EXPERIMENTAL PRODUCTION OF CHRONIC RENAL INSUFFICIENCY

by

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PREFACE

In the preparation of this thesis, the author intended to present a very simple method of producing azotemia, or eventually uremia in the dog. Its sole claim to uniqueness rests in the fact that the ameroid constrictor is used in the ureter for the first time, and this sets forth experimental considerations which the author believes should be readily available to experimentalists in various fields.

The experimental procedure presented in this work becomes enhanced with interest when its potential is recognized as an intermediary to other experimental interests, chief among which at present being homotransplantation.

Personal gratitude and indebtedness is expressed to those whose advice and assistance have helped in the development of this study.

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CHAPTER I

INTRODUCTION

The production of acute uremia in the experimental animal has been achieved with much success. Various procedures that deal with its production have been described. There are articles in medical literature that have been written on the production of an azotemia or uremia that follows a chronic pattern, yet none deals with it as a process that is prolonged, gradual and sustained, maintaining the experimental animal relatively free of untoward signs and symptoms of the condition produced.

The azotemic or uremic state, besides gaining intense interest lately because of the new concepts and excellent devices that have been introduced with the purpose of correcting this condition, assumes a level of importance in the fields of tissue transplantation and immunology. It has been found to exert paradoxically favourable effects on the survival of tissue homotransplants because of its effect in the alteration of the host's immunological response.

In an attempt to alter this response, it was thought worthwhile to investigate a procedure which could be used to produce chronic azotemia and/or uremia experimentally and, once the technique was mastered, to test the acceptance of renal homografts in the azotemic or uremic animal. Studies of the mechanism of the prolonged graft survival associated with azotemia or uremia, and investigative work dealing with its potentiation appear approachable on an experimental basis in dogs.

The results of the procedure to be described and some of its modifications, were observed by gross, microscopic and laboratory examinations.

Although the method does not apply to the human, it is earnestly hoped that this work may, in due time, shed some light on the different problems that may arise in the management of chronic obstructive renal disease, and more so, to the prolonged survival of homografted tissue, a biologic barrier which has become the great challenge of the day to the experimentalist, the surgeon, the immunologist, and the biochemist.

Historical Notes:

Much of the literature pertaining to the production of azotemia and/or uremia deals with this state as the end result of the experiment, rather than being a point of departure for initiating further experimentation.

Renal insufficiency became established as the underlying mechanism for the development of the uremic syndrome as early as 1892 when Bradford experimentally produced this syndrome clinically and chemically by extirpating 75% of the kidney tissue of dogs. Pearce, in 1908, had similar findings. Bollman and Mann in 1927 achieved levels of blood urea nitrogen as high as 808 mgm per cent without uremic symptoms by implanting the ureters into the small intestines, but this level however, fell subsequently with a slow accumulation of creatinine, creatine, uric acid and other nitrogenous wastes. Uremic manifestations leading to death in two to three weeks correlated with the last mentioned substances rather than with urea. Smith in 1951, after extensive and detailed studies on the physiology of the renal mechanism, showed the complexity of urine formation, and has further discredited the notion that uremia was equivalent to the addition of urine to the blood.

Furthermore the patho-physiology associated with the anatomic changes in the kidney and the resultant clinical picture have become correlated throughout the entire spectrum of renal insufficiency.

Working along the line of ureteral obstruction, Scott in 1912 produced hydronephrosis in a series of dogs by ligating their ureters either with a rubber band, where he produced an incomplete occlusion, or by ligating the latter with silkworm gut, thus making the obstruction complete. Naturally, subsequent experimentalists became engaged in the problem of restitution of the artificially occluded ureter, and thus determine the reversibility of the hydronephrosis produced. Hinman working on dogs, cats and rabbits in 1934, ligated and divided the ureters as close to the bladder as possible, and when the time came to remove the obstruction, the ends of the ureters were freed of their ligatures and were implanted in the bladder. He found that these animals did not survive a complete ureteral obstruction of longer duration than 2 to 3 weeks if the opposite kidney was removed at the time of the implantation. Following Hinman's monumental experiments on hydronephrosis, others carried on along this vein. Fylling (1951), has shown that by

doubly ligating a rat's ureter with silk-suture, the rats could survive after 30 days of occlusion. Kerr (1956), obstructed the ureters of dogs for 4 weeks, and noted maximum recovery of the kidney in 30 days, as determined by glomerular filtration and effective renal plasma flow. Widen (1957), presented the results of his studies after total, unilateral, temporary urinary stasis of varying duration which he produced on dogs. He concluded that total obstruction to drainage rapidly leads to damage of the kidney, and the morphologic, angiographic, and functional changes are proportionate to the duration of the induced stasis. Risholm et al (1960), studied the pressures in the ureter and renal pelvis after experimental ureteric occlusion in the dog. They observed that in the ureter, pressure-induced contractions occurred and did not cease even when the intraureteric pressure was high, but that in the pelvis, no motility was recorded as reflected in pressure oscillations. McDonald and Calams (1960) created strictures in the ureters of 12 dogs by extracting heat from a specified area in the ureteric tissue with the use of CO_2 gas under pressure, and allowed the injury to develop over a 3 to 8-month period. Their main purpose was the study of ureteral regrowth. Their observations were, that ureterotomy of experimental

intrinsic ureteral strictures in the presence of continuous urine flow increased the time required for ureteral healing, and also increased the degree of periuteteral fibrosis. They also advanced the observation that smooth muscle regrowth occurred after ureterotomy of experimental strictures in the presence of a continuous flow of urine. In 1960, Sheehan and Davis produced partial ureteral obstruction experimentally by clamping the ureters of adult rabbits with Dieffenbach forceps for a period of 6 hours. By this procedure, these workers gave rise to an incomplete obstruction that developed, and which could be compensated to allow a normal flow of urine down the ureter. Weinberg et al (1962), obstructed ureters in young pigs by either sclerosing the vesical neck with injections of sodium morrhuate, or by placing a folded cellophane strip about the vesical neck and tied in place against a rubber catheter that had been previously introduced into the bladder. This group of workers produced megaloureter experimentally, and once it was produced, showed that peristalsis was intermittent and of poor quality. Furthermore, when the obstruction was relieved, a return to a normal sized ureter with proper function was achieved. Zimskind

and co-workers (1962) performed unilateral ureteral ligations on dogs and observed that complete unilateral obstruction of the canine ureter produced a non-functioning kidney by urography within 2 weeks, and that relief of obstruction by cutaneous ureterostomy restored prompt renal function by X-rays in kidneys occluded for as long as 8 weeks.

There is no evidence in the literature reviewed of ureteral obstruction being produced with the purpose of establishing the state of azotemia or uremia in the experimental animal preparatory to an eventual homotransplantation experiment. Mannick, Powers et al (1960) in conditioning their series of azotemic dogs for homotransplantation, attained this by infusing these animals' kidneys with a given quantity of a solution of the 6-aminonucleoside of Puromycin. These authors produced uremia in their animals with subsequent reduction of the immune response.

With the advent of the great challenge of tissue transplantation, and on the other hand, irreversible kidney diseases, the state of azotemia and/or uremia is being evaluated under a new and different light; new factors and concepts have evolved concerning the chemistries of immune processes. Dammin, Couch and Murray (1957), studied patients with chronic renal insufficiency who exhibited

prolonged survival of skin homografts, the longest span being 6 years. Mannick believes that the survival of the grafts is related to an 'anergy' that is specific for homograft antigens, since immune responses to ordinary bacterial antigens were not correspondingly depressed. Smiddy and co-workers (1961) produced an acute uremia in rabbits by performing unilateral nephrectomy, after which the pedicle of the other kidney was clamped for 2 hours. Besides confirming the prolonged survival of skin homografts in the uremic animals, they advanced the statement that the principal response to orthotopic skin homografts occurred in the draining lymph nodes.

Numerous reports indicate that antibody is formed in lymph nodes draining areas of intradermal or subcutaneous injection of killed bacteria or other antigens. Burnet (1941) obtained evidence that antibody formation occurs in lymphatic tissue near a site of antigen injection. Nisonoff and Pressman (1959) working on the immunological aspect, confirmed Karush's (1958) finding that urea binds with the antibody but that its effect on binding is reversible. Thus, it binds itself non-specifically to the antibody surface, and upon dialysis completely restores the binding capacity of the antibody. Merrill and colleagues (1955) supported the concept of

prevalent infection in patients with renal failure, blaming this on a suspected defect in antibody production. It was their observation that renal homografts survived longer in terminal uremic patients than would be anticipated in non-uremic patients, and suggested that dialysis of patients before grafting could possibly increase the rate of destruction of the homograft.

EMBRYOLOGICAL, ANATOMICAL, AND PHYSIOLOGICAL CONSIDERATIONS

The Kidneys:

Inasmuch as the state of azotemia and/or uremia deals primarily with the kidney as the progenitor of ensuing changes in its pathogenesis, it seems essential and appropriate to consider some of the highlights of its embryology, anatomy and physiology.

The ureters, the main structures that have to be dealt with in this work for the production of the desired condition of renal insufficiency, undoubtedly deserve likewise a comprehensive review.

The adult human kidney has the complex capacity of forming and eliminating urine, and this is reached through three stages of development in the embryo: the pronephros, mesonephros and metanephros. The metanephros persists as the functioning adult kidney while the pronephros and mesonephros degenerate.

The pronephros is the first of the three kidneys to develop in the embryo. Pronephric tubules begin to appear late in the third week of embryonic life. Patten (1948) claims that

about seven pairs of tubules develop from the nephrotomes of the developing embryo. The tubules are so disposed that one end drains the celomic cavity and the other opens into a common pronephric duct that empties into the cloaca. The comprehensive work done by Arey (1949) emphasizes the fact that there is no supporting evidence to prove that the pronephros functions in the human embryo. The duct persists as the mesonephric (Wolffian) duct while its tubules degenerate. The mesonephros develops after the pronephros.

The mesonephos or Wolffian body makes its appearance in the fourth week of embryonic life. It apparently develops from the same mass of mesodermic tissue as that of the pronephros. Mesonephric tubules form and communicate with the mesonephric duct. The mesonephric corpuscle or primitive glomerulus is formed when the blind end of a tubule cups itself about a knotty conglomeration of blood vessels from the dorsal aorta.

The temporary excretory function in the human embryo is taken over by the mesonephros, while the pronephros is absolutely functionless. The mesonephirc tubules degenerate at about the fourth month. Some tubules persist and eventually form the

efferent ductules, the paradidymis and aberrant ductules of the male; the mesonephric duct persists as the vas deferens. During the growth and disappearance of the mesonephros, the true or adult kidney develops.

The metanephros arises from two sources. Its collecting (distal) portion is formed by an outgrowth of each mesonephric (Wolffian) duct near the cloaca. The secretory (proximal) portion arises from the blastema of the Wolffian body, a mass of mesoderm.

The ureteral stalks from the metanephros enlarge at the distal end to become the pelvis of the kidney. The collecting tubules, major and minor calices as well, develop and form upon further growth and subdivision. On the average, there are three major calyces and six minor calyces. Pyramidal masses of straight tubules that radiate from the calyces are formed by the collecting tubules that have grown from the minor calyces which have branched and subdivided. The renal pelves and collecting tubules as well as the ureters arise from the same embryological structure, an outgrowth from the mesonephric (Wolffian) duct.

As the primitive ureter lengthens, it invaginates itself into a mass of nephrogenic tissue that surrounds the dilated end

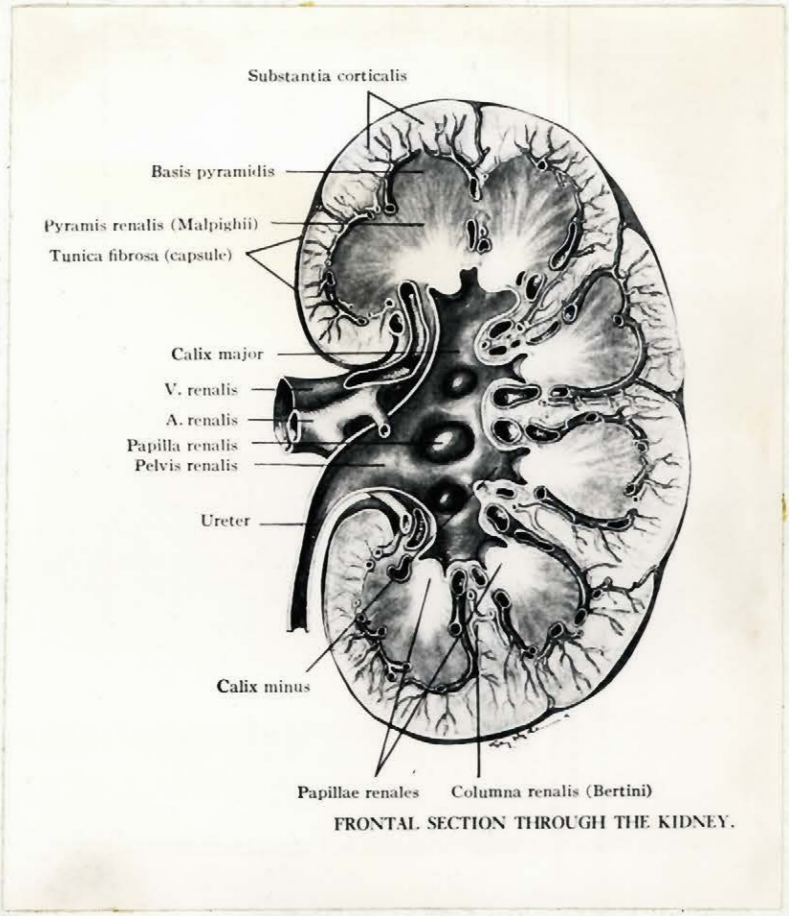


Fig. 1
Frontal section through the kidney. From Surgical Anatomy, B.J. Anson and Walter G. Maddock, 4th Edition, W.B. Saunders Co., 1958.

of the ureter like a cap. Secretory tubules are formed by masses of nephrogenic tissue at the ends of collecting tubules. These secretory tubules connect themselves with the collecting tubules, the blind end of each tubule becoming vesiculated. A knot of capillaries is enveloped in the cup-shaped end of this newly constructed tubule to give rise to a glomerulus, while the tubular lining forms a thin capsule that covers the capillary tuft and lines the cavity in which it lies. The thin capsule is known as Bowman's capsule.

The Nephron:

The relation of the glomerulus to the tubule has been expressed best by stating that the capillary tuft is thrust so far into the expanded but closed end of the tubule that the tuft has come to be enveloped by a double layer of tubular epithelium. The inner or visceral layer is closely applied to the capillaries, extending in between all the loops, and surrounding each loop almost completely. The outer or parietal layer forms a smooth spherical capsule about the tuft as a whole. The space within this capsule is continuous with the lumen of the tubule. The arrangement is in such a way that any fluid passing through the capillaries drains from the capsular space down the tubule.

Each tubule elongates, become convoluted, develops a long loop (Henle's loop) and finally connects with the end of a collecting tubule. In this manner the functioning renal unit or nephron is completed.

Ascent and Rotation of the Kidney:

The kidney "ascends" because of the rapid spinal growth of the embryo, so that by the fifth month the upper pole is at the level of the eleventh rib which is its position in normal adult life. The blood supply of the kidney is established at the level of the second lumbar vertebra during the eighth to ninth week and the renal pelvis has rotated from an anterior to a medial position. Certain more posterior arterial branches from the aorta supply the kidney in this process, but these normally dissapear.

Anatomy of the Kidney:

Each normal adult kidney weighs on the average about 150 grams, and the dog's kidney is in the range of 0.3% of its body weight.

The renal pelvis is actually the resultant enlargement formed by the ureter. It branches in a flower-like design into major calyces. The normal capacity of the human pelvis and calyces is four to eight cc. At the end of each cupped minor

calyx, one or more nipple-like projections mark the renal papilla into which fifteen to twenty papillary ducts open and allow urine to enter the calyx.

There are individual papillae at the apex of each renal pyramid, and the pyramids make up the medulla of the kidney. The renal cortex is plainly visible at the base of the pyramid since it appears as a zone that is lighter in color and with radial striations. These striations are composed of straight tubules that are continuous with those in the medulla. The glomeruli show up between striations as tiny red points. The glomeruli and tubular system of the kidneys seem to develop at the periphery of the cortex just beneath the capsule in their earliest stages of growth.

The true capsule of the kidney is fibrous, covers the surface of the kidney, and can readily be stripped away. The false capsule envelops all, and is discernible because it is canary yellow in color and soft in consistency due to the perirenal fat.

The false capsule of the kidney, separates the soft fatty tissue or perinephric fat from the paranephric fat. This fibrous tissue layer is commonly known as Gerota's fascia,

and is closed laterally by uniting with the retroperitoneal tissue, and does likewise above by attaching itself to the diaphragm. With this disposition of attachments, the kidney is free to move downward and medially where it does not meet any tissue impediment. Gerota's fascia is continuous ventrally across the mid-line, and its dorsal layer is continuous with the vertebral fascia.

Mitchell (1950) showed that the two layers of renal fascia are united medially where they cross the aorta and vena cava, and thus incorporate themselves with the connective tissue about the great vessels. There is also evidence to indicate that these two layers are likewise united inferiorly.

Position of the Kidneys:

The kidney lies in the fascia transversalis. A group of muscles in the posterior abdominal wall form the bed for the kidneys. These are: the psoas major, quadratus lumborum and transversus abdominis in that order from the medial to the lateral side below the twelfth rib. The diaphragm is also found behind each kidney above the level of the twelfth rib. These muscles are in a retroperitineal location.

Usually, the right kidney is a little lower in position than the left. The posterior aspect of the kidney is in close relation to the muscles that are attached to the bodies of the last thoracic and upper three lumbar vertebrae. The upper poles of the kidneys incline slightly anteriorly following the concavity of the diaphragm, and these are more medial than the lower poles. A thick fascial band, the lateral lumbocostal ligament, extends from the transverse process of the twelfth thoracic vertebrae to the last rib just posterior to the kidney.

Relations of the Kidney:

The left renal hilum is slightly higher and is crossed by the body of the pancreas as it passes to the left on the posterior abdominal wall. The adrenal glands are situated on the upper medial aspect of the kidneys. The anterior surface of the right kidney has in contact with it, in addition to the duodenum, which is separated from it only by connective tissue, the liver, the right colic flexure and a portion of the jejunum or ileum from above downwards. Both the liver and jejunum are separated by peritoneum from the kidney whereas the adrenal gland and the right colic flexure are in direct contact with its surface. The anterior surface of the left kidney is related to the stomach

medially and the spleen laterally. This area is covered by peritoneum. The jejunum is in contact with the medial half of the surface below the pancreas and is also separated from the kidney by peritoneum. The descending colon lies on the lower lateral part, and, with the pancreas and adrenal gland, has no peritoneum intervening.

Blood Supply of the Kidneys:

From the aorta, arteries arise and enter the medial surface of each kidney to form the hilum. Each of the renal arteries divide into several branches and enter the surface of the kidney between the branches of the renal vein in front and the renal pelvis behind. From the branches of the renal arteries interlobar arteries extend to the boundary of the cortex and medulla of the kidneys where they branch as the arcuate arteries which form arches across the bases of the renal pyramids. Then, from the arcuate arteries interlobular arteries extend through the convoluted part of the renal cortex where their fine branches enter the glomerular tufts.

The glomerular capillaries unite to form a single afferent vessel and a smaller efferent vessel. This supposed disparity in the size of the glomerular vessels is said to result in increased

pressure within the glomerulus. Each capillary tuft is enveloped by the visceral layer of Bowman's capsule. The efferent vessel leaves the glomerulus and divides into small branches that are distributed through the cortex and medulla among the convoluted and straight tubules.

Trueta and his associates (1947), found evidence of the existence of a vascular by-pass capable of cutting off the circulation to the glomeruli and transporting the blood directly into the blood vessels of the medulla of the kidney. They performed angiographic studies on animals and observed that under certain conditions the circulation through the renal cortex could be diminished or even, in some circumstances, totally arrested, while a circulation continued through the medulla of the kidney. The vasa recta, with their related blood vessels, constitute the medullary pathway through which the blood, when diverted from the renal cortex, is carried from the renal artery to the renal vein.

The efferent vessel of a glomerulus which lies nearest the medulla of the kidney breaks up into a group of more or less parallel straight vessels. They constitute the vasa recta. These vessels pass toward the apex of the medulla of the kidney.

Veins accompany the arteries of the kidney. The interlobular veins emerge from the hilum of the kidney to form the renal veins.

The renal pedicle:

The constituents of the vascular pedicle vary greatly, difference rather than similarity in their arrangement being the rule. Extra hilar arteries have been described in 43-65%. Renal arteries may arise from points as low as the hypogastric artery and as high as the aortic hiatus in the diaphragm. Venous abnormalities are less common (7 per cent). Usually the right renal artery arises from the aorta higher than the left although the right kidney is lower than the left. The right renal vein enters the vena cava at a lower level than the left in over one-half (55 per cent) of cases. The usual arrangement, then, is a higher origin of the right renal artery than the left, and a lower termination of the right renal vein than the left and a lower right kidney. The left renal arteries arising from the aorta to the left of the midline tend to be short while the right renal arteries are long. The left renal vein is longer than the right.

The renal veins lie anterior to the renal arteries with the renal pelvis the furthe st posterior. From above downward

the arteries lie just above the veins. Ronstron (1938) reports the fact that there is no general pattern in the branching of the renal artery as it enters the renal sinus.

The renal artery divides into three or four branches at the hilum. Two or three pass into the renal sinus in front of the pelvis and one, the retropelvic branch, passes over the upper border of the pelvis and courses downward under the edge of the posterior lip of the hilum. There are certain features of the renal circulation that are of special interest. Except for a small amount of blood which nourishes the capsule and interstitial tissue of the kidney, all the blood that enters the organ passes through the glomerular tufts. The arteries of the kidney are end arteries and do not anastomose.

Nerve Supply of the Kidneys:

The kidney is innervated by sympathetic and parasympathetic nerves.

Sympathetic nerves: Sympathetic fibers come to the kidney through the superior, middle and inferior splanchnic nerves from the sixth thoracic to the third lumbar segments inclusive. The greater and lesser splanchic fibers synapse in the semilunar ganglion whose post-ganglionic fibers, with fibers of the

vagus, form a renal plexus around the renal vessels. Fibers extend to the arterioles, and to the capillaries of the glomeruli and to the renal tubules.

Parasympathetic nerves: Parasympathetic fibers from the vagus nerve enter the kidney but their function is unknown.

The renal plexus: The renal plexus is composed of fibers which come from many sources and which vary considerably. The chief nerve supply of the kidney, however, is derived from four sources:

(a) The aortico-renal ganglion

(b) Middle and inferior splanchnic nerves

(c) Intermesenteric nerves

(d) Lumbar sympathetic chain

The most constant fibers seem to be those arising from the semilunar ganglion connecting with the aortico-renal region. These fibers sometimes receive a branch from the inferior splachnic nerve and often from the superior splanchnic nerve. The nerve fibers course along the superior border of the renal artery near its origin from the aorta. They then divide into two portions which run along the anterior and posterior walls of the renal artery. Fibers on the posterior walls are more numerous.

Fibers from the aortico-renal ganglion run chiefly along the anterior wall of the renal artery.

The middle splachnic nerve sends fibers to the aortico-renal ganglion and renal plexus. The inferior splanchnic nerve is inconstant. The middle splanchnic nerve arises from the last thoracic ganglion or from the portion of the sympathetic chain between the last thoracic and first lumbar ganglia and passes forward and inward to join the renal plexus. Its branches usually lie posterior to the renal artery. The superior splanchnic nerve and superior mesenteric plexus may send fibers to the renal plexus.

Several slender branches are given off by the intermesenteric nerves which lie between the origins of the mesenteric arteries. These branches pass to the aortico-renal ganglion or go directly to the renal plexus.

Branches from the first and second ganglia of the lumbar sympathetic chain go to the renal plexus and join fibers from the intermesenteric nerves.

The renal plexus also receives fibers from the vagus nerves and is connected by fibers to the opposite renal plexus.

The nerve plexus divides and forms secondary plexuses

where the renal artery branches. These secondary plexuses follow the arterial branches into the renal sinus sending some fibers to the renal capsule.

Best and Taylor (1950) emphasize the finding wherein stimulation or section of the nerves to the kidney affects the formation of urine only by vascular change, since the transplanted kidney secretes urine of the usual composition.

The renal plexus is composed of nerves from so many different sources that complete denervation of the kidney is difficult. Stripping of the renal artery, if possible, does not ensure complete denervation since the lower fibers from the intermesenteric plexus may remain untouched and continue to supply portions of renal tissue.

Lymphatics of the Kidney:

The kidney lymphatics may be classified as superficial and deep.

The superficial system is formed by lymph channels in the perirenal fat and beneath the capsule. A deep group of lymphatics surrounds the tubules and blood vessels in the interstitial tissue. There is a free anastomosis between these groups.

The deep lymphatics of the kidney drain into four to six large trunks at the hilum along the renal vessels and enter the lateral aortic lymph nodes. The perirenal lymphatics drain into the superior aortic nodes.

Goodwin et al (1956) recognized that fluid could pass from the renal collecting systems into the renal lymphatics and act to protect the kidneys under conditions of diuresis or urinary obstruction.

Physiology of the Kidney:

The kidneys are involved in a great variety of vital functions. Not only are they the principal excretory organs of the body responsible for ridding it of the waste products of catabolism, but they also play an essential role in the maintenance of homeostasis in the organism. The latter function involves the maintenance of acid-base balance, a normal electrolyte and water content of the blood and tissues, and a constant volume of the fluid compartments. In addition to these activities, normal kidney function is essential for the maintenance of the normotensive state and for normal hematopoietic function. Failure of the latter function results in the anemia so characteristic of renal insufficiency.

Despite the perpetual impact of the many distorting factors which tend to alter the normal composition and volume of the body fluids, the kidneys meet these complex requirements and function adequately depending always upon its structural integrity. Under normal conditions any deviation in the volume or composition of the body fluids will be corrected by the kidneys, but in the presence of renal dysfunction, this is impossible.

The excretion of urine:

It is now a well accepted theory that urine formation is the summation of simple physical filtration by the glomeruli, tubular reabsorption, and tubular excretion. The blood passing through the glomerulus is subjected to ultrafiltration through the glomerular membrane, in which process the filtrable constituents of the blood (water, sugar, amino acids, sodium, potassium, bicarbonate, chloride, sulfate, phosphate, urea, creatinine, and other diffusible molecules) pass into Bowman's capsule. Such non-filtrable molecules as protein and other colloidal substances and the formed elements of the blood are retained by the glomerular membrane. Only small amounts of these appear in the urine under normal conditions.

Wearn and Richards (1924) withdrew fluid from a single capsule of the frog's kidney by means of a fine quartz capillary pipette. They were able to withdraw the capsular fluid as fast as it was formed. Chemical analysis showed that this fluid contained no proteins.

Bowman's capsule communicates with the lumen of the attached tubule. The fluid formed by ultrafiltration through the glomerulus passes along the tubules and is subjected to

the processes of reabsorption, backward diffusion, and secretion which lead to the formation of urine, which is discharged into the collecting tubules. The hydrostatic pressure of the blood passing through the glomerulus is approximately 55 mm. of mercury. This exceeds the sum of the osmotic pressure of the non-filtrable constituents of the blood (25 mm.) plus the tissue pressure (10 mm.) by about 20 mm., which represents the filtration pressure. It is this pressure gradient, generated by the force of the heart beat, that is responsible for the formation of the glomerular fluid. The energy necessary for converting this fluid into urine is derived from metabolic processes occuring within the tubular cells.

Hayman (1927) and White (1932) showed that the blood pressure in the glomerular capillaries in amphibia is adequate to effect filtration against the colloid osmotic pressure of the blood. These facts have been applied to the glomeruli of mammals since these are inaccessible.

Normally about 650 ml. of plasma per minute pass through the kidneys of the average adult. Of this, about one-fifth (filtration fraction), or 130 ml. per minute, appears in the glomerular filtrate. Accordingly, during the course of 24 hours, almost

190 liters of filtrate are formed, of which all but about 1.5 liters, which is eliminated as urine, is reabsorbed by the tubular epithelium. By the selective action of the renal tubules, the excretion of water, electrolytes and other urinary constituents is controlled so as to maintain the normal volume and composition of the body fluids.

The constituents of the glomerular filtrate may be divided into three groups insofar as their fate during passage through the tubules is concerned. Glucose, the amino acids and most of the protein are practically entirely reabsorbed unless present in grossly abnormal amounts and are accordingly designated as "threshold" substances. Creatinine and other non-threshold substances are, on the other hand, completely excluded by the tubules. Urea, which is also a non-threshold substance, diffuses back to a considerable extent as a result, presumably, of the relative inefficiency of the tubular cells. Water, sodium, bicarbonate, chloride and potassium are retained or excreted in accord with the needs of the organism. By regulating the rate of elimination of these substances, the kidneys maintain a normal blood composition despite variations in the amounts of these elements.

The glomerular fluid which has essentially the composition of extracellular fluid, losses approximately 85% of its water, which returns to the blood through the proximal convoluted tubule. This so-called "obligatory" reabsorption of water is accompanied by reabsorption of glucose, urea, sodium, potassium and other substances. The remaining, approximately 20 ml. of glomerular ultrafiltrate formed per minute, is approximately isotonic or slightly hypotonic to the plasma. It has the same alkalinity (pH 7.4) and specific gravity (1.010) as the glomerular filtrate. As this fluid passes the thin loop of Henle, it is further reduced in volume so that only about 15 ml. per minute reach the distal tubule. It is here that the final reabsorption of water and salts, the secretion of potassium and ammonium, and the adjustment of the pH of the urine take place in accord with the needs of the organism. It is in the distal tubule that the water content of the urine, through the control of the antidiuretic hormone, and its sodium and potassium contents, through the action of the adrenal cortex as well as by hemodynamic mechanisms, are adjusted to the body's needs. The relative importance of these factors in regulating the rate of excretion of electrolyte is still disputable. Smith (1933) states however that tubular excretion

plays an important part in urine formation.

Ammonia is produced by the kidneys, a very important function of these organs, since ammonia can be employed in the excretion of acids in the urine thus saving fixed base. Ammonia is probaly formed in the distal tubules.

The complicated process of the formation of urine apparently is completed when the urine leaves the distal convoluted tubules and enters the collecting tubules. Thereafter, the process is one of transportation. This was beautifully explained and portrayed by Narath (1951).

The walls of the calyces, renal pelvis and ureter contain a layer of smooth muscle. Muscle fibers form a sphincter where the calyx joins the pelvis, and this prevents urine from flowing backward during contraction of the pelvis.

Normally, about 500 ml. of water must be excreted as the irreducible minimum in order to remove the catabolic waste products of the organism. When, as a result of renal insufficiency, the concentrating power of the kidney is impaired, a larger volume of a more dilute urine must be excreted in order to maintain a normal composition of the blood. Castro-Mendoza (1950) found that after nephrectomy, plasma volume is decreased

due to increased capillary permeability and extracellular fluid volume is increased. Extracellular fluid volume is also frequently increased in acute renal failure.

The process by which the kidneys respond to alterations in the volume and composition of the body fluids is not entirely known. Alterations in the osmolarity of the plasma perfusing the internal carotid artery by regulating the secretion of the posterior pituitary antidiuretic hormone (through the mediation of the hypothalamus) control the conservation of water. The exact mechanism of the reaction in the distal tubule by which the water content of the urine is determined is unknown. The antidiuretic hormone appears to have no direct effect on sodium or potassium excretion, although changes in the rate of excretion of solutes are accompanied by comparable changes in water excretion through the mechanism of osmotic diuresis.

The concentration of sodium in the extracellular fluid acts as a stimulus to the conservation or excretion of this ion, but is not the sole factor controlling this function. There is some evidence to indicate the existence of a neural center sensitive to changes in extracellular fluid volume. Alterations in plasma volume will affect the rate of excretion of sodium,

but whether this is mediated by the plasma volume itself or by the accompanying changes in cardiac output and its circulatory hemodynamics is unknown. That the latter is not the sole regulatory mechanism is indicated by the active reabsorption of sodium which occurs in cardiac failure and in degrees of sodium depletion which are not accompanied by obvious circulatory changes.

The mechanisms controlling the excretion of potassium are still poorly understood. Neither the level of potassium in the serum nor the intracellular content of this element can account adequately for the response of the kidneys to alterations in potassium metabolism. Potassium differs from sodium in that 15 to 20% of the filtered potassium is excreted as compared to only 1 to 2% in the case of sodium. In renal disease, potassium is excreted in excess of the filtered load. Recent evidence indicates that there is a competition between potassium and hydrogen and sodium ions in the distal tubules. The kidneys are unable to conserve potassium with the same efficiency as they do with sodium but they are able to maintain a high gradient between the urinary and plama contents of potassium. The excretory capacity thus may be maintained as long as urine volume does not decrease, despite marked reduction in glomerular filtration. Since potassium excretion

is roughly proportional to urinary volume, oliguria has been considered a prerequisite for elevation of serum potassium, but obviously this only applies to endogenous potassium. A limited capacity for potassium excretion in chronic renal insufficiency has been demonstrated by Winkler and co-workers (1941).

The reduction in the amount of functioning renal substance is usually the cause of functional disturbances occurring in renal failure, rather than the disordered function of the nephron. Loss of one kidney or bilateral renal disease usually entails little interference with excretory function until too few nephrons are left to maintain normal homeostasis.

Like all the other organs of the body, the kidney is dependent for its normal activity on the functional integrity of the endocrine organs. Of particular importance to renal function are the antidiuretic hormone of the neural lobe of the hypophysis, the adrenal cortical hormones and the parathyroid hormone. Other hormones undoubtedly also play a part in the maintenance of normal renal function, but their action is less obvious.

The antidiuretic hormone of the pituitary is essential for the reabsorption of water from the distal tubule; the adrenal

cortical hormone regulates the reabsorption of sodium and chloride in the proximal tubule; and the parathyroid hormone in addition to its action in mobilizing calcium from the bones also exerts an action on the kidneys concerned with the excretion of calcium and phosphate.

The nervous system, also plays a role in the regulation of renal function, but the exact mechanisms by which regulation operates are still poorly understood. Suffice it to say that the kidneys are abundantly supplied with vasoconstrictor sympathetic fibers from the renal plexus which terminate among the afferent and efferent arterioles, the tubular cells, the parietal layer of Bowman's capsule and the glomerular tuft. The blood supply to the kidneys can thus be effectively reduced and its hemodynamics altered through nervous stimuli. The fact that a denervated kidney maintains its excretory function is evidence that integrity of the nervous connections to the kidney is not essential for its activity. However, there is abundant evidence which indicates that nervous stimuli may modify renal function. This nervous effect may be postulated to occur as a result of: 1) a direct action on the secretory and reabsorptive action of the tubules; 2) in consequence of alterations in endocrine

function; 3) in response to vasomotor effects with their resultant alteration in renal hemodynamics; or 4) as a consequence of extrarenal activities to which the kidney responds passively.

The maintenance of neutrality of the body fluid constitutes one of the principal homeostatic functions of the kidneys. In disease, the breakdown of body protein augments the normal excess of acid ions, and this must be excreted in the urine. The pH of the blood is maintained constant by the buffer system carbonic acid: bicarbonate, the ratio of which in the plasma remains fixed at 1:20. The concentration of carbonic acid is controlled by the pulmonary ventilation; that of the bicarbonate by the kidneys. In order to conserve base (i.e., sodium bicarbonate), the kidney utilizes two mechanisms, namely, the excretion of sulfate and phosphate ions in the form of their acid salts and the synthesis of ammonia.

ANATOMY, EMBRYOLOGY AND PHYSIOLOGY OF THE URETERS

Development of the Ureter: The first or collecting portion of the metanephros enlarges and lengthens to form the ureter. These ureteral stalks enlarge at the distal end to become the pelvis of the kidney. The ureters, renal pelves and collecting tubules arise from the same embryological structure, an outgrowth from the mesonephric (Wolffian) duct.

Anatomy of the Ureter: The ureter is a rather thick-walled tube made up of three layers: an outer serosal coat of fibrous tissue in which are the main blood vessels, a middle or muscular coat, and an inner mucosal coat. The muscular coat is composed of three layers of smooth muscle. The outer and inner muscular layers are arranged longitudinally, the middle layer is circular. The innermost, mucosal coat of the ureter consists of stratified transitional epithelium on a submucosa of stron fibro-elastic tissue. The ureter increases in thicknes as it runs from the kidney to the bladder, mainly on account of the increase in bulk of the circular and longitudinal muscle layers, and more so towards the lower part where the third muscle layer is added (Kiil, 1957). Narath refers to this muscle layer or hull as Waldeyer's sheath.

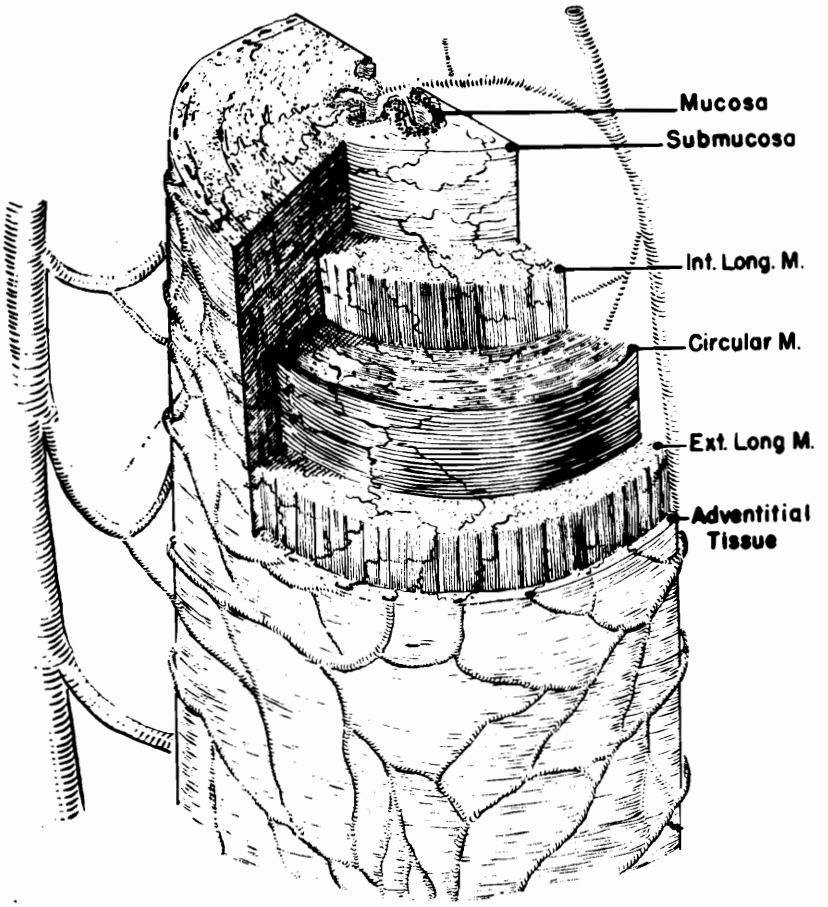


Diagram of the wall of the ureter

Fig. 2

Diagram of the wall of the ureter. From Essential Urology, F.H. Colby, 4th Edition. The Williams & Wilkins Co., Baltimore, 1961.

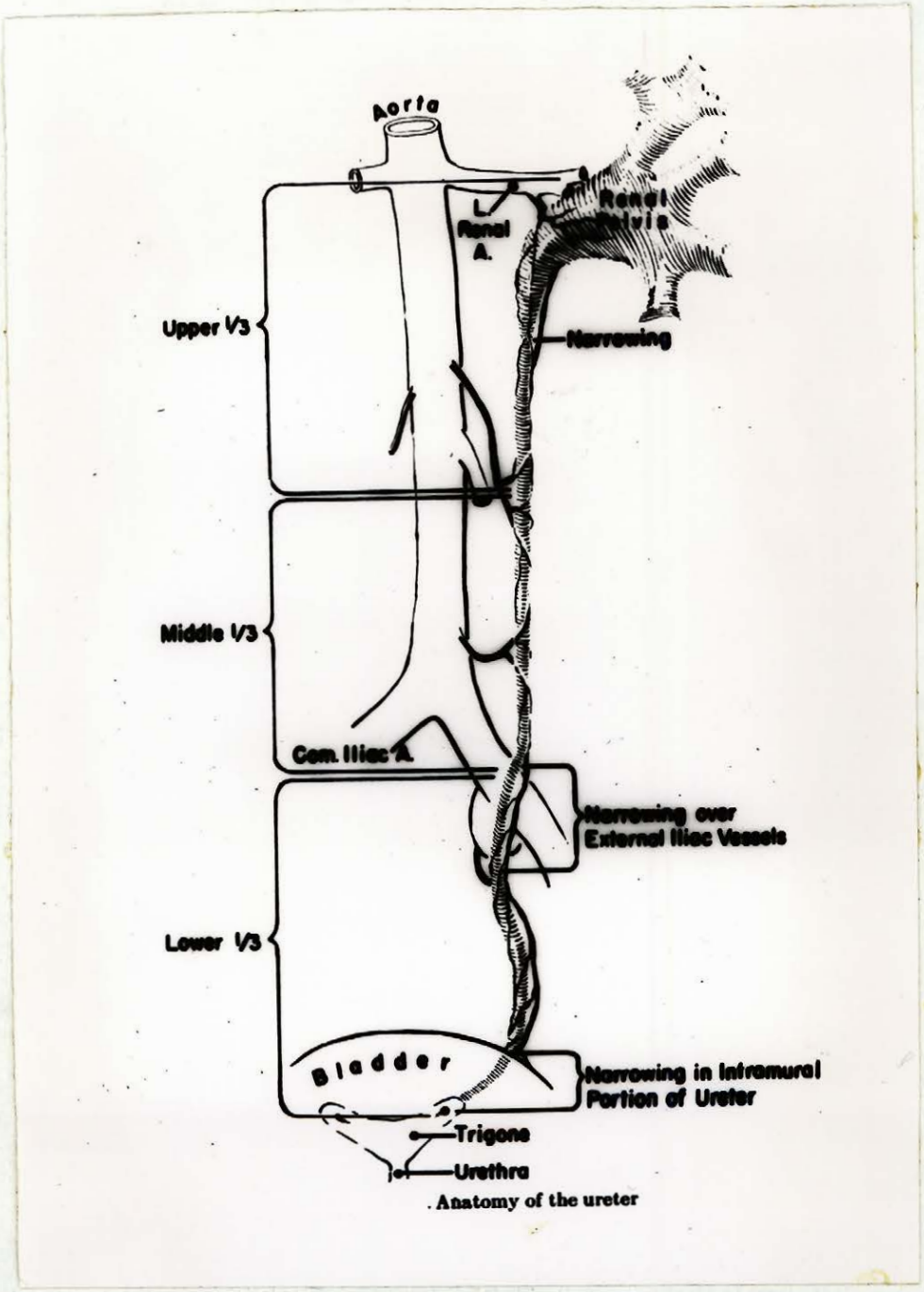


Fig. 3

Anatomy of the ureter. From Essential Urology, F.R. Colby, 4th Edition. The Williams & Wilkins Co., Baltimore, 1961.

The muscular wall of the intramural portion of the ureter is almost solely composed of longitudinal fibers.

The adult human ureter averages 27 to 30 cms. in length and is generally spoken of as composed of three parts: an upper third, middle third, and lower third. The ureter in the dog averages from 19 to 22 cms. and may also be subdivided in similar fashion.

As the ureter leaves the renal pelvis, it passes retroperitoneally through the perirenal fat with Gerota's fascia. The ureter then lies on the psoas muscle which it crosses as the ureter inclines toward the midline. Just before reaching the pelvic brim, it crosses the common iliac vessels at about their bifurcation, although the right ureter lies very close to the inferior vena cava. On the right side, the right colic and ileocolic vessels pass between the ureter and the peritoneum. The left ureter is crossed by the left colic and sigmoidal blood vessels.

The lower third of the ureter lies against the pelvic wall whose curve it follows as the ureter turns toward the midline to pierce the posterior wall of the bladder. As it crosses the pelvic floor, the ureters lie in front of the internal iliac artery, crosses

the obturator nerve and vessels and is surrounded by the venous tributaries from the vesical plexus that empty into the internal iliac vein. On the medial side, the ureter is crossed above by the vas deferens. The vas deferens first lies posterior to the ureter as the vas deferens leaves the ampulla of the seminal vesicle. Then the vas deferens forms a loop and crosses the ureter on its medial side as the ureter lies close to the posterior was of the bladder.

The ureter enters the bladder wall at an oblique angle just in front of the seminal vesicle or the ampulla. The intramural portion of the ureter is about 1 1/2 cm. long in the human bladder and 0.98 cm. in the dog's bladder.

Blood Supply of the Ureters: The blood supply of the ureters is of importance since the surgical interference with portions of it may result in sloughing of the wall of the ureter.

Arteries form a freely anastomosing plexus in the fibrous outer coat of the ureter. The upper ureter receives branches from the renal and spermatic, or ovarian arteries. In the middle third, branches come to the ureter from the spermatic or ovarian arteries. At its bifurcation, the aorta sends an important branch to the middle third of the ureter, or this vessel may come from

the common iliac artery. In its lower third, the ureter is supplied by small branches from the uterine, vaginal, middle hemorrhoidal, middle vesical and inferior vesical arteries.

The chief blood supply to the ureter is derived from long arteries which arise from either the lower end of the abdominal aorta just before its bifurcation, from the common iliac artery, or from the internal iliac artery. These ureteral arteries invariably arise from the medial side of these large vessels and cross the artery of origin to join the ureter on its medial wall. Here they divide and course superiorly and inferiorly for nearly the entire length of the ureter. These ascending and descending primary arterial branches anastomose with branches above from the renal, spermatic, or ovarian arteries, and below with branches from the uterine and vaginal arteries in addition to the branches which come to the lower ureter from the inferior vesical arteries and middle hemorrhoidal arteries. The arteries which supply the lower ureter reach it on the lateral side.

The long ureteral arteries give off many small branches which ramify around the ureter and anastomose freely with themselves and with the branches from other sources, such as the renal and spermatic branches.

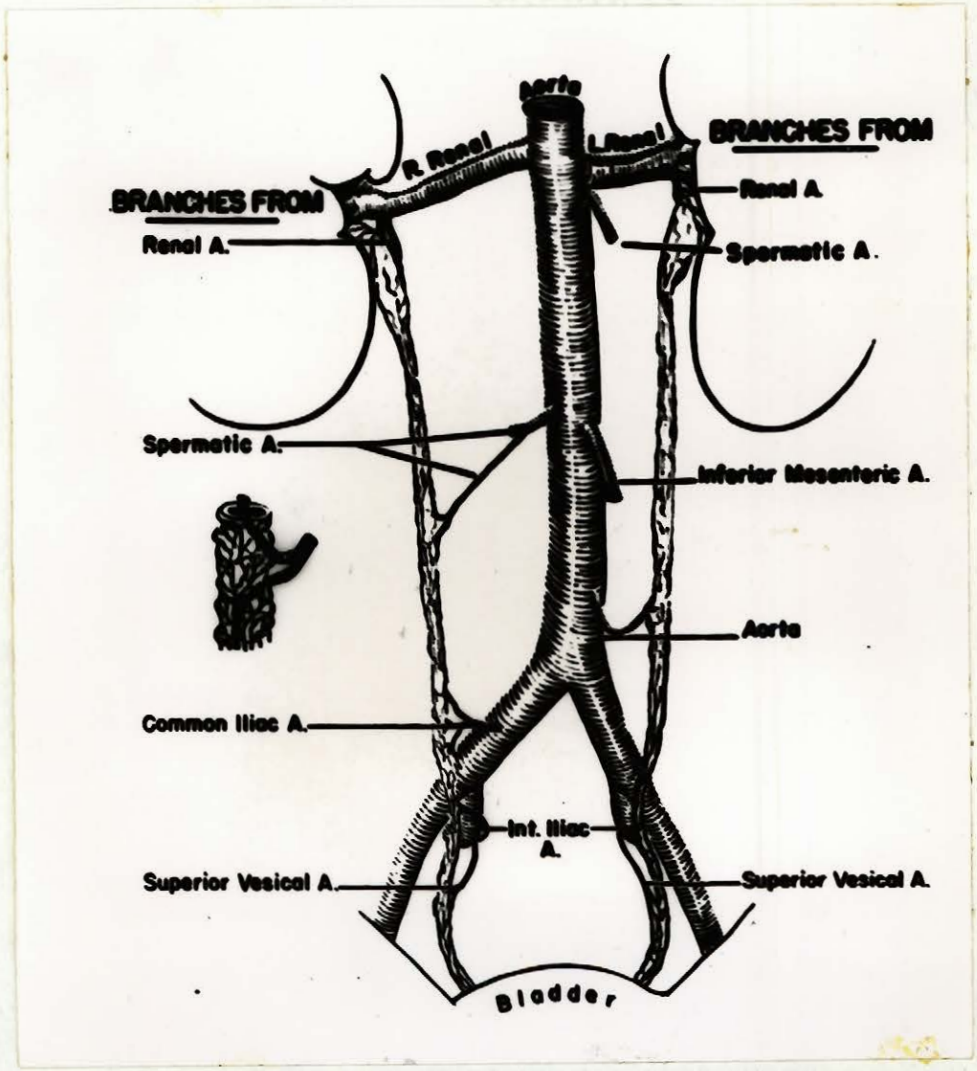


Fig. 4

Blood supply of the ureter. From Grant, J.C.B., An Atlas of Anatomy, The williams and Wilkins Co., Baltimore, 1951.

Veins in the submucosa of the ureter form a plexus from which larger channels pass to the adventitia and connect by free anastomosis with nearby veins such as the spermatic, ovarian, and renal veins above, and in the pelvis with the uterine, and vaginal veins and the vesical plexus.

Lymphatics of the Ureter: Lymph capillaries in the walls of the ureters give rise to lymphatic channels that pass diagonally outward through the musculature of the ureters.

These lymphatic vessels lie in the adventitial covering of the ureters. They then leave the ureters and go to the regional lynph nodes.

The regional nodes of the ureters belong to the lateral abdominal chains; common iliac, external iliac, and hypogastric groups of lymph nodes.

Nerve Sypply of the Ureter:

The ureter receives its nerve supply from three sources:

1. A superior group of nerves from the lower fibers of the renal plexus and intermesenteric nerves.
2. A middle group of nerves from the superior hypogastric plexus or from the upper end of the hypogastric nerve.
3. An inferior group of nerves from the lower end of the hypogastric nerve and the upper part of the inferior hypogastric plexus.

Superior Ureteral Nerves: The lower fibers of the renal plexus give off several delicate twigs which supply the lower part of the

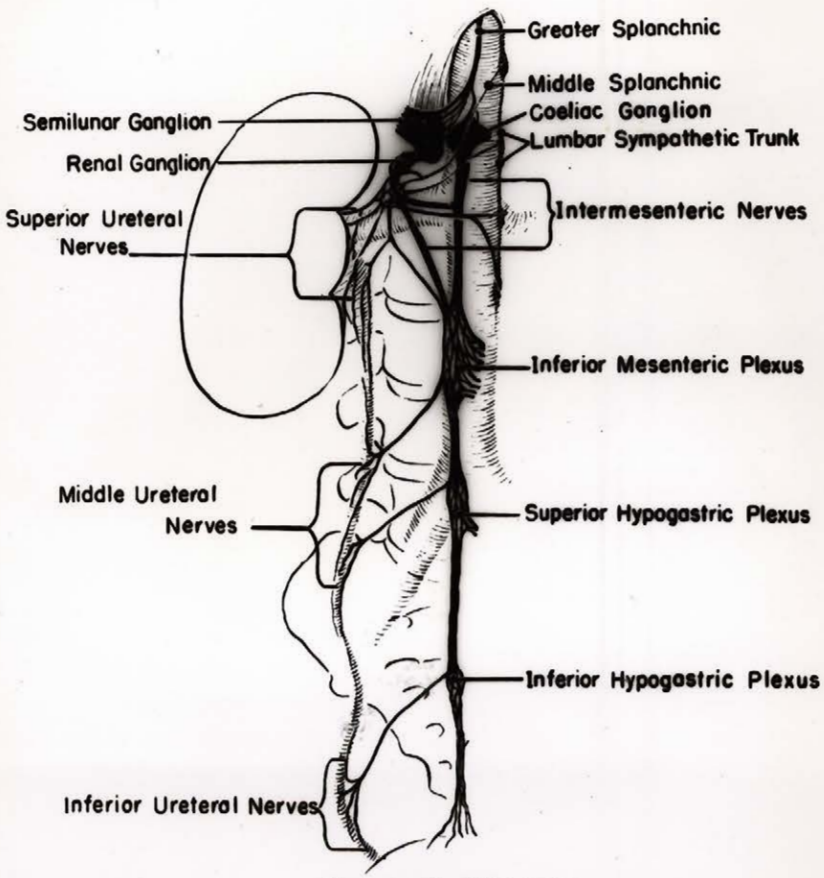


Fig. 5

Nerve supply of the ureter. From Essential Urology. F.H. Colby, 4th Edition. The Williams and Wilkins Co., Baltimore, 1961.

ureter. Fibers may also come from the intermesenteric nerves. There may be connections between these ureteral nerves and the spermatic plexus.

Middle Ureteral Nerves: The mid-portion of the ureter is supplied by branches from the superior hypogastric plexus or from the hypogastric nerve or plexus. These nerve branches follow the small ureteral artery which arises from either the common iliac or internal iliac artery. These nerves are closely associated with the spermatic nerve.

Inferior Ureteral Nerves: The lower end of the ureter receives its nerve supply by fibers from the hypogastric nerve and the inferior hypogastric plexus. These nerves connect fibers which supply the vas deferens and seminal vesicles.

Narath (1951) states that it is an accepted fact that there are no ganglia in the wall of the ureter, and that the only few ganglia found and demonstrated to satisfaction are near the juxtavesical part of the ureter. He therefore assumes that there is an automatism of the musculature of the upper urinary tract as firs claimed by Engelmann.

Physiology of the Ureter:

Peristaltic waves propel the urine from the kidney pelvis to the bladder. Contractions occur one to five times a minute,

depending upon the rate of urine formation. Reflux of urine from the bladder into the ureter is prevented by the oblique insertion of the ureter through the bladder wall, although it has been observed by previous investigators that when parts of the roof of the intramural ureters were removed, regurgitation occurred. Ureteral peristalsis is initiated as soon as urine flows into the renal calyces. As urine output increases ureteral peristalsis increases. The factor that is the normal adequate stimulus for rhythmic contractions of the ureter is urine volume mechanically stretching the muscle fibers of the ureter. Ureteral peristalsis appears to be entirely independent of the central nervous system and is unaffected by any known drugs except as they affect urine volume. Excised portions of the ureter continue to contract when deprived of any extrinsic nerve supply.

Narath (1951) and Murnaghan (1957), have stated that the peristaltic waves initiate in the calyces and proceed as unbroken waves of contraction down along the renal pelvis and ureter. On the other hand, Kiil (1957), expresses the view that the renal pelvis is a fairly passive reservoir with weak peristalsis which is independent of the ureter. The urine is "milked" from

there in installments by waves of contraction which start from the ureteric cone and continue down the ureter. Gould, Hsieh, and Tinckler (1955) have suggested that the lower end of the ureter has a task somewhat different from that of the upper and that the integration of tonic and peristaltic activity is such as normally to protect the nephron from the high basic pressures which arise in the lower segment when it is operating to handle large quantities of urine or against elevated intravesical pressures. Risholm et al, (1960 have concluded from their work on pressure and peristalsis studies in the upper urinary tract of the dog, that peristalsis of the ureter is at least in part dependent upon the raised pressure within that structure although in a patent ureter the pressure variations brought about by the contractions cease at a certain pressure level. They also advanced the statement that both respiration and blood pressure influence ureteric activity.

Lucas (1908), implied from his investigations that an increase in intrapelvic pressure has the effect of slowing down the circulation of blood through the kidney, producing some renal damage and favouring the trapping of organism in narrow capillaries.

In experiments on the isolated ureter it has previously and repeatedly been shown that ureteral activity is dependent on the temperature; the rate of contractions increased when the temperature was elevated 37 to 40 degrees; the ureteral activity ceased when the organ was cooled and reappeared when the organ rewarmed.

Definitions:

Medical literature frequently stresses the fact that the term "uremia" is vague, ill-defined, inconstant and unclear. Schreiner and Maher (1961) define this symptom complex as that which results from a dynamic imbalance between the organism's current metabolism and its appropriate renal functions. It is marked by aberrations in the volume and composition of body fluids, pathology in several important membranens and an array of patho-physiologic mechanisms still in the process of being defined. They state that its exact clinical signs and symptoms will vary with (a) biologic characteristics of the individual patient; (b) the etiology of the specific renal disease; (c) the time-course (rate) of development; and (d) the details of management.

These authors speak of "renal failure" to distinguish this entity from the previous, as an advanced quantitative reduction in renal function to a point where the kidneys are unable to maintain chemical homeostasis in the organism. Chemical changes are outside the accepted limits of "normal." These may be volume or "total body" changes as distinguished from those measured as concentration only. Renal failure is "acute" if it develops over a period of days or weeks, and "chronic" if it develops over a span of months or years.

General renal insufficiency is referred to as a quantitative reduction in renal function measurable by tests or manifested by transient episodes of renal failure occurring under the stress of diet or increased catabolism. This may be predominantly due to (a) loss of mass, as in infarction, nephrectomy or hypoplastic kidney; (b) loss of glomeruli, as in shock, capillary thrombosis, acute glomerulonephritis; or (d) as is most common, a mixture of these elements, the exact proportion of which may be difficult or at times impossible to define.

The absence, or at least the rate limitation, of an individual or combination of individual kidney functions, is known as "specific renal insufficiency". This implies either that the particular function can be specifically measured or has metabolic consequences

which can be delineated in the organism. This condition can be exemplified by renal hyperchloremic acidoses, renal glycosuria, idiopathic hypercalciuria, and aminoaciduria.

"Azotemia", as defined by these authors, is a retention of nitrogenous end products of protein catablism in the blood or body fluids. They classify azotemia as to its origin, thus: (a) urological, as with obstruction to the free flow of urine to the outside; (b) renal, when due to renal insufficiency; or (c) pre-renal, when due to the excessive ingestion or diffusion of nitrogenous materials, the contraction of body fluids or the presence of drugs, diseases or conditions which induce the excessive breakdown of body cells (e.g. adrenal cortical steroids, blood in the gastrointestinal tract, urea or amino acid infusions, multiple hematomata). True pre-renal azotemia may obviously be produced by such things as the infusion of urea or the ingestion of blood. Conditions characterized by measurable changes in the renal blood flow and usually classified as pre-renal azotemia include: (1) pernicious vomiting; (2) diarrhea; (3) duodenal drainage; (4) diabetic acidosis; (5) Addison's disease; (6) traumatic shock; (7) septicemia, particularly staphylococcal or gram negative; (8) burns; (9) myocardial

infarction; (10) liver disease; (11) gastrointestinal tract hemorrhage; (12) heat stroke; (13) advanced congestive heart failure; (14) the use of ganglionic blocking agents or other antihypertesive drugs in patients with rigid changes in the renal vasculature. However, these conditions should properly be classified as renal insufficiency since they contain a substantial element of renal functional impairment usually due to changes in the renal circulation.

In the experiments performed, whenever the animal exhibited only elevations in BUN and creatinine and was free of untoward symptoms, it was termed "azotemic". When the elevated blood nitrogenous constituents were accompanied by signs and symptoms, the animal was considered "uremic".

The Experimental Animal:

Healthy male and female adult mongrel dogs were selected in this study for the following reasons: the ease of their management, their great anaesthetic tolerance, their relative inexpensiveness as compared to primates and other larger animals, their accessibility and availability, their great body size as contrasted from other commonly used laboratory animals, the similarity in anatomy and physiology of their genito-urinary tract to that of humans, so that differences between the responses in the two species to different conditions are minor and functional. Thus, for the purpose of these experiments, the dog was thought to be more of the ideal experimental animal.

The dogs used ranged in weight on the average, between 10 and 20 kilograms, and were fed on a regular diet of Dr. Ballard's dog food, milk, and vitamins, regardless of their state of azotemia or uremia. These dogs were all vaccinated against distemper. The dietary habits especially the daily oral fluid intake, were closely observed.

These animals were kept in large room on the first post-operative day to allow them some degree of freedom. Whenever the weather allowed it, the dogs were kept outside for some time daily in specially arranged cubicles on the roof of the Donner Building.

The Ameroid Costrictor

This is a constrictor which was originally designed and used experimentally by Litwak and Vineberg (1959) for the purpose of producing occlusion of the coronary artery vessels. It is a plastic product made of casein, which due to its hygroscopic properties, swells up when it absorbs water or moisture. Casein plastic is prepared by mixing powdered Rennet casein with water. Rods of varying dimension are formed by extrusion under increased pressure and temperature. The rods are hardened for a prolonged period in formalin. The ameroid has a central lumen drilled to specific diameters ranging from 3 to 7 millimeters (0.110 to 0.2 inch). This substance is encased in a rigid steel jacket so as to prevent peripheral expansion and consequently to narrow the central lumen. Leading from the periphery into the central lumen is a communicating slot large enough for the introduction of a coronary artery, or, of particular interest in this thesis, a ureter into the central bore. The central lumina of all the ameroid constrictors that were used throughout the experiments to be mentioned were always of 3 millimeters (0.110 inch).

The ameroids are stored in a dessicator prior to their use, and are eventually sterilized in a container with vaseline an hour before the operation. The initial dessication storage prevents water absorption prior to sterilization and the vaseline forms a thin coating thus delaying the absorption of tissue fluids. In order for the ameroids to obtain more uniform vaseline impregnation, Litvak has recommended placing the vaseline-ameroid container in a hot water bath during this period of time. It has been observed that although ameroids are autoclaved satisfactorily, this apparently prevents vaseline impregnation to a lesser degree and consequently leads to a more rapid swelling and central luminal occlusion.

Vineberg et al (1959) state that a dry ameroid placed in saline reduces its central bore from 3 to 1.65 millimeters in 26 days. Vaseline-coated ameroids absorb less water and consequently their rate of swelling is diminished. Such ameroid sleeves reduce their central lumina from 3 to 1.98 millimeters in 32 days, and to 1.8 millimeter in 54 days. In cardiac experimental work, the slowly contracting vaseline-coated coronary artery ameroid constrictors have been used in order to allow more time for the re-establishment of cardiac circulation either naturally or by means of surgical revascularization procedures.

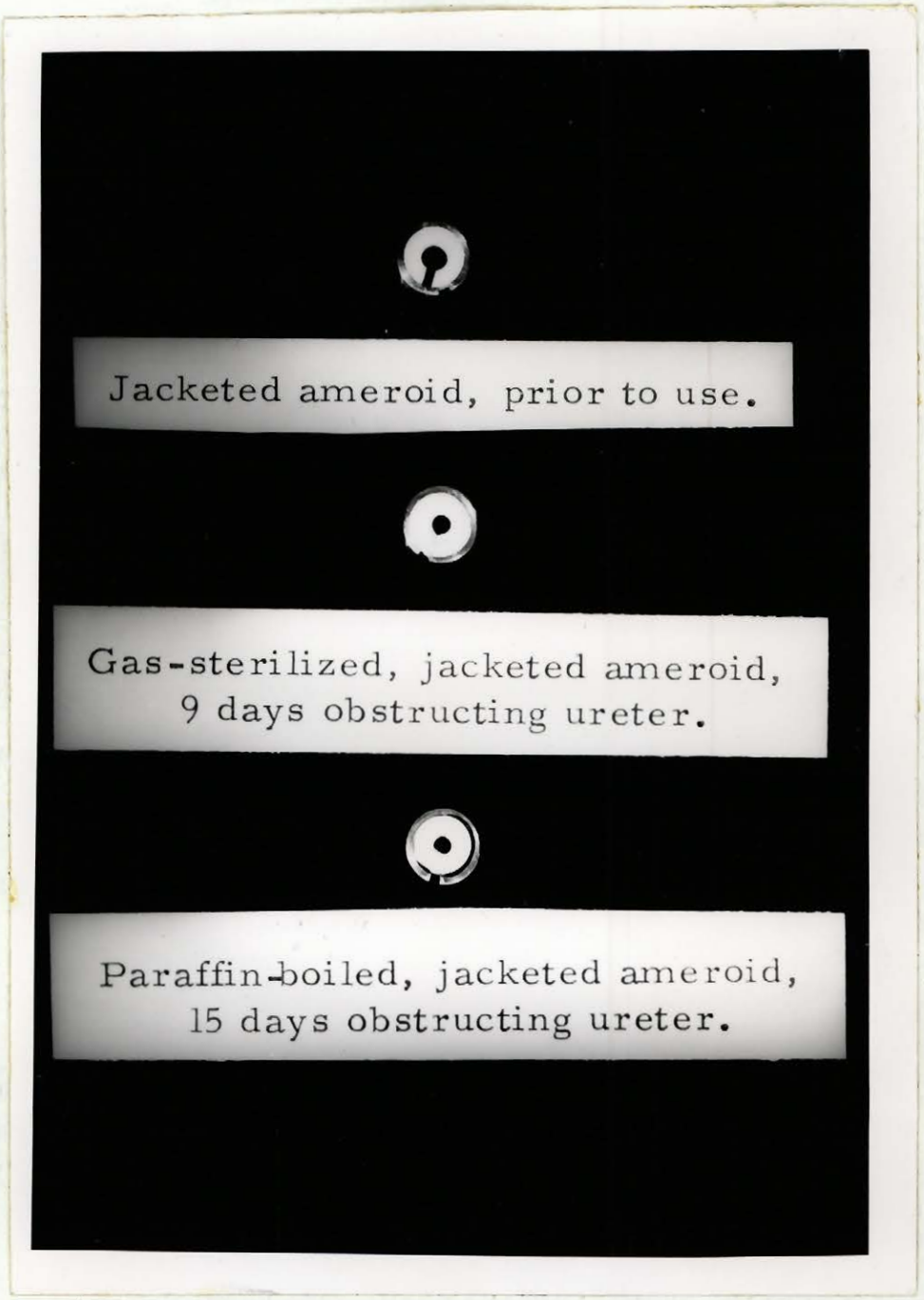


Fig. 6. Examples of jacketed ameroid constrictors used in experiments in this thesis. Top level: Jacketed ameroid constrictor prior to use, lumen measuring 3mm. in diameter. Middle level: Jacketed ameroid constrictor obstructing ureter for 9 days, lumen measuring 1.56 mm. in diameter. Botton level: Jacketed paraffin-boiled ameroid constricting ureter for 15 days, lumen measuring 1.33 mm. in diameter.

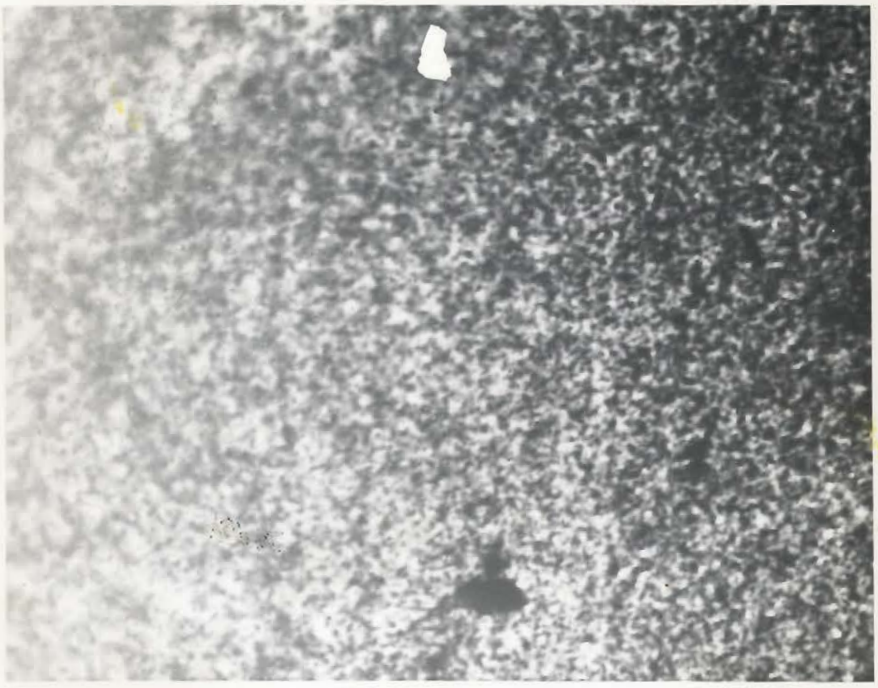


Fig. 7. Histological section of paraffin boiled ameroid core substance x 100

Unjacketed ameroids expand unevenly in both a luminal and a centrifugal direction, and due to the absence of a limiting device, the luminal occlusion is minimal.

Experimentally, the ameroid has been employed in various procedures wherein occlusive phenomena were expected as the end result. Initial work with them was suggested by Shipley, and thereafter has been utilized by different workers primarily for progressive occlusion of blood vessels like the aorta, coronary, hepatic, renal arteries, etc. This casein constrictor has also been used outside of the vascular tree as in the experimental occlusion of various structures of the biliary tree. It is in this thesis that its application to the ureters is described for the first time.

Laboratory Investigations:

The degree of azotemia and/or uremia in the experiments performed have been ascertained by the laboratory determinations of BUN and creatinine as guides. The determination of these blood constituents are relatively simple to perform and readily accessible in the laboratory. Additional information would have been desirable, but since the investigations were carried out with the aim of determining only the basic clinical

and biochemical changes that are altered in the condition produced, these were not done. Urinalysis and accurate urine volumes were not accomplished due to the great difficulty in collecting unadultered and exact volume samples from the dogs. Initial attempts proved hazardous in many respects, and this procedure was deemed unreliable. Emphasis was thus given only to the nitrogenous determinants of the blood.

It is essential that the BUN and creatinine, being the chief end products of protein catabolism which play an important role in the evaluation of the uremic syndrome, be described briefly.

The principal nitrogenous metabolite excreted by mammals which accounts for 85% of the urinary nitrogen, is urea. In the liver, when amino groups are dissociated from their original amino acids, they are synthesized by means of the urea cycle into urea. Chief among these relations is the hydrolysis of arginine to urea and ornithine, its synthesis having been recently reviewed by Ratner (1954).

It is of interest to note that urea is very well distributed among the tissues due to its high solubility in aqueous as well as in biological solutions. Likewise, its diffusibility through

most cell membranes is great, with the probable exceptions of the ureter and bladder. Levinsky and co-workers (1959), however claim that at very low urine flows, urea may cross the bladder.

Urea has been given an early emphasis in the pathogenesis of the uremic syndrome due to its liberal distribution in the tissues, the frequency of its measurement in normal and diseased states, and its historical priority. Its chemical significance has been agreed upon, whereas there is significant controversy on both the quantitative and qualitative aspects of its biological importance. Even the classical concept of urea being the end product of protein metabolism has been challenged. Chalmers and Synge (1954), have shown that ruminant animals can utilize urea for protein synthesis.

Urea has been definitely blamed as the cause of certain specific features in the uremic syndrome. Lieber and LeFevre (1959) related the urea concentration to gastric hypoacidity as seen in this syndrome. Bliss (1937), found that tartar from the teeth of nephrectomized dogs and uremic patients was responsible for the formation of ammonia from the hydrolysis of urea by urease in the saliva, and which in turn caused the ulcerations of the cheeks and tongue so often seen in uremic patients.

Creatinine is one of the major derivatives of protein catabolism. It is chemically the anhydride of creatine. In vivo, it is principally formed from creatine phosphate which is a major constituent of most body cells but is found in higher concentrationr in the larger sources of endogenous protein, namely muscle cells.

For the synthesis of creatine, the principal amino acids involved are glycine, arginine and methionine. Creatine is converted to creatinine in the muscles by the removal of water and the formation of an intramolecular peptide linkage. Creatinine may be considered a typical end product of protein metabolism. Schreiner and Maher (1961), state that creatinine excretion is a much more constant phenomenon than urea excretion, and that endogenous creatinine determination is a useful clinical test for the quantitative assay of renal damage. They further state that the dialysis of creatinine is different from the dialysis of urea, and is usually a more accurate biochemical guide to the degree of uremia than urea. Although the serum creatinine rises somewhat later than the blood urea nitrogen in renal insufficiency, it is less likely to be affected by such extraneous factors as the rate of diuresis and protein intake.

The following table has proven most useful in the evaluation of laboratory levels of nitrogenous catabolic substances in the experiments performed:

TABLE I

DOG Nitrogenous Constituents (mg./100ml.)	Mean Values	Standard Deviation	Complete Range		95% Range	
			High	Low	High	Low
NPN	27.5	6.5	52.4	15.3	42.4	16.7
Urea	14.7	4.8	21.6	7.0	20.3	7.5
Creatinine	1.2	0.47	2.7	0.3	2.6	0.4

From "Blood Components in the Dog: Normal Values" by M.H. Carr and P.R. Schloerb, J. Lab. & Clin.Med., 53: 646 - 652, 1959.

Operative Procedure:

The animals were not put on any special regimen nor did they receive medications of any form preoperatively.

Anaesthesia consisted of intravenous Sodium Pentobarbital in a dosage sufficient to produce surgical anaesthesia(60 milligrams of the anaesthetic per 5 pounds of body weight).

The dog was placed on the operating table in the supine position, all four extremities firmly bound to the edges, thereby permitting a good abdominal exposure. The abdomen was shaved with an electric razor and scrubbed with tincture of metaphen 1:200 solution (Nitromersol Tincture, N.F.) and sterile drapes were applied.

A median low abdominal incision of suitable lenght is made and carried down eventually opening up the peritoneum. The intestines are retracted carefully cephalad and laterally with moist, saline-soaked intestinal packs. The ureters are then identified and traced to their distal thirds where a small incision is made in the posterior peritoneum immediately lateral to the ureters. A gall-bladder forceps is slid through this incision underneath the ureter towards its medial aspect, where the peritoneum is also incised. The ureters are mobilized caudally for

for a length of about 2 centimeters. Two loose sutures of 2 - 0 silk are inserted to replace the clamp and serve for traction purposes. With the ameroid constrictor grasped firmly with a gall-bladder forceps, the ureter is gently tented upwards and its long axis is introduced carefully within the ameroid's lumen. The intestines are replaced in their original location, and abdominal incision is closed in layers.

Operative Technique:

In these experiments, the purpose has been primarily the interruption of urinary flow through the ureters. In addition, nephrectomies were performed in some instances. Several varied techniques were used in an effort to answer questions regarding:

1) What type of ameroid constrictor was most suitable for a desired degree of azotemia and/or uremia.
2) What group of animals made azotemic or uremic survived and would provide optimal conditions for an eventual kidney homotransplantation.
3) What particular technique produced the least harmful effects on the animals.

The experimental animals have been divided into groups according to the procedure employed.

Group I. Jacketed ameroid constrictors applied to both ureters.

Group II. A jacketed ameroid constrictor applied to one ureter only.

Group III. A jacketed ameroid constrictor applied to one ureter, and a contralateral nephrectomy.

Group IV. A jacketed ameroid constrictor applied to one ureter, and an unjacketed counterpart applied to the other ureter.

Group V. Unjacketed ameroid constrictors applied to both ureters.

Group VI. Unjacketed ameroid constrictor applied to one ureter only.

Group VII. An unjacketed ameroid constrictor applied to one ureter, and a contralateral nephrectomy.

Kidney Homotransplantation:

Employing the method used by most of the workers in kidney homotransplantation, the kidney homograft was transplanted to the receipient's iliac fossa. This was performed with the most aseptic technique possible under the circumstances. Upon opening the recipient's abdominal cavity, vascular continuity of the kidney homograft's pedicle was achieved by an end-to-end anastomosis of the donor's renal artery or arteries to the terminal branches of the recipient's middle sacral artery, and an end-to-side anastomosis of the donor's renal vein to the recipient's common iliac vein,

The homograft ureter was implanted into the receipient's urinary bladder via a submucosal tunnel, after it has been ascertained to have dripped urine from its free end. The time lapse from clamping of blood supply of the donor kidney to its restoration in the recipient was always in the vicinity of 50 to 60 minutes. The abdominal wall was closed in layers upon completion of the anastomosis and confirmation that circulation had been reestablished in the homograft.

RESULTS OBTAINED

Group I. Jacketed ameroid constrictors applied to both ureters.

Four dogs were subjected to this procedure. In all four animals it was generally observed that at about the third or the fourth day after the procedure, they appeared ill, often refusing food and water. Extreme apathy was the rule; the animals would lie flat and when made to walk, presented unsteady gait. Vomiting and diarrhea were a common occurrence, as was the fast loss of subcutaneous tissue. Urinary output gradually became scanty on the third and fourth day after the operation. These dogs presented a fast and steady rise in their BUN and creatinine values, starting already on the second or third post-operative day. They all expired within a period of one to two weeks, with BUN levels ranging between 30 and 91 mg per cent, and creatinine levels of 3 to 4.2 mg per cent.

At autopsy, the kidneys and ureters above the constrictors presented varying degrees of hydronephrosis. The renal pelves and calyces were dilated; the ureteral wall above the site of obstruction appeared edematous and thickened, while the segment below the ameroid appeared normal or even atrophic. There was considerable fibrous proliferation around the ameroids, yet when these were carefully removed from the ureters and the ureteral

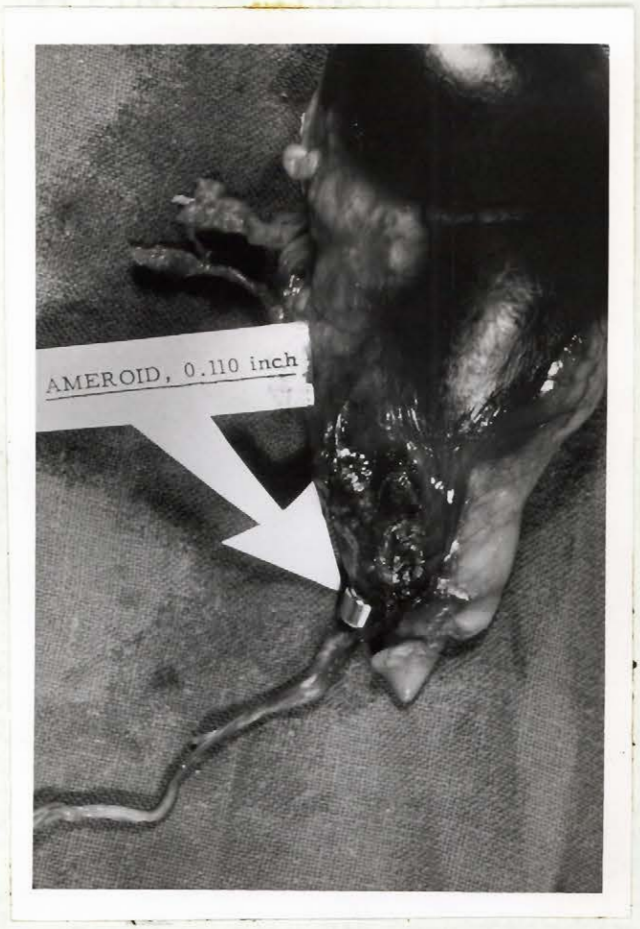


Fig. 8. Gross appearance of hydronephrotic kidney obstructed with jacketed ameroid constrictor for 2 weeks.

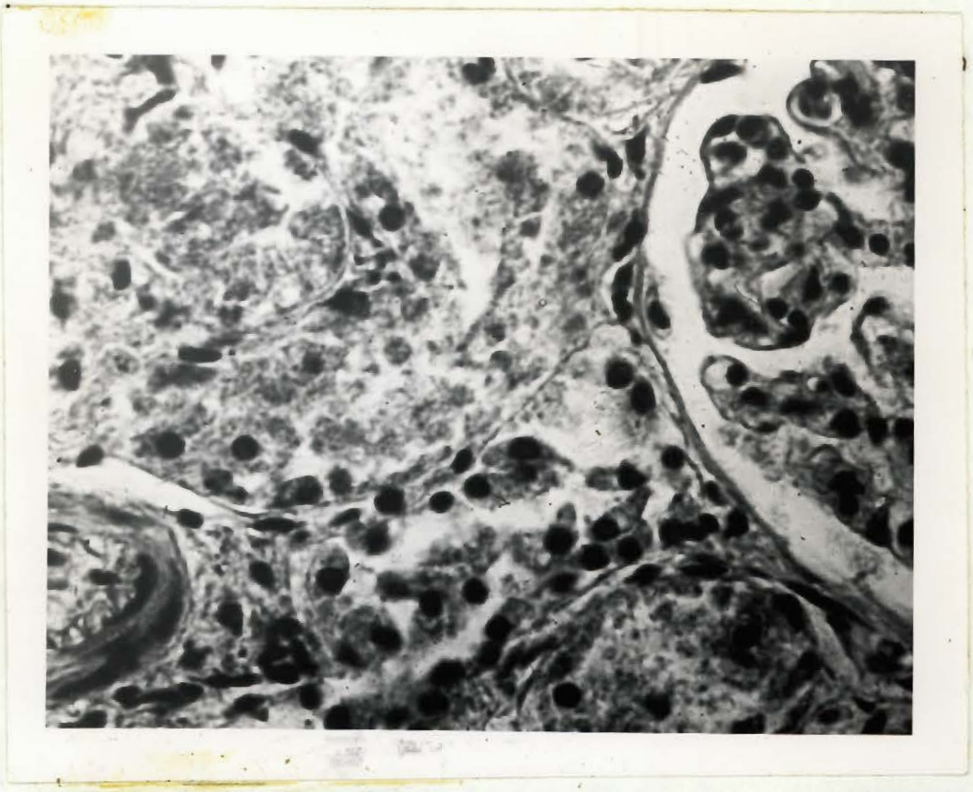


Fig. 9. Histological section of renal cortex of hydronephrotic kidney obstructed with jacketed ameroid constrictor 2 weeks. (Group I) x 420.

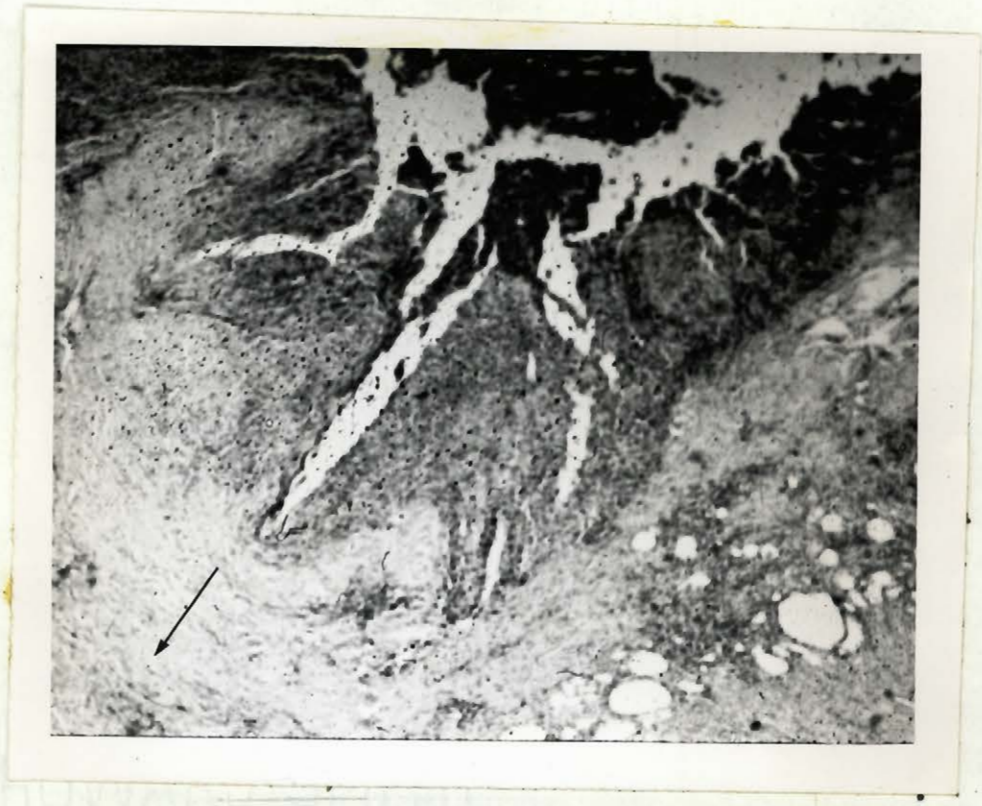
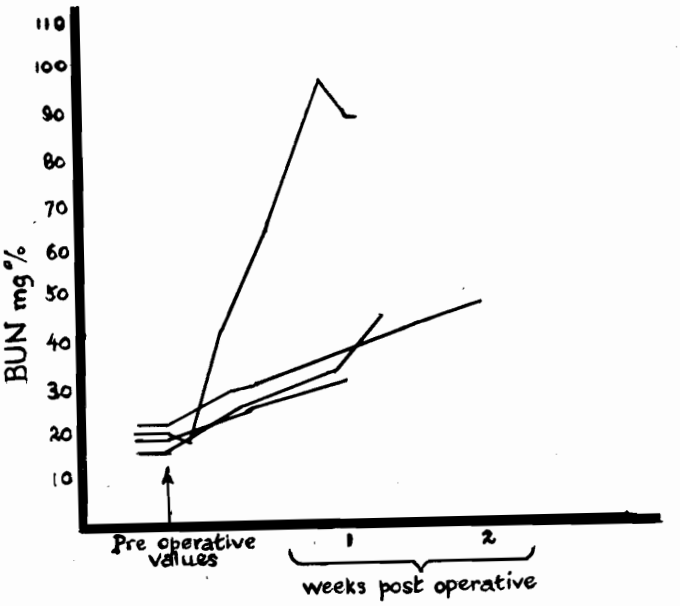


Fig. 10. Histological cross section of a ureter obstructed with a jacketed ameroid constrictor for 2 weeks, showing ameroid substance infiltrating the wall with reaction around it. x 80.

Blood Urea Nitrogen and Creatinine Levels in Group No. I
(Jacketed ameroid constrictors applied to both ureters)



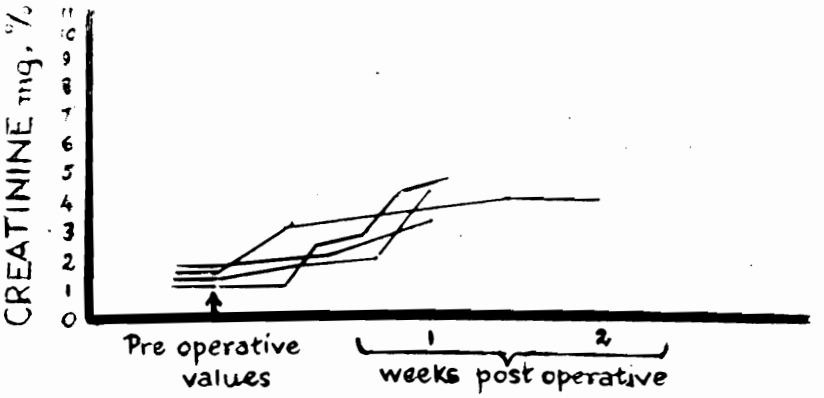


TABLE II

lumina opened, it was observed that the latter had narrowed considerably although patent enough to admit a No. 19-gauge needle.

The kidneys on sectioning oozed profusely with malodorous purulent material consistent with that found in infected hydronephrosis. The renal pelves and calyces were dilated, and the renal cortex appeared thinned out.

Microscopically, the typical atrophy of the tubules as compared to the relatively intact glomeruli, was present.

Group II. Jacketed ameroid constrictor applied to one ureter only.

In this group, the dogs were practically asymptomatic and apparently healthy, with the exception of one animal who died of a bronchopneumonia in the fifteenth week. Urinary output was apparently satisfactory postoperatively. BUN and creatinine values had very minimal and slow rise in the animals sacrificed fourteen to fifteen weeks after the procedure.

The highest BUN reading was 19 mg. per cent, and that of creatinine was 1.8 mg. per cent. All these dogs showed a marked hydronephrosis of the kidney corresponding to the obstructed ureter, whereas the contralateral kidney appeared slightly

hypertrophied or essentially normal in appearance. On sectioning the hydronephrotic kidney, the renal cortex would be thinned out to a mere shell with numerous lobulations in the pelvic part. The fluid in all the cases was fetid, yellowish in color, and markedly turbid, presenting the picture of a frank pyorephrosis. The gross findings in the obstructed ureter were very much like those found in the previous group of animals.

Histological sections of the obstructed ureter and kidney were again very similar to those of Group I, although on the contralateral kidney, the picture was one of compensatory hypertrophy in varying degrees.

Group III. Jacketed ameroid constrictor applied to one ureter, and a contralateral nephrectomy.

The dog that was made uremic with this procedure was utilized as a kidney donor for a homotransplantation procedure. It was observed that the BUN level rose from an initial 22 mg. per cent at operation, to 90 mg. per cent in a period of seven days; the creatinine values from 1.1 mg. per cent to 4.2 mg per cent, respectively. This animal developed profuse, repeated vomiting and salivation on the third day post-operatively. It showed marked apathy, loss of weight, refusal to eat or drink, and developed tremors of the extremities. These manifestations

became progressive up to the seventh day after the procedure, when the animal expired. Its urinary output was practically nil.

The remaining kidney, although hydronephrotic, was observed to be smaller than the specimens taken from the previous two groups. The hydronephrotic fluid was of the same color, consistency and odor as in the other groups. The ureter, above the site of obstruction was dilated, and below it, collapsed. Its ureteral lumen, although markedly diminished in caliber, maintained patency.

Histological sections of the kidney showed tubular atrophy and dilatation, occasionally cystic, more marked than in the previous groups. The glomeruli had likewise suffered some atrophic changes and hyaline cast formation.

Group IV. Jacketed ameroid constrictor applied to one ureter, and an unjacketed counterpart to the other ureter.

This group of four dogs had a relatively slower rise in BUN and creatinine levels than the preceding group. In a period of thirty days, the original BUN levels ranging between 15 and 20 mg. had risen to levels between 44 and 98 mg. per cent. Unfortunately, these animals died within a period of four to six weeks, two of them from pneumonitis, and the other two dying suddenly from causes undetermined.

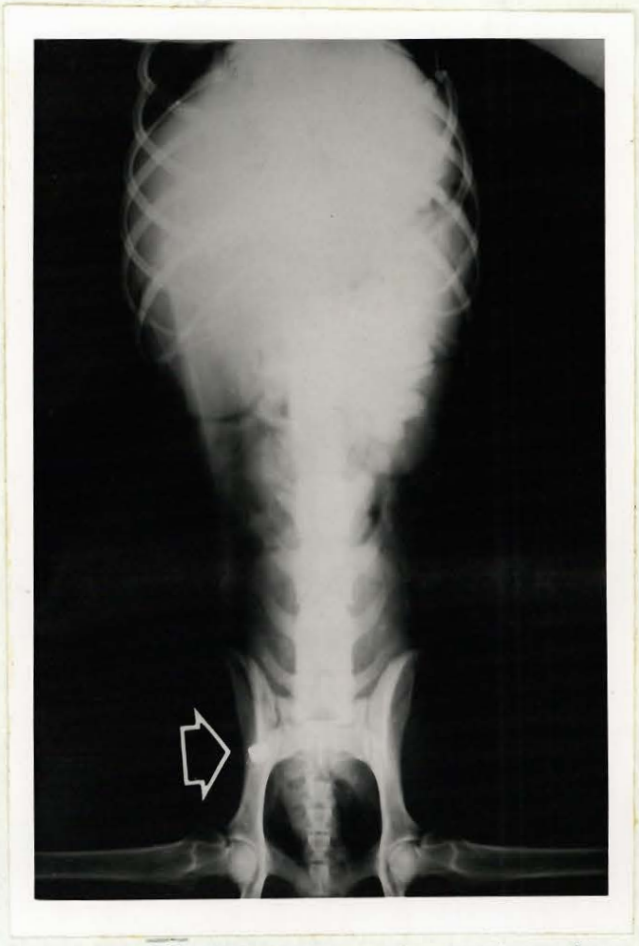


Fig. 11. Radiograph of illustrative example in Group IV.(A jacketed ameroid constrictor is applied to one ureter, and an unjacketed counterpart to the other). Arrow shows jacketed ameroid in the right ureter.

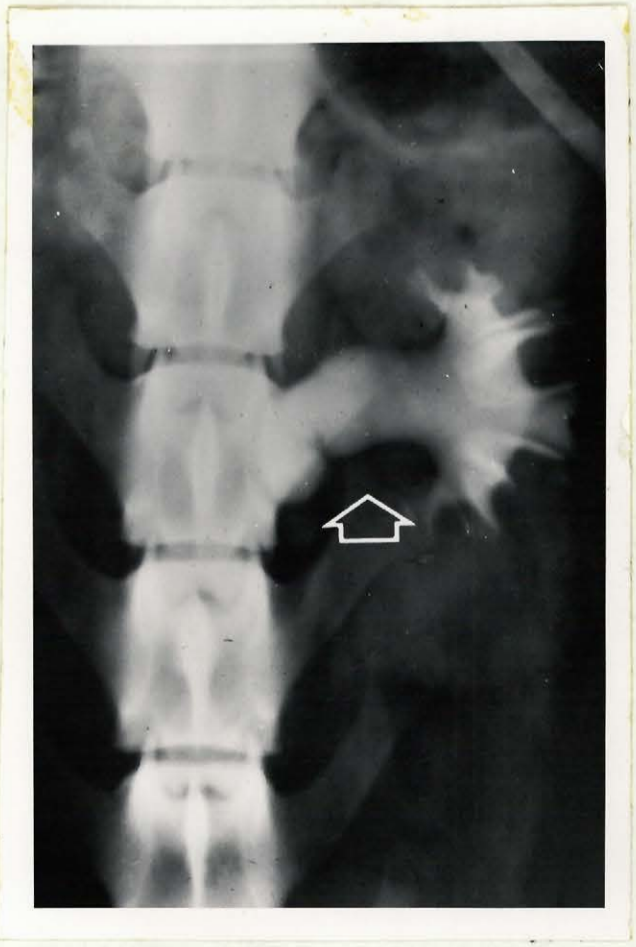


Fig. 12. Intravenous pyelogram of same dog in Group IV. (A jacketed ameroid applied to one ureter, and an unjacketed counterpart to the other). This picture shows kidney obstructed with the unjacketed ameroid. Arrow points to dilated ureter and pelvis.

They all presented the expected findings of bilateral hydropyonephrosis, more marked in the kidney whose ureter was obstructed by an unjacketed ameroid constrictor. The ureteral lumina at the site of obstruction were patent. A larger patency was observed in the ureter where an unjacketed ameroid constrictor produced the interference ot the urinary flow. The proximal ureters were dilated and edematous in all cases.

Tubular atrophy and dilatation as well as glomerular atrophy and hyalinization were salient features in the hydronephrotic kidneys obstructed by the jacketed ameroid constrictors. This same finding but to a lesser extent was present in the kidneys obstructed by the unjacketed ameroid.

Group V. Unjacketed ameroid constrictors applied to both ureters.

There were five dogs used for this procedure. It is interesting to note that their BUN and creatinine values had a very slow progressive rise. From BUN levels of 15 to 20 mg. per cent on the day of operation, determinations done 15 1/2 to 17 weeks post-operatively, rose between 15 and 45 mg. per cent. Creatinine values likewise had a steady slow increase

from normal values to readings between 4.8 and 6.0 mg. per cent. These dogs behaved normally in all respects, had a good food and fluid intake up to the time they were sacrificed, and their loss of body weight, if any, appeared minimal. Their urinary output seemed to be normal at all times. One dog developed a scaly dermatitic eruption over the inner thighs about the fourth week after the operation, but outside of this, behaved normally.

When sacrificed, the kidneys of four dogs presented a dusky appearance and were very minimally enlarged, the difference in size between the kidneys being insignificant. On section, the pelvis was appreciably larger in size and the renal cortex was but slightly thinned. The proximal ureter was very slightly edematous and enlarged, and the site of the ameroid-core application felt like a small smooth knob due to the proliferated fibrous tissue that had been formed around it. On opening this area, the ameroid-core appeared in most cases to have disintegrated markedly and merged with the surrounding fibrous tissue, since the former could not be identified readily as it appeared as small isolated deposits of brittle,

semi-calcareous material. The ureteral lumina had considerably decreased in caliber, but were patent in all the cases.

One of the dogs sacrificed at the end of the fifth week after the ameroid-core application presented severe hydropyonephrosis of both kidneys and proximal ureters, unlike the slight enlargement of the kidneys of the animals included in the same group. When sectioned, the renal cortex was extremely thinned out by the exaggeratedly enlarged pelvis that contained the increased amount of the pyohydronephrotic fluid. In life, this dog did not manifest any untoward sign and behaved like any of the dogs in the same group. Its BUN and creatinine levels followed the same general pattern of slow progressive rise as in the others. The microscopic picture in this case was very similar to that presented by kidneys whose obstruction was produced by means of a jacketed ameroid constrictor.

The general histologic picture in the majority of these animals coincided with those found in kidneys obstructed by the ameroid-core, although it was evident that the degree of tubular dilatation, as well as glomerular destruction appeared somewhat less. There was some dilation of Bowman's spaces with occassional small inflammatory foci in the renal pelvis.

Fig. 13. Illustrative example of the gross appearance of kidneys and ureters found in dogs from Group V. (Unjacketed ameroid constrictors applied to both ureters.

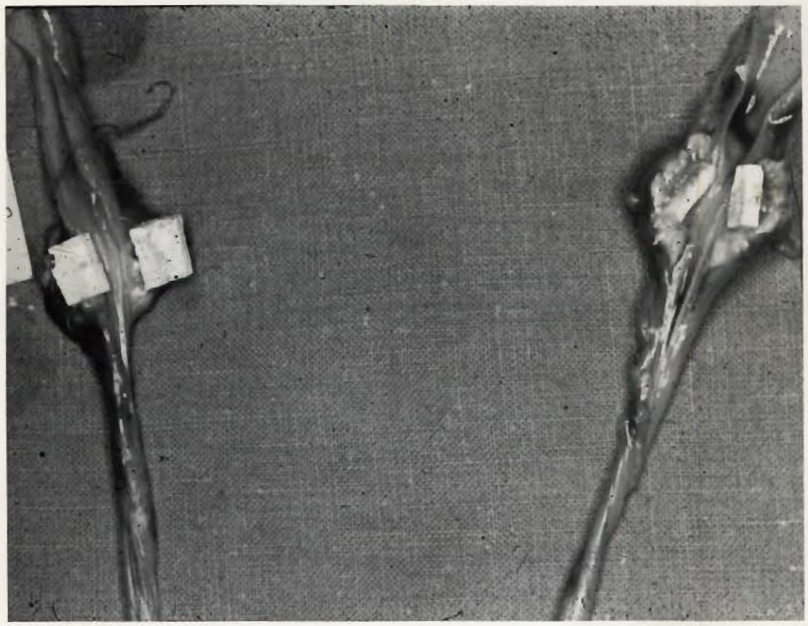


Fig. 14. Close-up view of the same ureters at the point of ameroid core application and obstruction. Note dilatation, edema and hypertrophy of proximal segment as contrasted from distal. Ameroid core is enveloped in thick mesh of fibrous tissue as seen on the right. Lumina both patent.

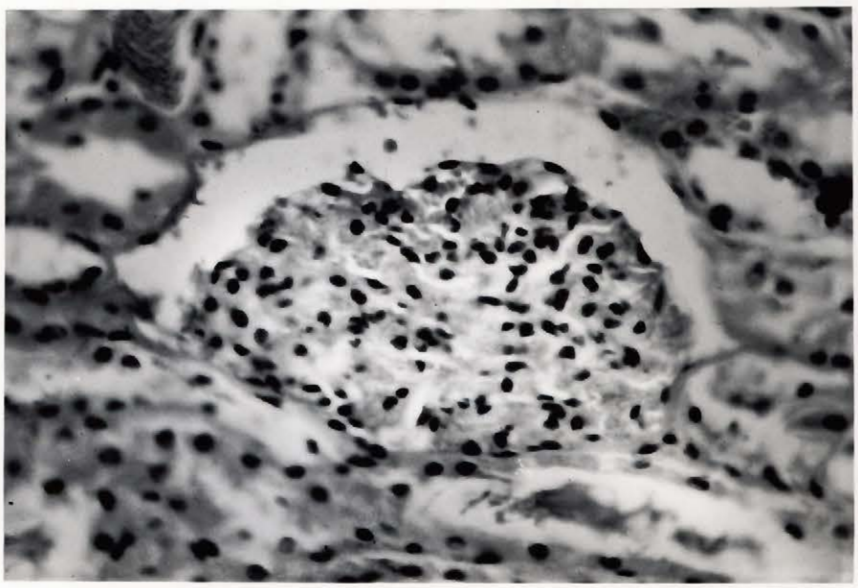
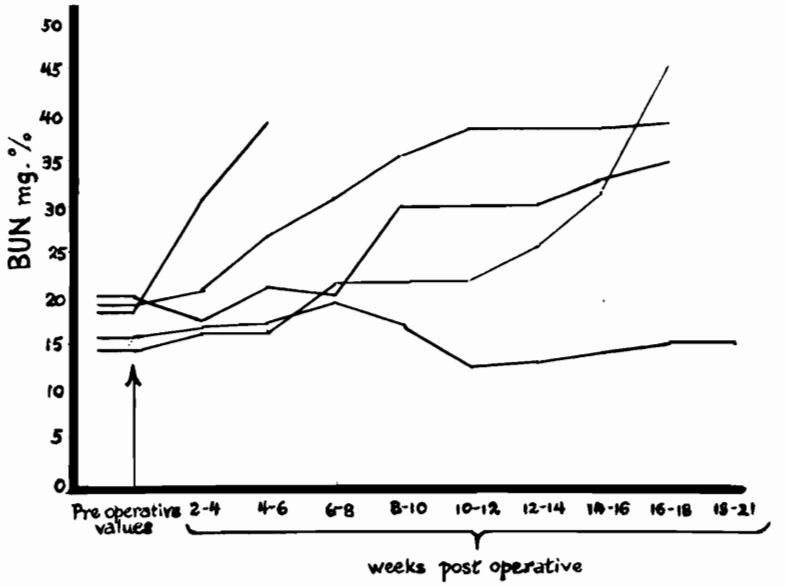


Fig. 15. Histological section of kidney after ureteral obstruction with unjacketed ameroid constrictors applied to both ureters (Group V) for 16 1/2 weeks. x 450.

Blood Urea Nitrogen and Creatinine Levels in Group No. V
(Unjacketed ameroid constrictors applied to both ureters)



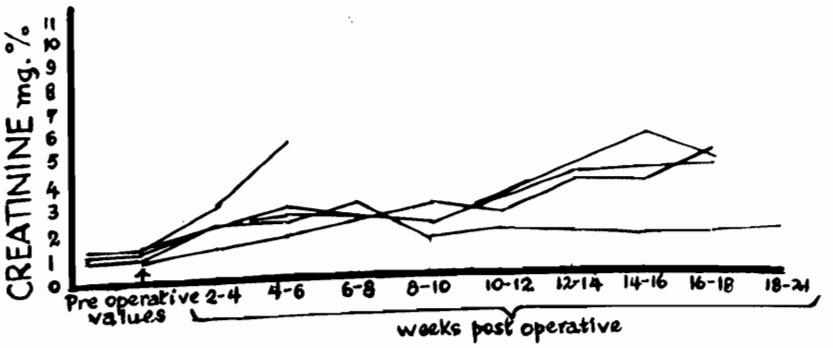


TABLE III

Group VI. Unjacketed ameroid constrictor applied to one ureter only.

There were four dogs in this group. It was noted that when these animals were sacrificed five to six weeks after the operation, their BUN and creatinine levels were in the range of 18 to 20 mg. per cent and 0.8 to 1.0 mg. per cent above their pre-operative values. As was expected, these dogs were free of any unwanted signs.

At autopsy, the obstructed kidney was hydropyonephrotic, manifesting the usual lobulations in its enlarged pelvis. The renal cortex was in most cases, ironed out to a mere shell, and when sectioned, there was a profuse ooze of fetid, turbid fluid. In one instance, the fluid appeared like diluted anchovy paste.

The ureter showed its usual proximal thickening and dilatation, and a normal or slightly atrophied segment distal to the ameroid-core location. The ameroid-core would always be hidden in a smooth knob of fibrous tissue easily identifiable from the proximal or distal ureter. In two instances, the site of ameroid-core application felt hard and smooth to touch as compared to the rest of the ureter, although the usual fibrous

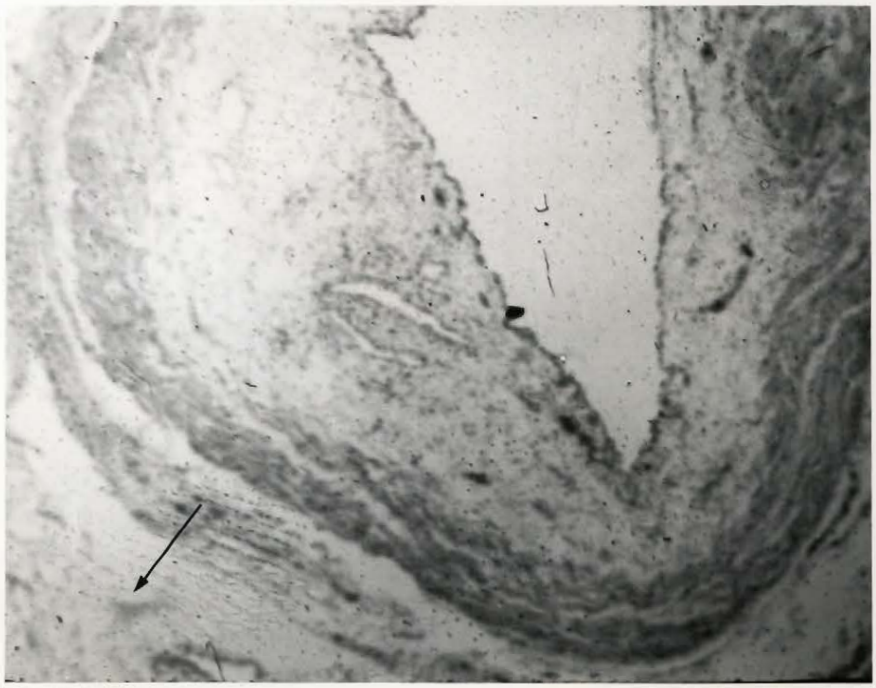


Fig. 16. Histological cross section of ureter obstructed with an unjacketed ameroid constrictor for 16 1/2 weeks, showing ameroid substance well defined around the ureteral wall. x 22.

knob could not be demonstrated. On opening this area however, the definite narrowing of the lumen would always be constant. Ureteral lumina in all the cases were appreciably diminished in diameter at this site.

The histological picture of the affected kidney was essentially the same as that described in the kidneys blocked with unjacketed ameroid constrictors.

Three contralateral undisturbed kidneys showed evidence of hypertrophy, while one kidney did not show hardly any abnormality in its histological structure.

Group VII. Unjacketed ameroid constrictor applied to one ureter, with a contralateral nephrectomy.

There were three dogs in this group, and two of them became donors and azotemic recipients for the kidney homotransplatation procedures. One dog was sacrificed six weeks after ameroid-core application and contralateral nephrectomy. Its BUN reading was 42 mg. per cent and the creatinine level had risen to 4 mg. per cent. This animal did not present any uremic manifestations at this stage. The animals in this group failed to show any diminution of urinary output.

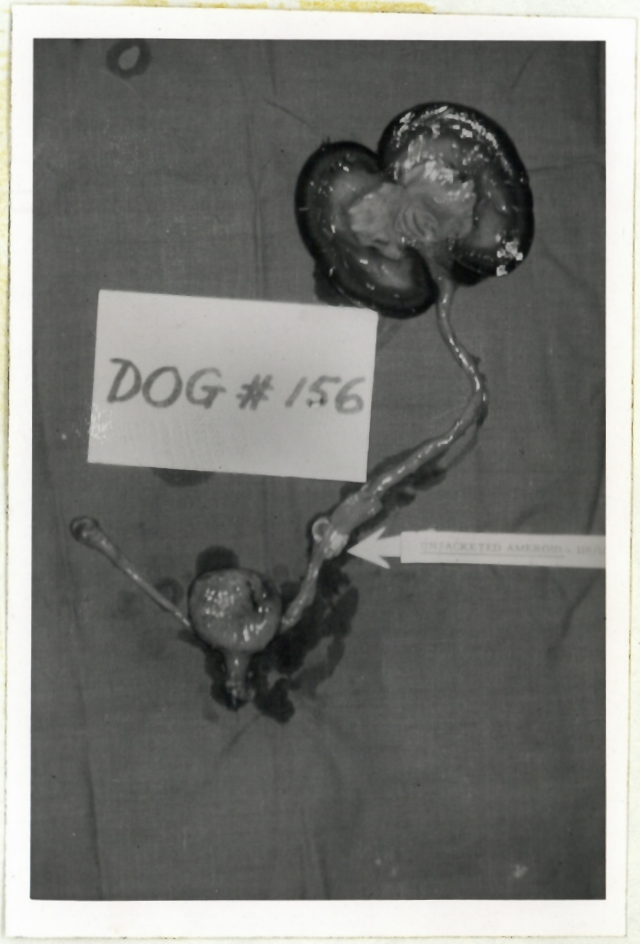


Fig. 17a.

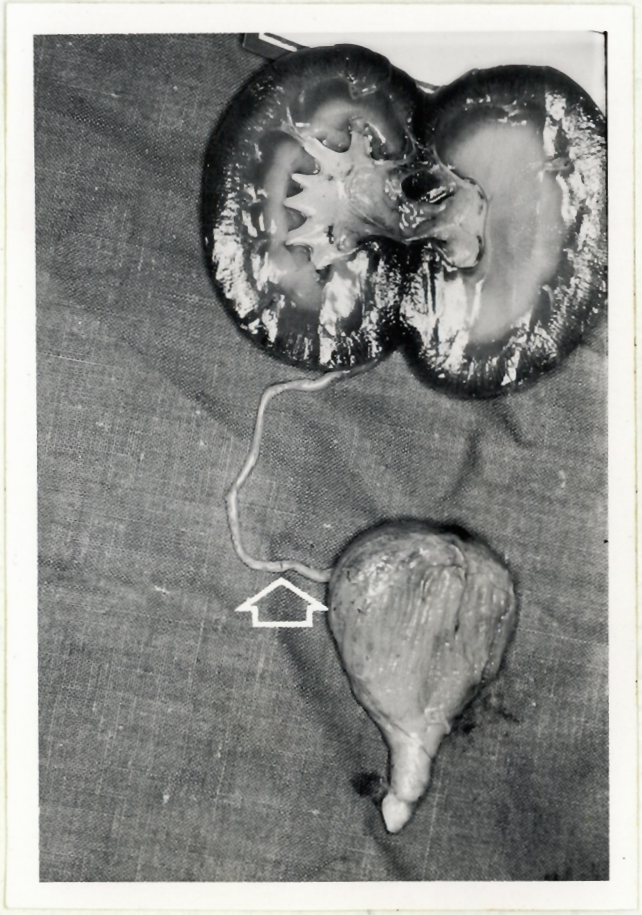


Fig. 17b.

Illustrative appearance of kidneys and ureters after 6 weeks of obstruction found in dogs from Group VII. (Unjacketed ameroid constrictor applied to one ureter, and a contralateral nephrectomy). Note that in Fig. 17b. the usual fibrous knob around the ameroid cannot be seen due to absorption of the ameroid core.

Fig. 18. Close up view of the same ureter shown in Fig. 17a, of ameroid core application and obstruction. Note patent lumen.

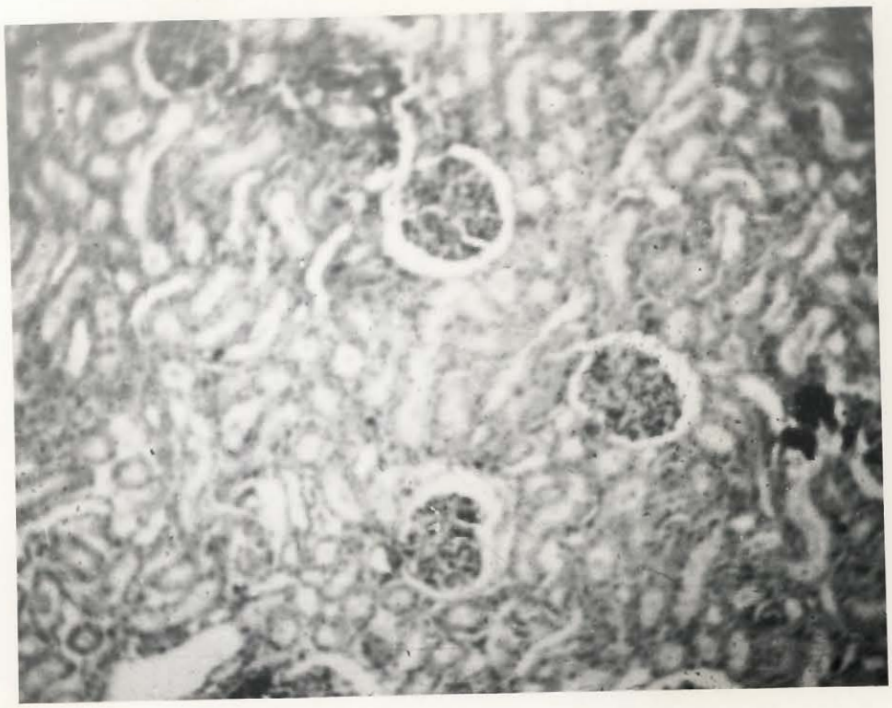


Fig. 19. Histological section of kidney after application of unjacketed ameroid constrictor to its ureter, and a contralateral nephrectomy at 6 weeks. x 120.

The gross appearance of the kidney and proximal ureter was that of a massive hydronephrosis. On cut section, the renal cortex again was markedly thinned out, and the pelvis showed the characteristic lobulations seen in severe hydronephrosis. The fluid therein, although urinous in smell, was turbid and of a yellowish-green color.

The microscopic picture tallied with kidney findings in cases of infected hydronephrosis.

Attempts at kidney homotransplantation.

With the level of azotemia established in the host, it was thought valuable to investigate and probe into the azotemic or uremic state as a predisposing factor to an effective homograft "take".

Employing the method used by most of the workers in this field, the kidney homograft was transplanted to the donor's iliac fossa. Transplantation procedures were performed on one uremic and three azotemic recipients from normal donors. All of these recipients had a unilateral nephrectomy prior to this procedure. The uremic recipient died on the first post- operative day from a slow venous leakage at the site of the anastomosis.

The first two azotemic hosts lived for 21 and 22 days

respectively after the operation. Despite the fact that both of the donor kidney ureters were secreting urine prior to their implantation into the host bladder, these kidneys were found to have undergone extensive rejection changes at the time of death. The renal homografts in both cases appeared very much the same. They were both pale, enlarged, and their surfaces presented sharp fissures. On section, it was hard to distinguish the pelvis from the cortical substance since they had merged into a dirty gray, caseous, homogenous mass. Their renal vessels had thrombosed, and the ureters were collapsed. These dogs manifested an array of symptoms and signs of disturbed metabolism within the first post-operative week. (Copious salivation, vomiting, diarrhea, progressive cachexia, tremors, and finally, coma.)

The last azotemic host had had a nephrectomy and an unjacketed ameroid applied to the contralateral ureter. The BUN was 49 mg. per cent and creatinine 2.0 mg. per cent at the time of operation, and the dog was completely asymptomatic. A homologous kidney was transplanted to its iliac fossa, and a free flow of urine was observed from the cut end of the donor ureter before its incorporation ot the host bladder. Excretory

pyelogram done on the eight post-operative day revealed good function in an otherwise slightly hydronephrotic homotransplanted kidney and ureter. The animal seemed to be thriving satisfactorily. To corroborate this finding, a laparatomy was performed two days later. On opening the abdominal cavity, the kidney homotransplant appeared slightly enlarged, viable, and presented a slightly dilated ureter. The renal artery was strong and pulsating, the renal veins were patent and the suture lines in the blood vessels were intact. Following this last surgical intervention, the dog gradually became anorexic and lethargic, and persistently lay on the floor. Vomiting, which in the beginning was slight, became continuous and intractable. The azotemic levels of BUN and creatinine had dropped a little but never to normal levels. This animal died on the twelfth day after the homotransplantation procedure. At autopsy the homotransplant and its corresponding ureter had not changed much in appearance since exploration, but in addition, there was a frank, profuse suppurative peritonitis.

The microscopic picture was that of a prominent tubular disease showing evidence of hyaline casts within the tubules. Scattered foci of acute and chronic inflammatory cells were

observed throughout the kidney, as well as evidence of hemorrhage and inflammation in the peripelvic fat. These changes were consistent with a pyelonephritis and septicemia.

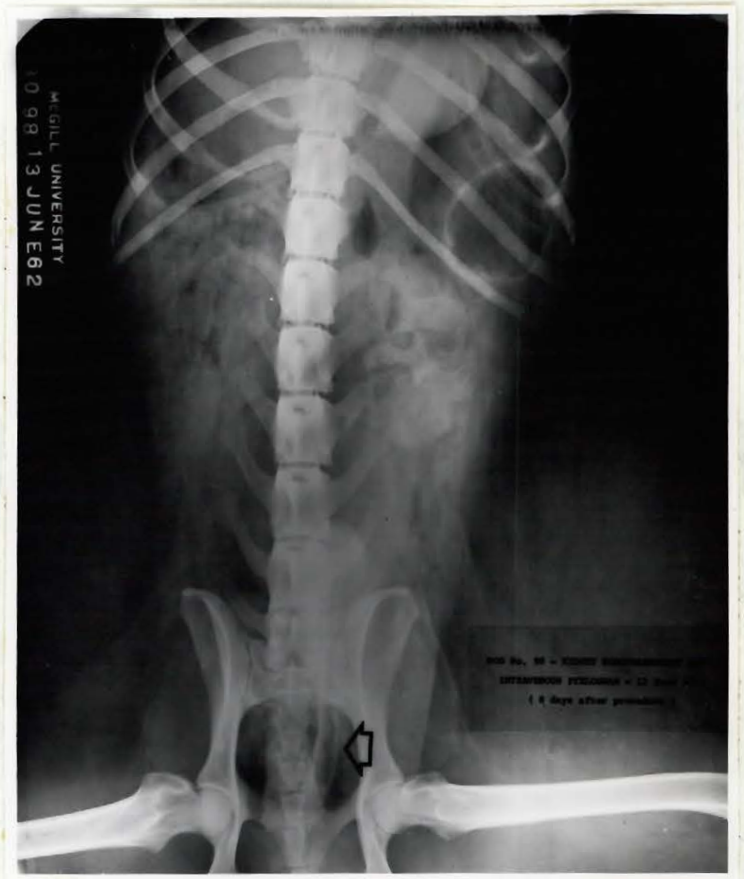


Fig. 20. Intravenous pyelogram of azotemic recipient dog (#98) showing adequate function of homograft on the 8th post-operative day.

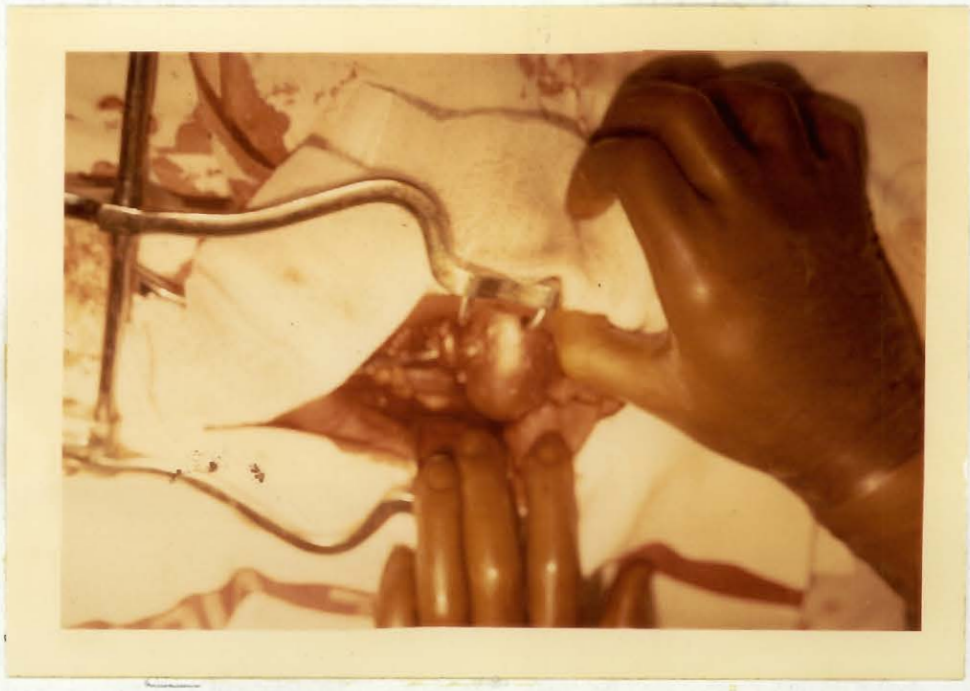


Fig. 21. A functioning kidney homotransplant as seen at laparotomy 10 days after transplantation procedure. (#98).

Fig. 22. Histological section of kidney homograft done at autopsy 12 days after the procedure. x 120.

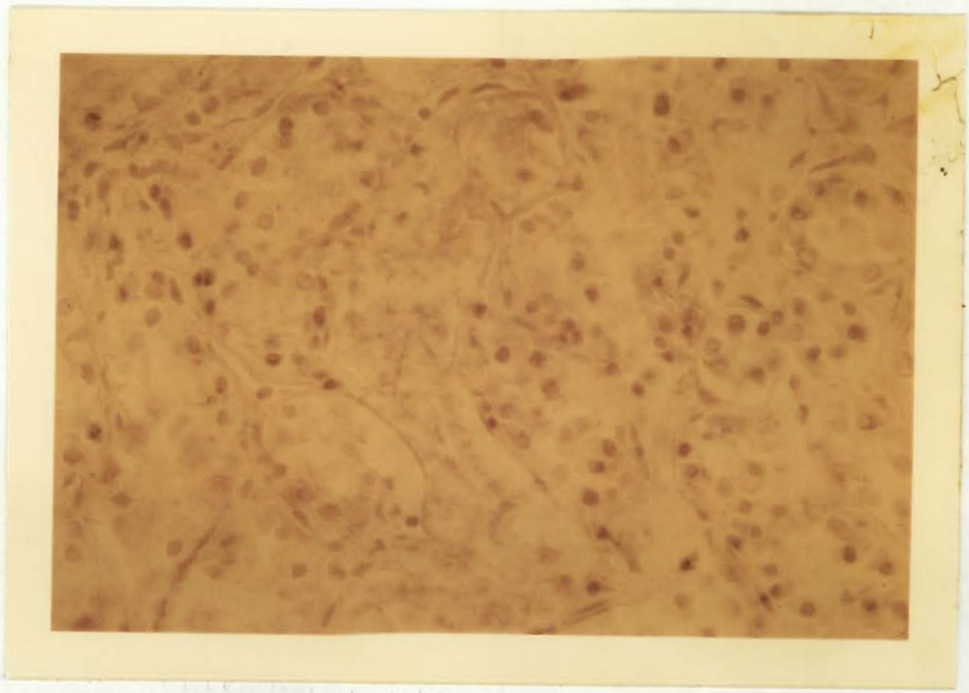


Fig. 23. Same histological section seen under high power (x 400).

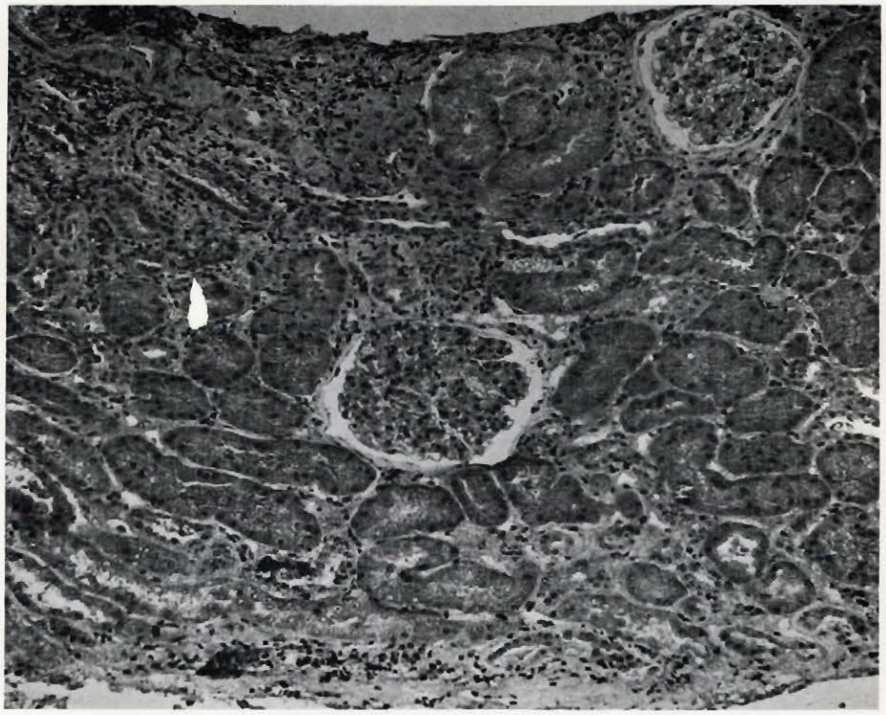


Fig. 23-B. Histological section of homotransplanted kidney showing rejection changes (x 160)

Fig. 24. Dog # 98, azotemic recipient immediately before pyelography on the 8th post-transplantation day. Note apparently normal and healthy appearance.

DISCUSSION

The discussion of the results of the procedures performed will be focused on two topics, namely: (a) the contribution of ameroid constrictors in the experimental production of chronic renal insufficiency; (b) the role of this type of induced chronic renal insufficiency relative to the prolongation in the survival time of kidney homografts.

The present investigation has shown that the degree of azotemia or uremia produced in the dog may be accelerated or retarded, as the case may be, by the use of the suitable ameroid constrictor. Furthermore, the rate of acceleration can be enhanced by combining the latter technique with nephrectomy.

The functional disturbance from the constricting action of the ameroid upon the ureter, possibly stems from the damage produced by the expanding ameroid constrictor to the ureteral muscle cells, or to the nerve cells of fibers. Sheehan and Davis (1961) considered the ureteral disturbances produced by external pressure agents as a type of "achalasia" wherein the ureter dilates above the constricted segment. By do doing, progressive hydronephrosis eventually is established, and intrapelvic pressure is increased permanently. The

kidney continues to secrete urine, though probably in a lesser volume than normal. This urine escapes continuously from the renal pelvis by means of a "pyelovenous backflow" as postulated by Hinman and Lee-Brown (1924). Domingues (1940) and Adams (1960) confirmed Hinman's findings by affirming that pyelovenous communications are always established, as proven by their work. Narath (1940) offers a different interpretation based on anatomical studies he has performed on the minute musculature of the fornices, calyces and papillae. He believes that the term "pyelovenous backflow" be restricted only to the traumatic production of sinovenous ingression. In the terms of Hinman et al, it may be justified to presume that the backflow increases considerably as the intrapelvic pressure rises, so that the pressure cannot exceed a certain upper level. This level is sufficiently low to allow the kidney to continue to secrete urine until the renal cortex finally becomes atrophied and functionless. Östling (1942) claims that the intrapelvic pressure in hydronephrosis is only 2 to 5 mm. Hg. higher than in the normal pelves.

Kill (1957) noted that the total ligation of the ureter of the dog only occasionally leads to atrophy of the kidney without development of hydronephrosis. This suggests that reflux is

less easily established in dogs. In man however, canals of reflux are evidently not produced to a sufficient extent, and the excretory ducts of the kidney are obstructed by the renal-pelvic counter-pressure, so that renal atrophy is produced following the cessation of renal function. Ureteral obstruction produces damage, first to the distal tubules, then to the proximal tubules, and eventually to the glomeruli, as found by Reisman et all (1957) in his experiments. In dogs, the damage produced is reflected in a diminished clearance test that is proportional to the duration of obstruction. Thus, it is safe to state that ureteral obstruction damages the kidney roughly in proportion to the degree and duration of obstruction.

A review of the literature gives evidence that detailed research into the pathogenesis of hydronephrosis is difficult to perform on animals because the common laboratory ones (dogs, rabbits, guinea pigs and rats) possess unipapillar kidneys as contrasted to the human renal pelvis which is multipapillar.

An establishment hydrophrosis will invariably lead to stagnation of urine, and thus, infection. Anatomically, the

ureter provides a direct route of invasion from bladder to kidney because the subepithelial tissue of the bladder, ureter, and renal pelvis is continous with the interstitial tissue of the kidney. Physiologically the obstructed ureter increases the susceptibility of the kidney to infection because ureteral dysfunction generally involves besides obstruction, a vesico-ureteral reflux. The ureter is not an inert tube but an active muscular structure, and recognition of this fact leads to a dynamic, rather than a static concept of the process that develops.

Besides the infection that commonly follows ureteral obstruction, substances in the blood derived from protein or protein metabolism manifest cummulative changes in response to the renal impairment. Principal among these end products of protein catabolism is urea. The possibility still remains that urea in high concentrations is directly toxic to some specific organs or to the organism in general.

Creatinine levels are likewise elevated since any of the factors which tend to promote a nitrogen retention might well affect its rate of formation. Similarly, uric acid, blood ammonia and other nitrogenous wastes are elevated.

It is well known that there are also disturbances in the acid base balance, as well as in the fluid and electrolyte metabolism.

In the present studies, it was observed that in Group I responding to a faster rate of bilateral ureteral constriction produced by the jacketed ameroids, the rise in BUN presented an accelerated response. It was evident that up to the third or fourth day, urinary excretion appeared within normal limits, and from then on, a gradual decrease in the output was noted. This deficiency in urinary output may well be explained by the constriction offered by the expanding casein plastic core limited by its rigid unyielding steel jacket. These animals showed signs and symptoms of clinical urinary insufficiency at about the same time that the urinary output gave evidence of impairment. It is to be expected that besides the concomitant rise in creatinine values, serum potassium would also be increased since this inorganic cation is elevated in any form of untreated, progressive renal disease. The dogs became frankly acidotic, and expired in one to two weeks consequent to the obstructive phenomenon created. In addition, the abnormal

accumulation of these nitrogenous waste products plus the de rangement in the normal electrolyte pattern, body fluids, and an imbalance in the "milieu interieur" contributed to the death of these animals.

Although it would have been ideal to perform urine composition and volume studies in these dogs, it has been suggested in the literature that in renal failure, the kidneys are unable to maintain homeostasis, and therefore the urine is not a reliable index of the endogenous metabolism and exogenous intake. As a matter of fact, it has been stated that the impaired ability to vary its composition in response to metabolic and dietary variations is the most characteristic urinary change of renal failure.

In contrast to the animals in Group I, (wherein jacketed constrictors were applied to both ureters,) those of Group V, (wherein unjacketed constrictors were applied bilaterally), it was demonstrated that BUN and creatinine levels rose much slower than in the first group. These dogs were free of any untoward signs and symptoms of renal insufficiency. At no time did they ever show any changes in their dietary habits. Their fluid intake was

adequate at all times. There were no changes observed in their daily urinary output. All these observations seem to stem from the fact that the ureteral constriction produced is not as great as that found in Group I, because of the unrestrained expansion of the unjacketed ameroid. The casein-plastic core expands both centrifugally and centripetally, thus offering the ureter within its bore a relatively minimal impediment to urinary flow. This, however, did not prevent the blood nitrogenous constituents to accumulate and rise, although as shown in Table II , it took a span of 15 1/2 to 17 weeks for the BUN and creatinine levels to reach the azotemic planes. In Group I, Table III shows the more accelerated elevation of these constituents that took place in a span of 2 weeks. An evaluation of the over-all results of dogs in Group V has led to the conjecture that unjacketed ameroid constrictors applied to the ureters produce the least objectionable findings in the experimental animals as proven by their clinical behaviour, physiological manifestations, laboratory findings and histopathological evidences. For some inknown reason, one dog in this group developed massive hydronephrosis, although devoid of any other associated clinical or

laboratory findings of an expeditious renal insufficiency. Then, one is led to suspect the possibility of some pre-existing congenital abnormality in the cellular structure of the affected kidneys, or perhaps some discrepancy in the composition of the ameroid core substance. At autopsy, it was impossible to determine a previous congenital structural anomaly because of the marked hydronephrotic distortion that had transformed the kidneys into mere fluid-distented sacs. It was also not feasible to make any observations on the ameroid cores since they had merged extensively with the surrounding fibrous tissue that had formed around them.

It is clear and obvious in Groups II and VI that when an ameroid constrictor with or without a jacket was applied to one ureter, the undisturbed contralateral kidney had to become hypertrophied by obligatory compensation and take over a great deal of the load of the obstructed kidney. The behaviour of the dogs was normal. Their nutritional status did not suffer any changes and their fluid intake was adequate at all times. BUN and creatinine levels practically remained within normal limits. Histological sections of the involved kidneys in both groups gave

essentially the same hydronephrotic picture. In the group obstructed with the unjacketed ameroid constrictor, while most of the contralateral undisturbed kidneys showed evidence of hypertrophy, one of these kidneys maintained an almost normal histological structure although grossly manifesting some enlargement. It seems feasible in this case to explain this finding by applying Hinmman's "pyelovenous backflow" theory to the obstructed kidney. The trapped urine continuously leaves the kidney pelvis to find its way into the renal veins. By so doing, the contralateral kidney apparently has undergone minimal structural changes in compensation.

When the ureter was constricted with a jacketed ameroid and a contralateral nephrectomy performed, as done in Group III, fast, progressive rise in the BUN and creatinine levels were observed, with the animal exhibiting anuria in the third day post-operatively. The animals would not tolerate any food or water, persistently lay on the floor, developed tremors of the extremities, salivated profusely and vomited repeatedly. One dog was frankly acidotic, azotemic, and died in a period of one week. The kidney

at autopsy, although hydronephrotic, did not attain the size of those in the other groups, probably because the rapidly expanding jacketed ameroid constrictor did not allow it to undergo active adaptive changes to the abrupt post-operative modification of function. On the other hand, the dogs in Group VII, where an unjacketed ameroid constrictor was applied to one ureter and a contralateral nephrectomy done, did not exhibit any manifestations like those in Group III. They appeared and acted very much like normal dogs although their BUN and creatinine levels rose progressively. Their urinary output was seemingly unimpaired. Their status was such that they were considered to be the most satisfactory hosts for kidney homotransplantation procedures.

The dogs included in Group IV, where an unjacketed and a jacketed ameroid constrictor were applied to each ureter respectively, presented a picture very much like those in Group I, where jacketed ameroid constrictors were applied to both ureters. All these dogs died within a period of four to six weeks, from causes probably contributed by the renal dysfunction that was being produced. Nevertheless, they all revealed the fast rise in their BUN and creatinine levels. Varying degrees

of hydronephrosis were revealed at autopsy. The kidney whose ureter was constricted by the unjacketed ameroid usually appeared larger.

All the necropsy specimens of chronic hydronephrotic kidneys gave evidence of varying degrees of pyelonephritis. The relationship between urinary obstruction and infection is well supported by much evidence in the medical literature.

It was observed that the method of ameroid application to the ureters as described in this work, is relatively free from immediate post-operative complications. The events that fall are purely due to the eventual constrictive action of the ameroids, and the infection that supervenes from stasis.

Kidney Homotransplantation:

As soon as the conditioned recipients were felt to conform with the ideal picture of chronic renal insufficiency, whether azotemic or uremic, kidney homotransplantation procedures were performed.

In 1961, Morrison, Maness and Tawes, performed experiments to prove skin homograft survival in rats with chronic renal insufficiency, and clearly showed significant prolongation. Other workers performed skin homografts between animal partners before or during a kidney homotransplantation procedure. This was done in order to obtain an index of rejection changes that may occur in the homotransplanted kidney

or changes that may soon be anticipated, since skin homografts present visible rejection phenomena readily. Skin homotransplants between kidney host and recipient were not done during these transplantation procedures since reports in the literature, which were lately reaffirmed by Hamburger (1962), stated that skin grafts are unsuitable because of the preimmunization danger.

Although rejection changes were noted in the kidney homotransplants in two azotemic recipients, it is of significance to note that one kidney homotransplant continued functioning for 12 days following transplantation. There were practically no gross findings at autopsy that would warrant the diagnosis of a rejection reaction as seen in the other recipients. To begin with, an intravenous pyelogram done on the eighth day showed faint visualization of the homograft and its corresponding ureter. This observation was corroborated at laparatomy that was performed two days after. Unfortonuately, peritonitis ensued following this last surgical intervention

resulting in the animal's demise. It should be noted that in this particular animal, the degree of BUN increase was not very high and the creatinine level was likewise only slighty increased. Although there was little evidence of homograft rejection at 12 days, it can certainly be stated that this finding is beyond the range of changes expected at this time.

Survival of a homograft kidney after 12 days can happen in unconditioned hosts, and this particular case may belong to this category.

This acceptance may in part be due to a direct depression of the immunological reactivity of the lymph nodes draining the graft, and again, the non-specific stress of the operation may contribute to adreno-cortical stimulation. If systemic or topical cortisone acetate administration will prolong survival of skin grafts in rabbits, as observed by Smiddy et al (1961), then it would not be difficult to surmise that prolonged kidney homograft survival time be mediated through an overactive adrenal gland, or glands.

The gradual evolution of experimental events involved in this work, from the induction of chronic renal disease to its utilization in homotransplantation has, for the most part, given indirect evidence, and a more detailed approach seems to be indicated in the assessment of more specific parameters. These findings suggest several aspects of further research, and on the present evidence, it is tempting to extend these basic observations. It would be interesting to know what effect dialysis would have on the azotemic or uremic homotransplant host, if the blood nitrogenous waste levels were maintained at a certain threshold plane; what the different immunological studies

done on such animals would show; and other significant minor facets that may arise from the induced condition. Recognition of the fact that some gaps still have to be filled is made, and were it not for the limitation of the time element, a clearer insight into the causative relationship that exists between chronic renal insufficiency and effective kidney homograft "take" might have been realized.

SUMMARY

1. A reliable and simple technique for the production os slow progressive renal insufficiency has been described.

2. Unjacketed ameroids have been observed to yield the optimum results desired as regards the gradual, delayed and prolonged effect of the induced renal insufficiency. This particular constrictor deserves more investigational work, since it offers speculation on future research.

3. Hydronephrosis in varying degrees always occurred subsequent to the ureteral obstruction produced, and infection was a common factor associated with it.

4. A homologous kidney transplant survived a span of 12 days in a mildly azotemic host. This animal recipient was conditioned with a combination of an unjacketed ameroid constrictor applied to one ureter and a contralateral nephrectomy.

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