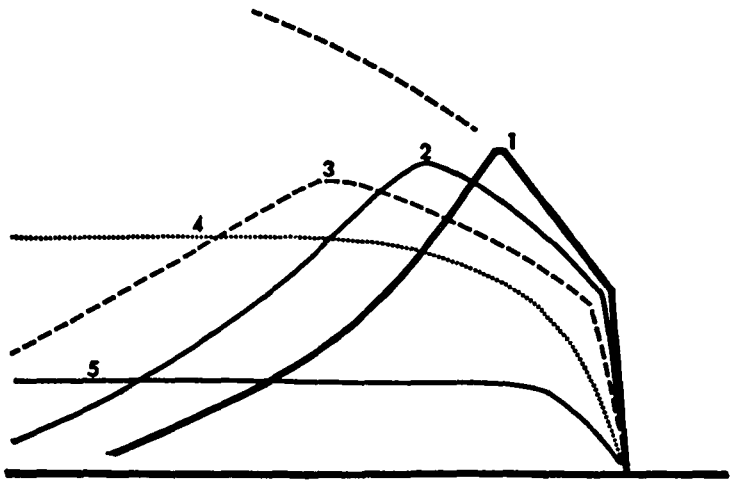
The classification was devised by taking grade 1 as normal renogram, and grade 5 being the tracing of complete tubular necrosis ( see figure 13).




| TIME AXIS | AMPLITUDE AXIS | ANGLES |
|---|---|---|
| XE -- $T_{max}$ | AC -- Amplitude of 1st segment | ① -- slope of 2nd segment |
| EF -- $T_{½}$ | AB -- " " " 2nd " " | ② -- mean slope of 1st & 2nd segments |
| $T_{max}/T_{½}$ -- Ratio of $T_{max}/T_{½}$ | AC/AB - Ratio of 1st segment: 2nd segment | |

Fig. 12.

Renographic parameters measured.




RENOGRAM CURVES

FIGURE 4. Five main types of renogram curve, used for renogram analysis and determination of renogram index per patient.

After; Dossetor, MacKinnon, Gault, MacLean.

Fig. 13.

Main types of renogram curve.

Intermediary grades show varying degree of renal damage. Grade 6 is given for back ground radiation count only.

GRADE I. NORMAL RENOGRAM

1. Well pronounced first, second and third segment.
2. Transit time less than 5 minutes.
3. Slope of second segment more than $50^{o}$.
4. Third segment concave upwards (double exponential with an unknown variable ).

GRADE II

1. All segments well differentiated.
2. Transit time 5 - 7 1/2 minutes.
3. Slope of second segment $30^{o}$ - $50^{o}$.
4. Third segment still concave upwards.

GRADE III

1. All segments can still be differentiated, but peak second segment becomes rounded.
2. Transit time 7 1/2 - 10 minutes.
3. Slope of second segment less than $30^{o}$.
4. Third segment loses exponential curvature.

GRADE IV

1. Differentiation between various segments lost.
2. Transit time less than 10 minutes.
3. Second segment rounded and no straight tangent present to measure the angle.
4. Third segment becomes a straight horizontal line.

GRADE V

1. No differentiation between various segments.
2. Transit time cannot be measured.
3. Second segment disappeared.
4. Third segment remains a horizontal straight line.

GRADE VI (Back ground radiation only)

A vascular spike with no evidence of renal activity.

CORRELATION OF RENOGRAMS WITH SERUM CREATININE LEVELS

The renograms were graded according to the classification described. The merits and demerits of the classification will be discussed in the next chapter. Figure 17 shows renogram index plotted against the simultaneous serum creatinine levels. Multiple readings at the same point are plotted with circumferential circles or sectors. The mean serum creatinine for each grade has been plotted with a heavy dot and horizontal bar. The verticle box denotes standard deviations for each mean serum creatinine level. Table 6 depicts the same data in numerical form.

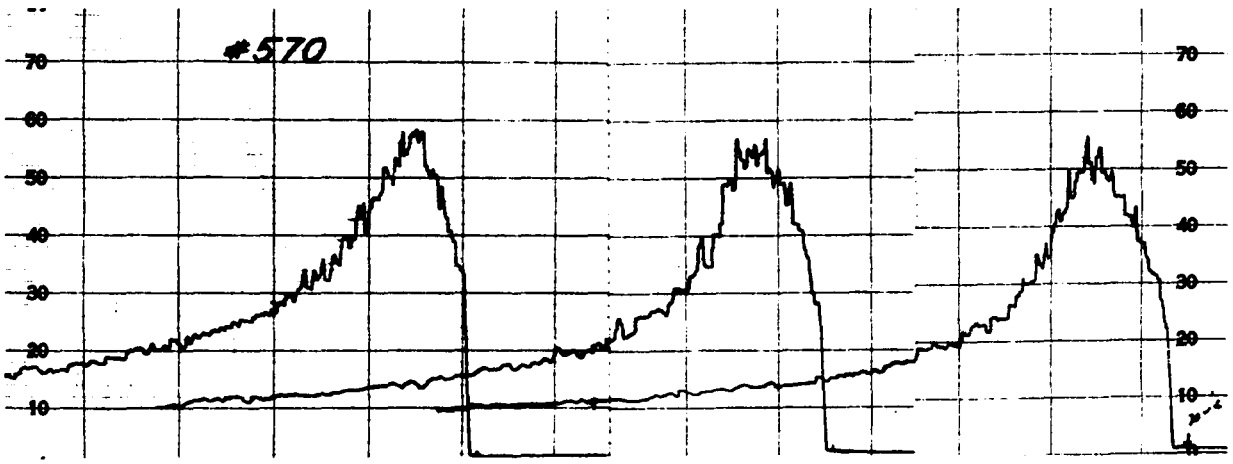



Fig. 14. A.

Normal renograms,
(control group)
From right to left: Preoperative, 1 day post-operative and
7 days post-operative respectively.


Fig. 14. B.

Reversible ischemic damage.
( 1 1/2 hour ischemia group)
From right to left: Preoperative, 1 day post-operative,
7 " " "
28 " " "

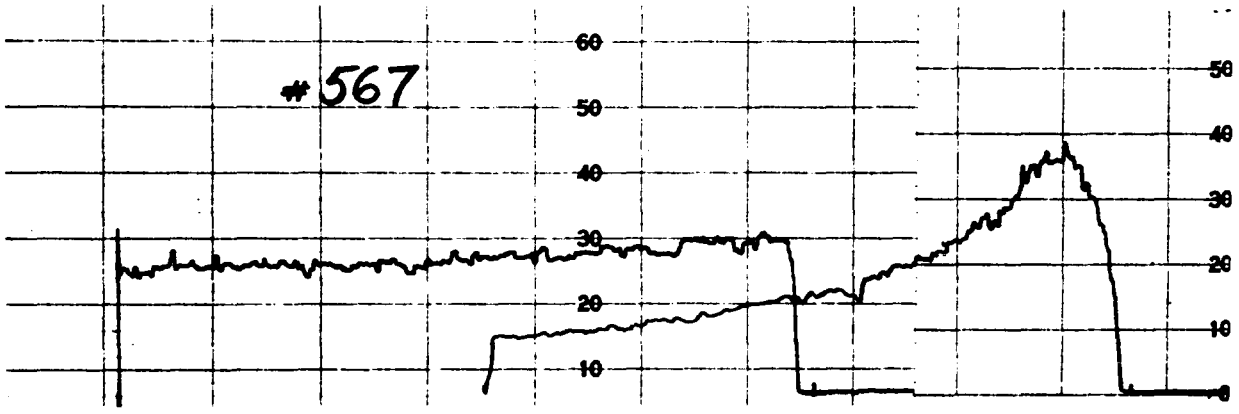



Fig. 14. C.

Irreversible ischemic damage.
( 2 hour ischemia group )
From right to left: Preoperative, 5 days post-operative,
( animal died on 8th. day. )

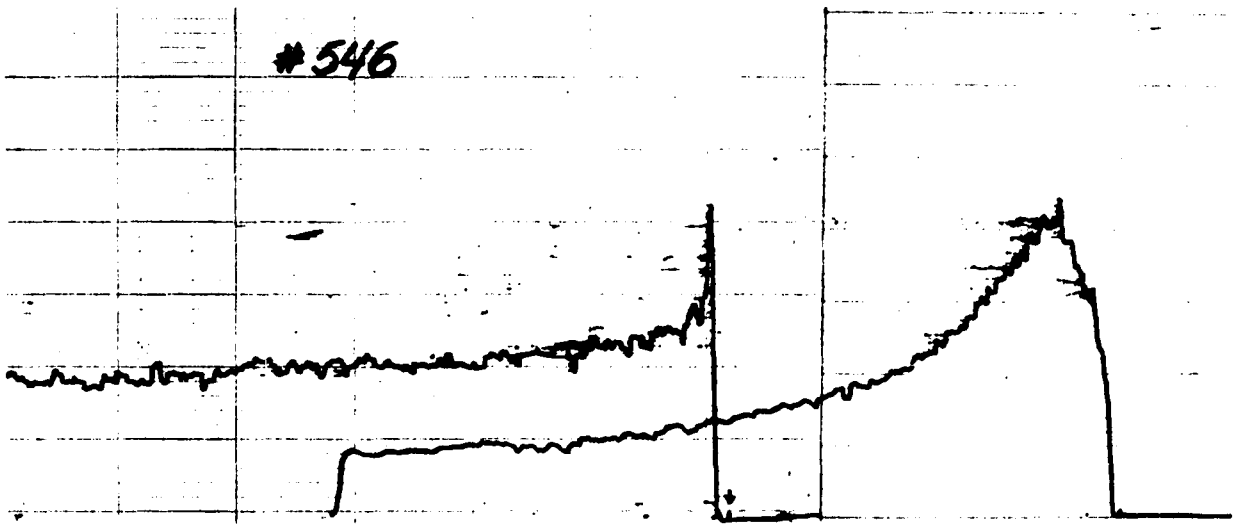



Fig. 14. D.

Irreversible ischemic damage.
( 2 1/2 hour ischemia group )
From right to left: Preoperative, 3 days post-operative,
( animal died on 5th. day. )

## POST ISCHEMIC RENAL CONSISTENCY

The renal consistency after 15 minutes of restoration of renal arterial blood flow, was measured by palpation. Taking grade 4 as normal pre-operative renal consistency, it was graded on a scale of 5 according to the degree of firmness, for comparison with post-operative renal function. 1. Flabby, 2, Soft, 3. Subnormal, 4. Normal. 5. More tense than normal.

## MORPHOLOGICAL CHANGES

There were only 16 animals dying from acute renal failure, and thus available for study of changes directly due to ischemic injury. The sacrificed dogs showed a totally different picture and are discussed under a separate heading.

## DOGS DYING OF ACUTE RENAL FAILURE

Gross appearance of kidney: Whatever the duration of ischemia there did not seem to be any difference in appearance of the kidney. In all cases the kidney was markedly enlarged and edematous. A greyish brown lesion of infarction and areas of hemorrhage could be noticed on the surface. Artery and vein were always carefully dissected, and in no case was vascular occlusion found. During autopsy, the position of the kidney was also specially noticed, to rule out the possibility of torsion. Some amount of calcification was frequently present, but in two animals, dying on the 8th and 9th day, there was extensive calcification of the cortex.

Cut surface: On section, the cut surface bulged out. The most obvious

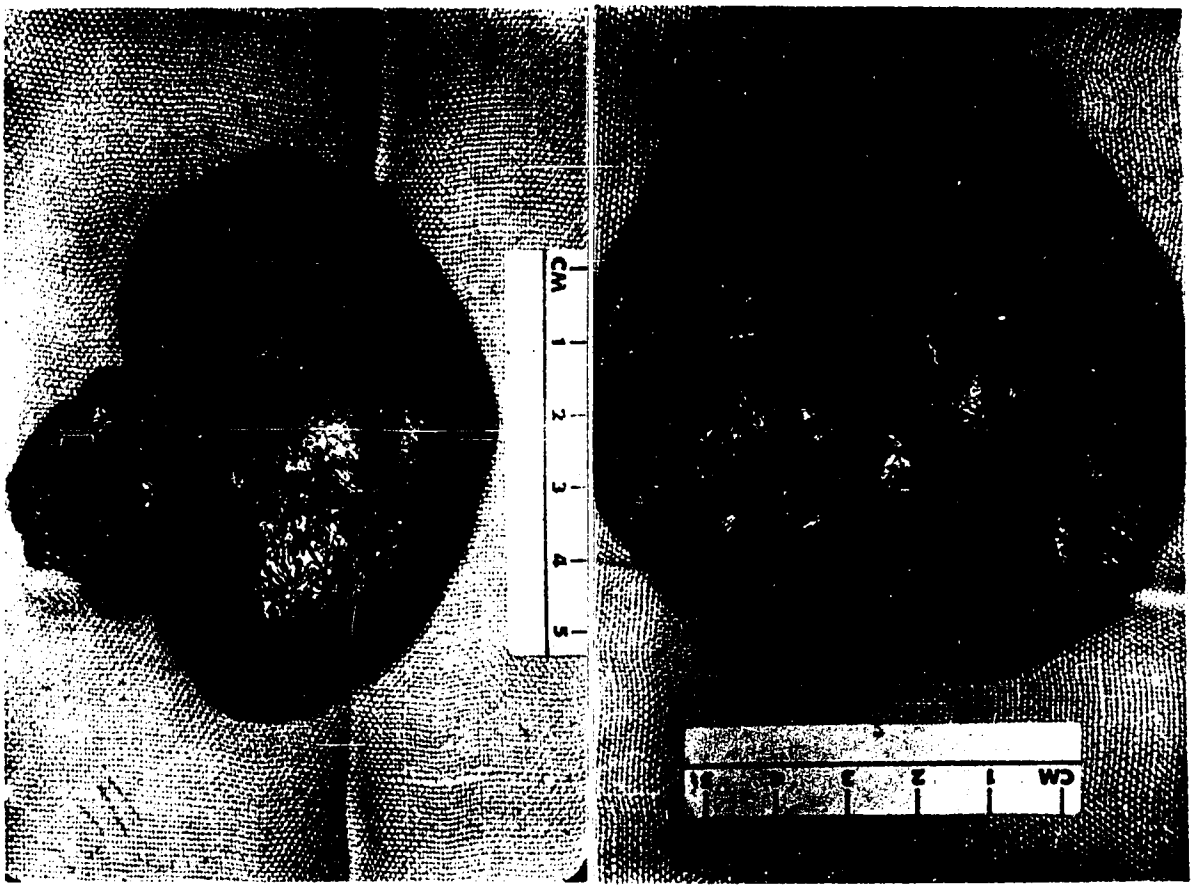



Fig. 15. A.

Renal necrosis after 3 hours ischemia,
( with gross hemorrhage in medulla at
the lower pole)

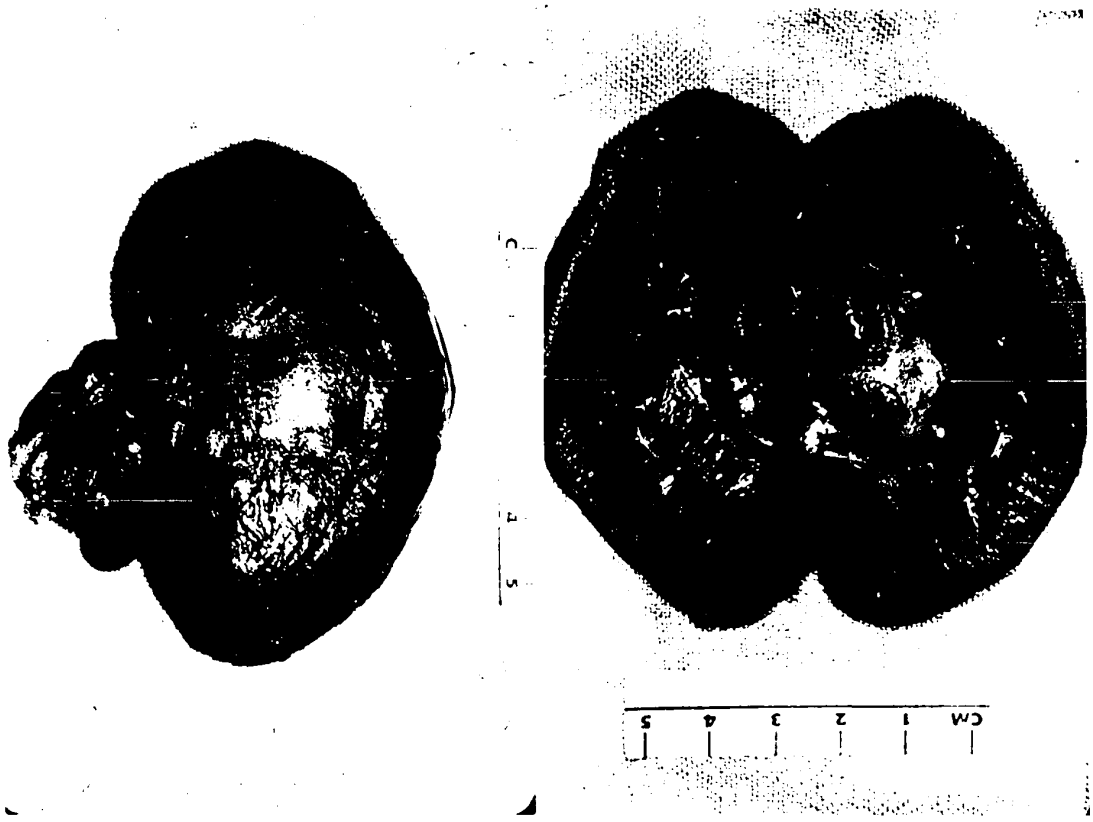



Fig. 15. A.

Renal necrosis after 3 hours ischemia,
( with gross hemorrhage in medulla at
the lower pole)


Fig. 15. B.

Gross edema of the kidney after 2 hours renal ischemia, ( animal died 7 days after the operation)


Fig. 15. B.

Gross edema of the kidney after 2 hours renal ischemia, ( animal died 7 days after the operation)

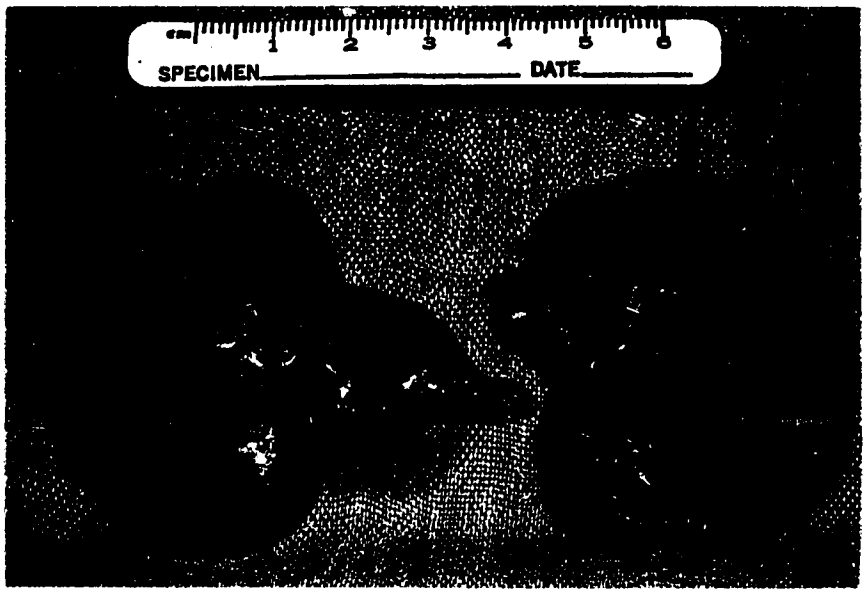



Fig. 15. C.

Survivor after 2 hours of renal ischemia,
( animal sacrificed after 28 days )

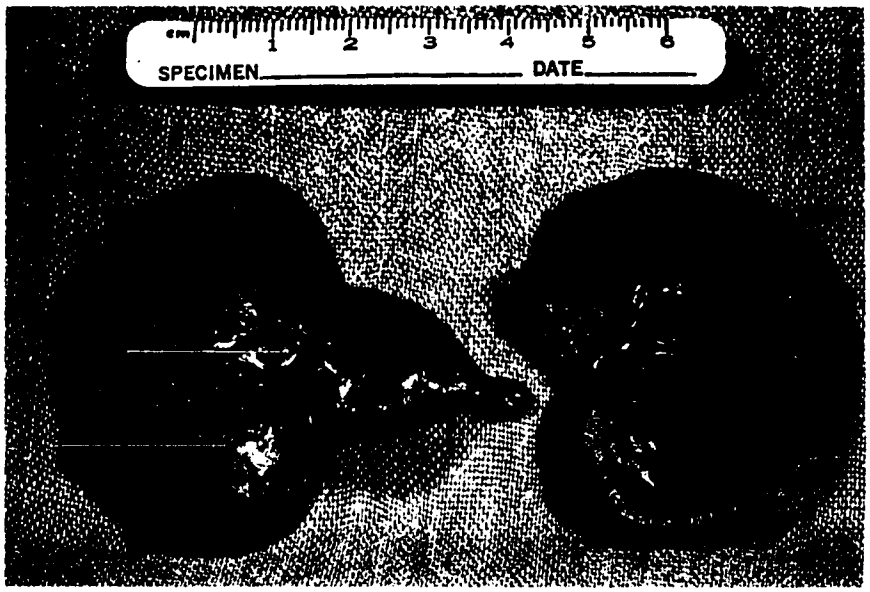



Fig. 15. C.

Survivor after 2 hours of renal ischemia,
( animal sacrificed after 28 days )

change was a pale cortex, in sharp contrast to deep congested medulla. The typical wedge shaped infarct rarely occurred. Most commonly irregular lesions of yellowish grey, or dark brown colour were noticed. These patches were frequently surrounded by diffuse greyish red hemorrhagic border. In some animals the whole cortex of the kidney was infarcted and stippled with granules of calcium.

Microscopic appearance: The striking features on microscopic examination were as follows:

1. Multifocal distribution of the lesion with complete sparing of parenchyma in the intermediary areas.
2. There was a surprising discrepancy between severity of acute renal failure and only modest microscopic changes. It was difficult to estimate the approximate length of ischemia from microscopic appearance.
3. The lesion involved predominantly, tubules and interstitial tissue. Glomeruli were almost completely spared.

Glomeruli: The renal corpuscles were usually found morphologically intact. The only change noticed occasionally was collapse of capillary loops, with absence of red blood cells.

Tubules: The tubules involved, frequently lay deep in the cortex. The changes varied from cloudy swelling, coagulation necrosis to complete necrosis of the cells. The general architecture was often preserved. Initial changes were edema of cells and vacoulization of the cytoplasm. The nuclei became

Fig. 16. A.

Section of kidney showing
medullary congestion. x 5
( 2 hour ischemia, animal died on 8th. day.)

Fig. 16. A.

Section of kidney showing
medullary congestion. x 5
( 2 hour ischemia, animal died on 8th. day. )

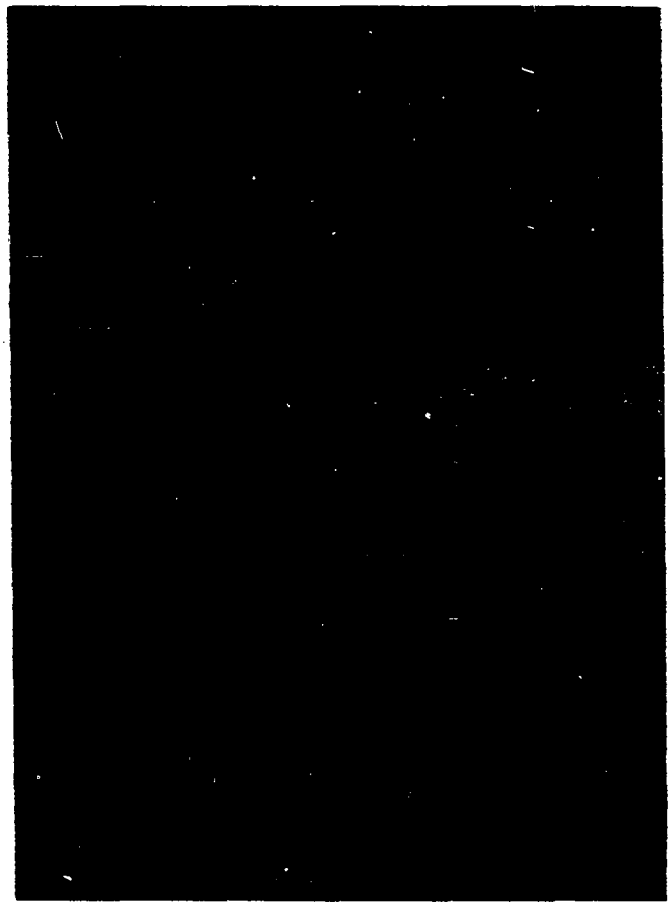


Fig. 16. B.

Section of kidney showing multiple focal distribution of the lesion with relative sparing of the glomeruli. x 35

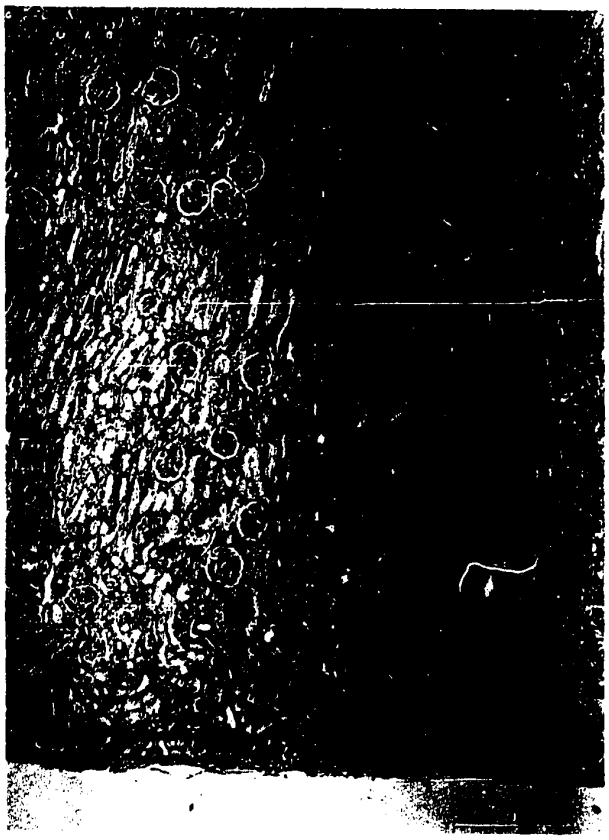


Fig. 16. B.

Section of kidney showing multiple focal distribution of the lesion with relative sparing of the glomeruli. x 35

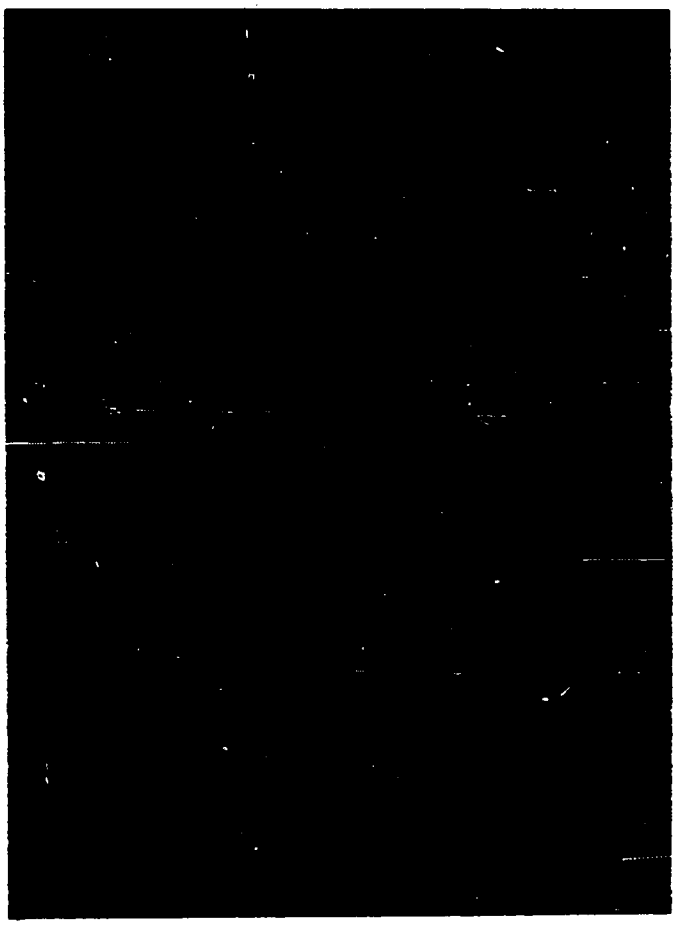


Fig. 16. C.

Section of kidney showing necrosis of the tubular cells with preservation of the general architecture. There is moderate interstitial infliltration. x 100

Fig. 16. C.

Section of kidney showing necrosis of the tubular cells with preservation of the general architecture. There is moderate interstitial infliltration. x 100

pyknotic, shrunken, and finally karyorrhextic. Eventually the nuclei disappeared completely. The cytoplasm was eosinophilic and became granular. In no section was typical tubulorrhexis of Oliver noticed. However, suspicion of tubular wall damage was present occasionally, and debris was noticed inside the lumen of the tubules. Several areas showed tubular cast.

Interstitial tissue: There was marked interstitial edema constantly present in dogs dying between 5 - 10 days. Several areas showed frank hemorrhages. The periphery of necrotic foci showed cellular infiltration. Scattered areas of calcification were present in the cortex.

Surviving animals: The surviving animals showed quite a different appearance of the kidney. The capsule was increased in thickness. The kidney was usually covered with adhesions. The size was frequently normal or hypertrophied. The surface was scarred at places.

Cut surface: The gross architecture did not seem much changed, except that several areas of scarring and depression were noticable. Surprisingly, there was little calcification present in these kidneys.

Microscopic appearance: There was considerable subcapsular fibrosis. There was also evidence of increased collagen deposition in foci in the parenchyma. Interstitial tissue showed increased number of fibroblasts which were larger in size and spindle shaped. Tubular cells were also hypertrophied and projecting well in the lumen of the tubules.

# DISCUSSION

## COURSE OF CHANGES IN SERUM CREATININE CONCENTRATION

Serum creatinine was the most significant of the parameters by which function could be assessed on a day to day basis. The analysis of the results yields significant information on the following aspect of the renal ischemia.

1. Prognostic indices
2. Reversible versus irreversible renal damage
3. Recovery of glomerulo tubular function
4. Validity of serial estimation of serum creatinine

### 1. PROGNOSTIC INDICES

#### A. Response peak of serum creatinine

Maximum level of serum creatinine compatible with reversible renal damage was less that 7mg %, irrespective of duration of renal ischemia. Death occurred ultimately in all experiments where serum creatinine level reached higher than 7 mg%. The prognostic value of serum creatinine level has been previously pointed out by Myers and Killian who reported that patients whose creatinine rose above 5 mg%, had a poor prognosis and ultimately died after a variable period of time. This finding was published before the advent of hemodialysis. Several other reports also support serial serum creatinine estimation as an accurate assessment of renal function and prognosis. Several other observations were made regarding the peak level of serum creatinine.

## EXPLANATION: COURSE OF CHANGES IN S CREATININE CONCENTRATION

The time taken by serum creatinine to reach the peak level was proportional to the duration of renal ischemic damage. The peak occurred earlier in shorter periods of ischemia, and later when duration of ischemia was longer. All experiments in which renal damage was reversible, the peak level of serum creatinine had passed by the 5th post-operative day. Thus, animals that did not show improvement after the 5th day, carried a very poor prognosis.

B. Duration of ischemia

Complete arterial occlusion for two hours proved to be the minimum lethal dose in this series of experiments. Hamilton et al. considered 3 hours as the lethal dose, but in their method they did not divide all the capsular and anastomosing vessels in the pedicle. The two situations, therefore, cannot be compared. Porch et al. found that there was uniform mortality after they clamped the renal artery for 3 hours. The importance of collateral circulation through these vessels has been proved in several subsequent studies. Morris et al. compared the effect of two hours of renal ischemia, by occluding only the renal artery in one group, and renal artery and aorta proximal to renal artery in another group. Glomerular filtration rate in the former group dropped 68%, whereas, in the latter group it decreased to 13% of control.

2. REVERSIBLE VERSUS IRREVERSIBLE ISCHEMIA

Irrespective of the length of ischemia, both irreversible and reversible renal damage, seemed to follow a set pattern. In irreversible ischemia there was a steep rise of the post-operative creatinine level which continued until death occurred. Because the average number of days of

survival was greater in the 2 1/2 hour ischemia group as compared to the 3 hour group, the former reached a higher level of serum creatinine before death. Maximum level of serum creatinine recorded was 17. 4 mg%.

In the case of the reversible ischemia, the rise of serum creatinine level was gradual and proportional to the extent of damage. Divergence of course was apparent, sometimes as early as the second post-operative day, and the latest, by the third or fourth day when the extent of rise could be clearly distinguished from the irreversible damage. This is quite apparent in figures 3 and 4. As mentioned earlier, recovery if at all to occure, started by the 5th post-operative day. If an animal did not show a drop of serum creatinine level, its chances of recovery were small.

3. RECOVERY OF GLOMERULO TUBULAR FUNCTION

The time of recovery for a given duration of ischemia was defined as the time required for serum creatinine level to come back to the control level. In our case, we have estimated the time of recovery by testing (using T test) the significance of the difference between the average serum creatinine levels of the control, and various groups at different time periods. The results of the T test are shown in table 4, along with estimated recovery time.

1. The serum creatinine level of the 1/2 hour ischemia group shows no significant difference from the creatinine levels of the control group by the 11th day.
2. The 1 1/2 hour ischemia group remains different from the control group up to 21 - 25 day period. Therefore, recovery

occurred after 25 days.

3. The 2 hour ischemia group remains significantly different from the control group throughout the period of follow up, therefore, it never showed a complete recovery during the period of study. A similar experience has been reported by Badenoch, Scarf, and Hamilton. In general the longer the duration of ischemia, the longer it took for complete recovery.

4. VALIDITY OF SERIAL ESTIMATION OF SERUM CREATININE

Table 3, column day 0, shows a statistically significant uniform pre-operative serum creatinine level which provides an adequate baseline for subsequent serial determination of serum creatinine.

POST ISCHEMIC RENAL CONSISTENCY

This parameter was subjective, as objective measurement could not have been made without proper instrumentation for measuring blood flow. The observer was aware of the length of time of ischemia, therefore, the measurement could have been biased. It was not possible to obtain an independent opinion. Nonetheless, grading of post ischemic renal consistency was carried out because of its potential clinical value.

From table 5, it is evident that post-operative survival was roughly porportional to the consistency of the kidney following resumption of arterial blood flow. The consistency changes are probably brought about by the shunting of renal blood flow from the cortex. Although no claim of correlation of renal functional efficiency with post ischemic consistency is made, this possibility should be

## RELATIONSHIP OF COURSE WITH POST ISCHEMIA RENAL CONSISTENCY

| Grade of Consistency | No of Animals | Mortality | SURVIVAL | |
|---|---|---|---|---|
| | | | With Serum Cr. 2 mgm% | With Serum Cr. 2 mgm% |
| 1 Flabby | 5 | 5 | - | - |
| 2 Soft | 14 | 11 | 1 | 2 |
| 3 Sub-normal | 14 | - | 3 | 11 |
| 4 Normal | 9 | - | - | 9 |
| 5 More tense than normal | - | - | - | - |

actively investigated on a scientific basis with an electromagnetic flow meter, or Krypton or Xenon washout studies. Such information would be clinically useful to prevent the transplantation of kidneys which do not perfuse well, even on the operating table and thus, lower the overall failure rate. Under specific criteria these kidneys could be removed before closure of incision without any consequences. Changes in renal consistency might also detect technical problems of vascular anastomosis which can be corrected immediately.

## RENOGRAMS

Serial renograms were performed with the aim of answering the following questions.

1. What are the changes brough about by ischemia in the renographic tracing?
2. Can the ischemic effect in a renogram be graded quantitatively?
3. Can the renographic interpretation be correlated with corresponding serum creatinine levels?
4. What are the renographic differences between reversible and irreversible ischemia, and are renogram changes useful in determining the prognosis of kidneys with various times?
5. What are the clinical applications of a renogram? Can it be used as a screening procedure in checking functional integrity of donor kidneys for transplantation?

## RENOGRAPHIC CHANGES SUGGESTIVE OF ISCHEMIC DAMAGE

The most significant change in the tracing appeared to be progressive flattening of the second segment. The amplitude of the second segment de-

creased, and the junction between the second and third segment became less sharp, proportional to the decrease in renal function. This has been observed by Luke, Briggs and Kennedy as to rounding of the peak. With further reduction of renal function the second segment flattened into a horizontal line. This is referred as a "non functioning curve", or grade 5 in our classification. Considerable disagreement exists concerning pathophysiological mechanism that brings about this change. However, this discussion is not within the scope of this paper.

In figure 12 a multitude of parameters is given which were routinely measured to detect any pattern consistent with renal function. On the whole, these parameters did not change proportionately to functional change. The only two measurements that could be compared with renal function were the slope of the second segment of the renogram and the $T_{max}$ or transit time.

Meanwhile, a semiqualitative classification was carried out as described. It is to be stressed that this classification should be treated only as such. It has been devised without adequate mathematical analysis. It is only a way of qualifying the changes in configuration which are most noticeable after acute glomerulo-tubular damage. Using this method of grading, the renal index was plotted against simultaneous serum creatinine estimations. Figure 17 shows that the margin of error (standard deviation) in anticipating serum creatinine levels, was much less in renograms of grade 1, but progressively increased with the shift to the right (towards grade 5 - 6).

Table 7 shows the significance of the difference of serum creatinine levels

associated with various renograph grades. For example, renogram grade (as expressed by corresponding serum creatinine level) is significantly different from grades 2, 3, 4, 5, and 6. Whereas, renogram grade 5 can only be differentiated from grades 1, 2, and 3, but is not significantly different from grades 4 and 6. In general, renograms representing better renal function are more differentiable than, renograms representing poor renal function.

A significant point noticed in this correlation was that the renogram tracings looked worse when the serum creatinine levels were still rising. Therefore, in the acute phase of damage the renogram reflected the true extent of renal damage more accurately than the concurrent serum creatinine. This finding may have some implications in ante-mortem evaluation of cadaver kidney for transplantation. In these cases the serum creatinine levels may be misleadingly low despite fresh renal damage.

DIFFERENTIATION BETWEEN REVERSIBLE AND IRREVERSIBLE DAMAGE

This question was of particular interest, because of its practical importance. The following observations were made.

1. Some of the animals after 2 1/2 hour and 3 hour ischemia period showed a tracing similar to the background vascular radiation (grade 6). These animals never recovered, and died within a few days.
2. Survival was directly proportional to the prominence of the second segment of the tracing. All dogs with grade 2 and 3 renograms, and most of grade 4 tracings survived.

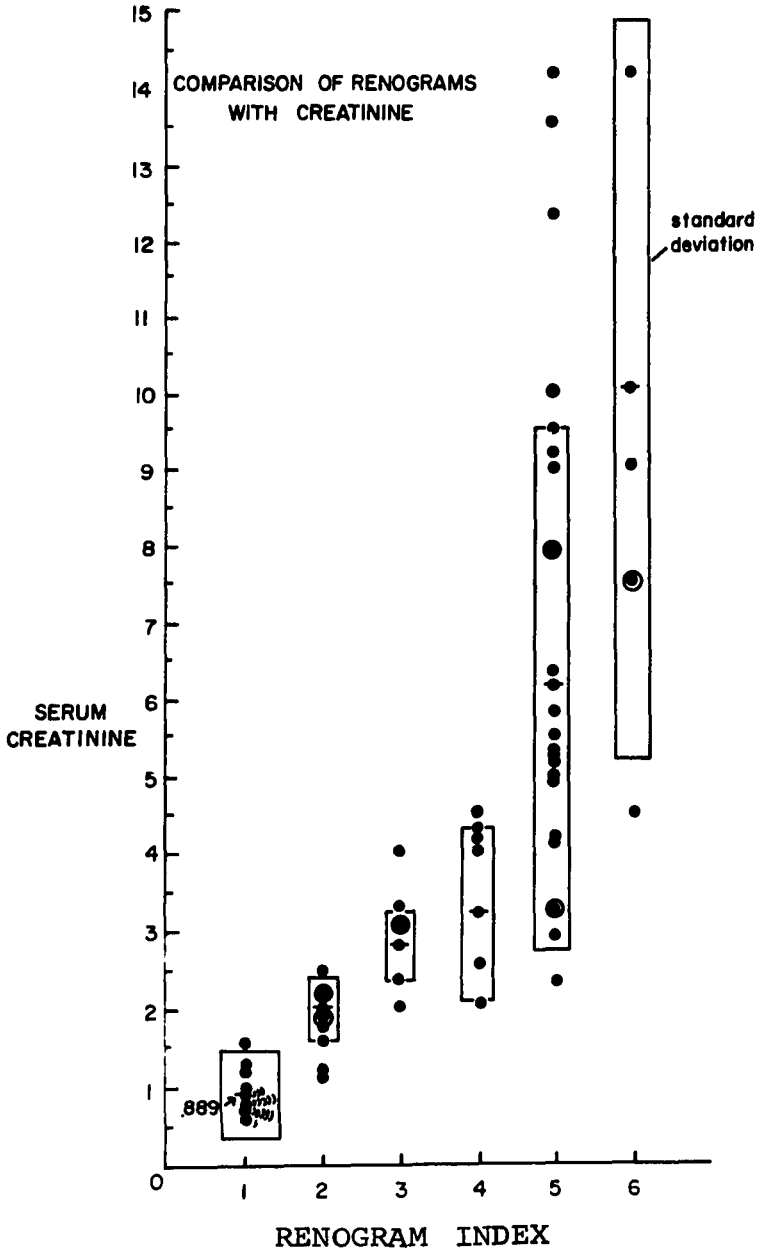



Fig. 17.

Comparison of renogram index with serum creatinine.

3. The prognosis was most difficult to predict in the bulk of renograms labelled grade 5. Although there was no evidence of tubular function, no prediction could be made regarding reversibility. In these cases serial renograms showed late development of the second segment, and eventual recovery to a varying degree, occurred. Unfortunately, this information did not come earlier than the serum creatinine levels and therefore, was of no practical value. In contrast to the rising slope of serum creatinine, the downward course went more or less hand in hand with the renogram index. This was probably due to the fact that the recovery process is so slow that no lag in serum creatinine level occurs.

SCOPE OF USE FOR EVALUATION OF RENAL TRANSPLANT DONORS

Cadaver donors represent a special problem requiring a different approach for evaluation of renal function. Donors are usually patients with terminal illness who are often too ill to transport, or anesthetized for the purpose of differential renal function studies. Kidney function is changing from moment to moment (probably going from bad to worse) and tests such as an endogenous creatinine clearance, which does not give an idea of immediate renal function, is unreliable. Under these conditions renograms have a distinct advantage in spite of the present inadequate methods of evaluation. Also the renogram can be repeated moments before harvesting the kidney and the results are available instantly. Furthermore, the test can be repeated after completion of the vascular anastomosis. Any functional deficit sustained during this time will be

## COMPARISON OF RENOGRAMS WITH ENDOGNEOUS CREATININE

| Renogram Index | No. of Renograms | Mean Creatinine | S. D. | Range |
|---|---|---|---|---|
| 1 | 61 | .89 | ±.27 | 0.4 - 1.7 |
| 2 | 9 | 2.01 | ±.39 | 1.3 - 2.5 |
| 3 | 3 | 2.8 | ±.45 | 2.4 - 3.3 |
| 4 | 4 | 3.2 | ±1.12 | 2.0 - 4.3 |
| 5 | 21 | 6.15 | ±3.42 | 2.3 -14.1 |
| 6 | 6 | 10.0 | ±4.8 | 4.5 -17.4 |

SIGNIFICANCE OF DIFFERENCE OF CREATININE LEVELS ASSOCIATED WITH RENOGRAM INDEX

| Renogram Index | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | x | .001 | .001 | .001 | .001 | .001 |
| 2 | x | x | .02 | .02 | .01 | .001 |
| 3 | x | x | x | N. S. | N. S. | N. S. |
| 4 | x | x | x | x | N. S. | N. S. |
| 5 | x | x | x | x | x | N. S. |
| 6 | x | x | x | x | x | x |

evident by comparison with the pre-transplant tracing.

## CRITICAL APPRAISAL OF RENOGRAMS

To sum up in the words of Klapproth, "renogram reflects a complex kinetic mechanism involved in the transit of the isotope through the whole urinary tract". Superficially it appears to be a very simple test, but the same simplicity is the reason for its inadequacy and inaccuracy. Most of the confusion that surrounds its interpretation and quantitation is due to the multiple variables (e. g. blood background count) involved, which have not been properly investigated and quantitated. Therefore, we cannot expect uniformity of results when we have not standardized the data input. The latter, however, is a tedious problem and will involve complex mathematics and further research in basic mechanism of external radiation monitoring, and renal kinetics involved in handling of radioactive excretory substances. Efforts in this direction are already being made with simulation models, using digital and analogue computers. Until this is available, we cannot expect an adequate quantitative expression of renographic function, and such was not the purpose of this study. Renographic review was added to the project only to establish validity of the test in existing form. Within the limits, the renogram is a very useful tool for evaluation of renal function. It has offered a radically different approach to the problem, and has distinct advantages not offered by other tests. This is the only test which can be carried out speedily, safely, without administration of anesthesia which gives information on differential function of renal blood flow, parenchymal function and patency of urinary tract.

Its greatest disadvantage is the lack of a standardized method of interpretation. In the current form a qualitative method of visual expression, by an experienced person, is probably more reliable than measuring individual parameters. This has been shown in this study, when a variety of parameters of the tracing were correlated with renal function, not one of them comes to a statistically significant uniformity.

## SUMMARY

The ischemic tolerance of the canine kidney has been determined as follows:

| | |
|---|---|
| with 1/2 hour renal ischemia. | No evidence of detectable renal damage. |
| with 1-2 hours renal ischemia. | A variable amount of reversible renal insufficiency. |
| with more than 2 hours renal ischemia. | Progressive renal failure. |

The longer the duration of ischemia, the longer is the time taken for recovery.

Serum creatinine concentration above 7 mg% is indicative of irreversible acute renal failure when untreated.

## RENOGRAM

1. With the present state of knowledge, a qualitative analysis of renogram is still more reliable than an attempt at quantitative assessment.

2. With worsening of renal function the renogram becomes increasingly more uninterpretable.

3. The renogram is more reliable than the serum creatinine level in determining the severity of the acute phase of renal damage.

4. Predictions of reversibility of renal damage cannot be made from the renographic tracings.

## BIBLIOGRAPHY

1. ALLEN, A. C.

"The Kidney in: Medical and Surgical diseases", second edition, p. 380-397.

Grune and Stratton, New York, 1962.

2. BADENOCH, A. W., DARMADY, E. M.

"The effect of temporary occlusion of the renal artery in rabbits and its relationship to traumatic uremia"

J. PATHOL. & BACTERIOL. Vol. 59: p. 79, 1947.

3. BERNSTEIN, L. H., SCHILEGEL, J. O., O'DELL, . RM.

"Differential renograms for evaluation of renal tubular function"

SURG. FORUM. Vol. 181: p. 533, 1967.

4. BLOCK, K., SCHOFNHEIMER, R.

"Studies of protein metabolism: The metabolic relation of creatine and creatinine studied with isotopic nitrogen"

J. BIOL. CHEM. Vol. 191: p. 111, 1939.

5. BROD, J., and SIROTA, J. H.

"The renal clearance of endogenous 'creatinine' in man"

J. CLIN. INVEST. Vol. 27: p. 645, 1948.

6. CALNE, R. Y.

"Renal transplantation", second edition, p. 155.

Edward Arnold Ltd.

7. COHN, H. E., MOSES, M.

"The critical interval in cadaver kidney transplantation: Function of canine renal autotransplants after variable periods of ischemia"

SURG. Vol. 60: p. 750-753, Sept. 1966.

8. CRAWFORD, B.

"Depression of the erogenous creatinine insulin thiosulphate clearance ratio in man by diodrast and PAH"

J. CLIN. INVEST. Vol. 27: p. 171, 1948.

9. DORE, E. K., TAPLIN, G. V., and JOHNSON, D. E.

"Current interpretation of the sodium iodohippurate $1^{131}$ Renocystogram"

J. A. M. A. Vol. 185: p. 925-932, 1963.

10. DOSSETOR, J. B.

"Creatininemia versus uremia"

ANN. of INTERNAL MED. Vol. 65: p. 1287, 1968.

11. DUFF, G. L., and MURRAY, E. G. D.

"Bilateral cortical necrosis of the kidneys"

AMER. J. M. Sc. Vol. 201: p. 428, 1941.

12. EDWARDS, K. D. G., WHYTE, A. M.

"Plasma creatinine level and creatinine clearance as tests of renal function"

AUSTRALIAN ANN. MED. Vol. 8: p. 218, 1959.

13. GAULT, M. H., DOSSETOR, J. B.

"Evaluation of renal function without collection of urine"

ANN. INT. MED. (Editorial) 1327: p. 69, 1968.

14. GIUSTINA, G.

"Diagnosis of renovascular hypertension: Comparison between renogram and separate renal function tests."

J. CARDIO-VASC. SURG. Vol. 9: p. 71-76, Jan. 1968.

15. GOODWIN, D. A.

"Renal function using hippuran $I^{131}$ and neohydrin HG $^{203}$ "

McGill Thesis, 1963.

16. HAMILTON, P. B., PHILLIPS, R. A., HILLER, A.

"Duration of renal ischemia required to produce uremia"

AMER. J. PHYSIOL. Vol. 152: p. 517, 1948.

17. HARE, K., GOLDSTEIN, H., BARNETT, H. L., McNAMARA, H., HARE, R. S.

"Renal excretion of creatinine in man"

FED. PROC. Vol. 8: p. 67, 1949.

18. HIRAKAWA, A., and CORCORAN, A. C.
" $I^{131}$ 0-iodohippurate excretion and quantitative formulation of radio isotope renogram as indices of bilateral and unilateral renal function"
J. LAB. CLIN. MED. Vol. 61: p. 795-807, 1963.

19. HOBERMAN, H. D., SIMS, E. A. H., PETERS, J. H.
"Creatine, creatinine metabolism in the normal adult studied with the aid of isotopic nitrogen"
J. BIOL. CHEM. Vol. 172: p. 45, 1948.

20. KEELER, R.
"Composition and function of rat kidneys after normothermia ischemia"
CAN. J. PHYSIOL. & PHARMOCOL. Vol. 46: p. 739-743, Sept. 1968.

21. KNUDSEN, E., and SOE, HOJBERG, K.
"Simulation of the radiohippuran renogram: Test on an analogue computer"
J. APPLIED RADIATION & ISOTOPES. Vol. 18: p. 639-646, 1967.

22. KAPLOWITZ, J. M., MITCHELL, J. F., BLAHD, W. H.
"The radioisotope renogram: A comparison of qualitative and quantitative interpretation"
J. A. M. A. Vol. 192: p. 1032, 1965.

23. LUCK, R. J., WILSON, R. E., FELDMAN, D., VAYO, B.
"Numerical evaluation of $I^{131}$ renograms in homotransplanted kidneys"
BRIT. J. UROL. Vol. 38: p. 371-381, 1966.

24. LUCKE, B.

"Lower nephron nephrosis"

MIL. SURG. Vol. 99: p. 371, 1946.

25. LUNDGREN, G., EKMAN, L., HANSON, E., MAGNUSSON, G., NORDSTROM, M.

"Renographic and auto-radiographic studies during rejection in canine renal allografts"

ACTA. CHIR. SCAND. Supplementum 382, 1967.

26. MAGNUSSON, G.

"Kidney function 'studies' with $^{131}I$ - tagged sodium ortho-iodohippurate"

ACTA. MED. SCAND. Suppl. 378, 1962.

27. MAXWELL, M. H., LUPU, A., TAPLIN, G. V.

"Radioisotope renogram in renal arterial hypertension"

J. UROL. Vol. 100: p. 376-383, 1968.

28. MEADE, R. C., and SHY, C. M.

"The evaluation of individual kidney function using radio-iodohippurate sodium"

J. UROL. Vol. 86: p. 163, 1961.

29. MOORE, F. D.

"Metabolic care of surgical patient"

W. B. Saunders Company, Philadelphia, p. 660.

30. MORRIS, G., HEIDER, C., and HANDLEY, C.

"The effect of various types of prolonged renal ischemia on renal function"

CLIN. RES. PROC. Vol. 4: p. 42, 1956.

31. MYERS, V. C.

"The prognostic significance of an elevated blood creatinine"

J. LAB. & CLIN. MED. Vol. 29: p. 1001, 1944.

32. MYERS, V. C., and KILLIAN, J. A.

"The prognostic value of the creatinine of the blood in nephritis"

AMER. J. MED. Sc. Vol. 157: p. 674, 1919.

33. NEELY, W. A.

"The effect of arterial venous and arterio-venous occlusion on renal blood flow"

S. G. & O. Vol. 108: p. 669-672, 1959.

34. NORDYKE, R. A., TUBIS, M., BLAND, W. H.

"Use of radio-iodinated hippuran for individual kidney function testing"

J. LAB. CLIN. MED. Vol. 56: p. 438, 1960.

35. PENNER, A., and BERNHEIM, A. I.

"Acute ischemic necrosis of the kidney: Clinico pathological and experimental study"

ARCH. PATH. Vol. 30: p. 465-480, 1946.

36. PHILLIPS, R. A., and HAMILTON, P. B.
"Effect of 20, 60 and 120 minutes of renal ischemia on glomerular and tubular function"
AMER. J. PHYSIOL. Vol. 163: p. 523, 1948.

37. PORCH, P. P., CHAMBERLAIN, N. O., and EDWARDS, W. H.
"The effect of temporary renal vascular occlusion in dogs"
SUR. FORUM. Vol. 10: p. 892-895, 1959.

38. O'CONNOR, V. J., LIBRETTI, J. V., and GRAYHACK, J. T.
"The early differential diagnosis of postoperative anuria using the radioactive renogram: An experimental study"
J. UROL. Vol. 86: p. 276, 1961.

39. O'CONNEL, J. M. B., ROMEO, J. A., MUDGE, G. H.
"Renal tubular secretion of creatinine in dog"
AMER. J. PHYSIOL. Vol. 203: p. 985, 1962.

40. OLIVER, J., MacDONELL, M., and TRACY, A.
"Pathogenesis of acute renal failure associated with traumatic and toxic injury: Renal ischemia, nephrotoxic drainage and the ischemuric episode"
J. CLIN. INVEST. Vol. 30: p. 1305-1429, 1951.

41. REESE, L.
"An experimental analysis of $^{131}$I iodohippurate renogram"
INVEST. UROL. Vol. 5: p. 265-275, 1967-68.

42. ROOF, B. S., LAUSON, H. D., BELLA, S. T., EDER, H. A.
"Recovery of glomerular and tubular function including PAH extraction following two hours of renal artery occlusion in the dog"
AMER. J. PHYSIOL. Vol. 166: p. 666, 1951

43. ROSLER, H.
"Total clearance of $^{131}I$ - ortho-iodohippurate as basis for quantitative radiorenography"
GERM. MED. MTH. Vol. 12: p. 533-538, 1967.

44. SCHEIBE, J. R., GIRALDI, E., VERMEOLEN, C. W.
"The effect of temporary renal vascular occlusion on kidney function"
SURG. Vol. 25: p. 724-729, 1949.

45. SHANNON, J. A.
"The excretin of insulin and creatinine at low urine flows by the normal dog."
AMER. J. PHYSIOL. Vol. 114: p. 362, 1936.

46. SHEEHAN, H. L., DAVIS, J. C.
"Renal ischemia with failed reflow"
J. PATH. BACT. Vol. 48: p. 105, 1959.

47. SMITH, H. W.
"The kidney", p. 182-194.
Oxford University Press, 1951.

48. SMITH, H. W., FINKELSTEIN, N., ALIMINOSA, L., CRAWFORD, B., GRABER, M.

"The renal clearances of subtituted hippuric acid derivative and other aromatic acid in dog and man"

J. CLIN. INVEST. Vol. 24: p. 388, 1945.

49. SPENCER, C. C., CALLENDINE, G. W. Jr., and VINCENT, D. J.

"Reproducibility of the radio-isotope renogram"

J. LAB. CLIN. MED. Vol. 57: p. 350, 1961.

50. STEWART, B. H., HAYNIE, T. P.

"Critical appraisal of the renogram in renal vascular disease"

J. A. M. A. Vol. 180: p. 454-459, 1962.

51. TAPLIN, G. V., MEREDITH, O. M. Jr., KADE, H., and WINTER, C. C.

"Radio-isotope renogram; external test for individual kidney function and upper urinary tract patency"

J. LAB. CLIN. MED. Vol. 48: p. 886-901, 1956.

52. TRINKLE, J. K., and KISER, W. S.

"Acute renal failure: Diagnosis of etiology by radio-isotope renography"

J. UROL. Vol. 91: p. 199, 1964.

53. VAN SLYKE, D. D.

"The effect of shock on the kidney"

ANN. INT. MED. Vol. 28: p. 701, 1948.

54. VAN SLYKE, D. D., PHILLIPS, R. A., HAMILTON, P. B., ARCHIBALD, R. M., DOLE, V. P., and EMERSON, K. Jr., "Effect of shock on the kidney" TRANS. ASSN. AM. PHYS. Vol. 58: p. 119, 1944.

55. WAX, S. H., FRANK, I. N., and McDONALD, D. F. "The usefulness of radioactive hippuran renogram in the differential diagnosis of azotemia" J. UROL. Vol. 88: p. 433, 1962.

56. WELLS, C., and KYLE, J. "Scientific foundation of surgery", p. 522. William Hernemann Medical Books Ltd., 1967.

57. WINTER, Chester C. "A clinical study of a new renal function test: The radioactive diodrast renogram" J. UROL. Vol. 76: p. 182, 1956.

58. WINTER, Chester C. "Quantitative qualitative interpretation of the radio-isotope renogram" J. AMER. MED. ASSOC. Vol. 192: (Editorial) p. 1089, 1965.

59. HEPINSTALL, R. H. "Pathology of the kidney", p. 646. Little, Brown and Company, Boston.