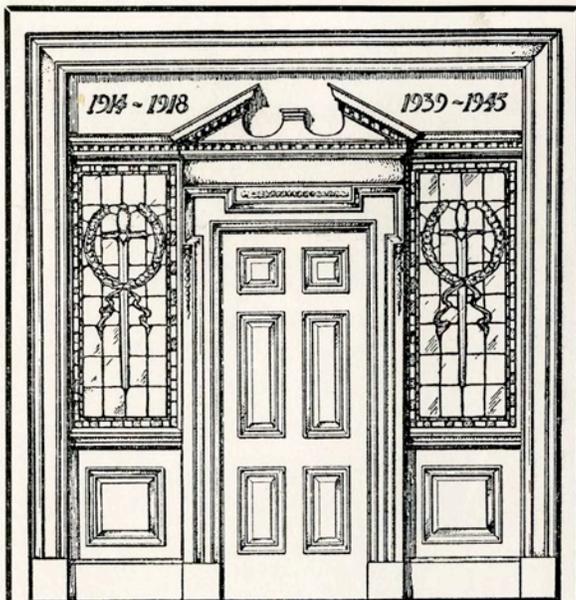


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THE TRANSPLANTATION OF THE  
MAMMALIAN KIDNEY

by

James Archibald

A thesis presented to the Faculty of the Graduate School of the  
University of Toronto in partial fulfilment of the requirements  
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## INTRODUCTION

For many years the idea of transplantation has engaged scientific minds. Perhaps the most publicized experiments in this field were testicular implantations in the human, the so-called "monkey gland" operations. To quote Markowitz (12) "This sensational charlatanry has served to discredit the field of organ transplantation in the eyes of the scientific world."

The true picture of transplantation would seem to be as follows: Organs transplanted in the same animal (autotransplant) survive for as long as nine months, according to Williamson (17), and perhaps is able to survive indefinitely. If an organ is transplanted by vascular anastomosis from one animal to another (homotransplant), it will survive for varying periods, averaging four to six days only.

However, from time to time varying reports as to the efficacy of the homotransplantation of organs has appeared in the literature. These reports range from those which deal with organs in which function has never been evident, to those which have reportedly functioned perfectly up to ninety days.

When the original work on the transplantation of mammalian kidneys was done, the sulphonamides and antibiotics were not available. It was therefore decided, in this series of experiments, to determine, if possible, the effect of penicillin upon the longevity of the transplant, since infection is almost impossible to prevent by surgical asepsis.

Loeb (10) has supported an hypothesis of organismal differentials, and has published several treatises on the biological basis

of individuality. He describes individuality as being of two types. The first is the mosaic type or the sum of organ and tissue characteristics or differentials. The second type is characterised by the presence of a chemical factor -- the individuality differential -- which makes the organs and tissues of one individual different from those of any other individual. Loeb called this difference the "organismal differential".

Some of the following experiments were designed to attempt to nullify this organismal differential. One method used was the storage of the donor kidney in a refrigerator at approximately 4°C. for twenty-four hours. In 1950, Selye (14) intimated that the adreno-corticotrophic hormone (ACTH) was beneficial in nephrosis. As nephrosis is most certainly a feature in the homotransplanted kidney, any agent which would serve to modify this reaction should increase the life of the transplant.

At the present time some cases of uraemia are treated by peritoneal lavage and the use of an artificial kidney. On the whole the treatment is palliative, according to Atchley (1). The therapy is one of replacement and sedation. Constant intravenous therapy is tedious and great care is required to supervise the well-being of the patient and to ensure against overloading the heart. In short, the treatment of these cases is unsatisfactory.

If homotransplantation of the kidney were found to be feasible and if the transplanted kidney would sustain life for even a few days, the clinical value of such a procedure would be established.

REVIEW OF THE LITERATURE

In 1905 Carrel and Guthrie (4, 6) reported the extirpation of an abdominal kidney from a dog and the subsequent transplantation of this organ into the animal's neck by anastomosing the renal vein to the external jugular and the renal artery to the carotid. This kidney functioned at least three days.

The urinary constituents were reported to be essentially normal, but the volume of urine secreted by the transplant was five times as great as that of the normal abdominal kidney.

Again in 1906, Carrel and Guthrie (5) reported a homotransplantation of both kidneys of a dog into the abdomen of a bitch by anastomosing the renal vein to the vena cava and the renal artery to the aorta.

During the time reported (eight days), the condition of the bitch was absolutely normal, as was the urine collected. No subsequent report could be found on this case. In 1906, Carrel and Guthrie (5) reported fourteen cases of transplantation of the kidney by vascular anastomosis, all with excellent results.

No information is given as to the ultimate fate of the animals, nor is there any histological discussion of the transplant beyond mention that gangrene was never observed in any case. In 1908, Carrel, as quoted by Simonsen and Sorensen (15), obtained his best results with massive homotransplantation in cats. The longest survival was thirty-six days. No histological examination is recorded. In three other cases, however,

histological examination revealed acute and subacute interstitial nephritis with plasma cell infiltration. No pathological changes were found in the parenchyma.

The work of Avramovici in 1924, as cited by Simonsen and Sorensen (15), records a kidney homotransplant which functioned for seventy-one days! Another case, in which the donor kidney was kept in an ice box for eight hours, functioned for forty days!

Williamson (16) in 1923 carried out a number of experiments on both autotransplantation and homotransplantation of kidneys in the dog. He believed that an autotransplant would function and maintain life for long periods of time. One of his dogs lived for nine months with a transplanted kidney in the cervical region as his only means of eliminating urinary waste products.

In homotransplanted kidneys Williamson found the average length of function to be four days, although in one case the organ functioned for ten days. It was his opinion that the failure of homotransplants was not due to mechanical factors.

Again, Williamson (17) in 1926 stated that "the location of the transplant does not materially lengthen the functional life or influence the terminal histologic picture", of the homotransplant. His work was done in order to ascertain whether transplantation into the abdominal cavity would increase the functional life of the transplant over that of the cervical region.

The histology of the homotransplants performed by Williamson

is of interest. He sectioned kidneys at varying times after transplantation and found that early sections showed oedema and hyaline material in the collecting tubules. Later slides showed early glomerular changes and lymphocytic infiltration, and little change in the tubular elements. Still later tubular degeneration occurred and lymphocytes either increased or remained constant in number. Polymorphonuclear cells began to appear. The last sections showed one of two changes, namely "a predominating glomerular type of injury or an overwhelming tubular injury".

Williamson concluded that the primary lesion found in these transplanted kidneys was a blood-borne agent and suggested that closer blood-typing of donor and recipient animal might be the solution to the problem.

Loeb (10) in 1930 pointed out that the nearer the relationship between the host and the transplant, the greater is the likelihood of the transplant functioning. In 1945, he pointed out that organismal differentials determine the fate and function of transplants. Thus autotransplants usually survive indefinitely, and homotransplants survive usually only for a few days. And heterotransplants (the transplantation of tissue or an organ from one animal into an animal of a different species) usually fail to function.

Markowitz (12) in 1949 suggested that homotransplantation of organs might be as feasible in humans as it is in dogs. He indicated that such an operation might be life-saving in cases of complete

temporary anuria, as in that following mercuric chloride poisoning. He also suggested that donor and recipient be of compatible blood groupings and that investigation be carried out on the possibility of using kidneys obtained at autopsy for homotransplantation. Markowitz further pointed out the advisability of (a) keeping kidneys refrigerated aseptically for varying periods of time in order to determine how long these organs remain viable, and (b) removing both kidneys from a dog, and, three days later when the animal is at the point of death, transplanting a kidney from another dog into its neck.

Blumenthal (2) in 1941 cited a series of experiments whereby, among other methods, the organismal differential of clotted rabbit plasma was destroyed by heating. The temperatures which he claimed to be most effective, ranged between 54°C. and 56°C. He stated further that "the influence of an initial homotransplant on a subsequent one is specific", and that freezing does not destroy the organismal differential.

Markowitz (12) in 1949 pointed out that the more closely related the donor and the host the greater is the success of homotransplantation. In accordance with this idea he suggested that in humans homotransplantation might be more successful in closely related blood groups.

Dukes (7) in 1947 quoted Olson as reporting that "in many cases, owing to the weakness of the natural isohaemagglutinins in dogs, the transfusion of incompatible blood will produce no symptoms in this

species". Olson did, however, propose a division of dogs into blood groups.

Lawler, West, McNulty, Clancy and Murphy (8) in 1950 reported the homotransplantation of a kidney in a human after ascertaining that donor and recipient were of identical blood types. This paper withheld comment on the success or failure of the operation until a later date. A later report by Lawler et al (9) in 1951 states that on subsequent examination the kidney tissue was alive but apparently not producing urine.

Simonsen and Sorensen (15) in 1949 reported on homotransplantation of the kidney in the cervical region in four dogs. "The transplanted kidneys functioned respectively for eleven, four, and four days. One kidney functioned for only a few hours. The removed kidneys showed necrosis of the tissue and thrombosis of the vessels. Histologically there was no particular infiltration with plasma cells or histiocytes".

These workers state further that in the two dogs treated with penicillin there was no evidence of infection. Of the two cases which did not receive penicillin one developed pyelonephritis and the other a subcapsular infection. Sticky occlusion of the ostium of the ureter they consider to be the greatest technical difficulty in their experiments.

Parkinson and Woodworth (13) in 1947 reported upon a series of homotransplants of kidneys in goats. Their site (the cervical region) and vessels used were identical with those which have been

previously mentioned by other workers using the dog. The method of vascular anastomosis differed, however, in that instead of suturing the vessels they used a vitallium tube to complete the anastomosis. They reported that these homotransplants ceased to secrete urine on about the tenth day. These workers also stored kidneys in a deep freeze unit for one week before autotransplantation at a temperature of minus 40°F. It was reported that one such kidney was still actively excreting clear urine thirty days post-operatively. Nothing was said about the composition of the urine.

The conclusions of Parkinson and Woodworth on this work were as follows:-

1. Vessel grafts of any type, whether fresh or frozen, survive a sufficient length of time to allow development of an adequate collateral circulation.
2. Autogenous renal transplants survive if the anastomosis does not break down, develop a collateral circulation, and survive indefinitely.
3. Homogenous renal transplants do not survive.
4. Homogenous vessels are less subject to lysis than homogenous parenchymal tissue.
5. Vessels supplying a homogenous kidney are lysed more quickly than a free homogenous vessel graft.

In 1949, Woodruff and Woodruff (18) pointed out that most workers in this field assume that the lymphocytes which accumulate in a homotransplant are responsible for its destruction. They felt that while this may be true, it may also be that the lymphocytes are merely a result of the necrotic graft or that the lymphocytes are not phagocytic

but rather the source of antibodies which cause the destruction of the graft.

### SURGICAL TECHNIQUE

Whether the kidney was an autotransplant or a homotransplant made no difference to the surgical technique. The technique used in all cases was that described by Markowitz (12) in his text book Experimental Surgery.

#### Size of Animal

Large dogs, approximately twenty kilograms in weight, were found to be preferable from a surgical viewpoint, because the vessels of the recipient and donor were large enough to facilitate anastomosis.

#### Preparation for Donor Animal

When an animal was to be prepared for a nephrectomy the hair on the abdomen was cut with electric clippers, preferably one day prior to surgery. This was followed by scrubbing the abdomen with water and tincture of green soap. The abdomen was dried with a towel and then swabbed with seventy per cent alcohol. This was followed by an application of two and one-half per cent tincture of iodine. This procedure was repeated immediately before operation, after the animal had been anaesthetized and placed upon the operating table.

#### Anaesthesia

Pentobarbital sodium was the anaesthetic of choice. It was administered to each animal intravenously, using the cephalic vein.

The dosage was approximately 65 mgm. per kilogram of body weight, although this rule was not strictly adhered to, preference being given to the obvious effect the anaesthetic had upon the individual animal rather than upon any calculated dose.

Occasionally ether was used as an anaesthetic. This method required more constant supervision. When ether was used, a plastic Magill tube was inserted into the trachea and the ether-oxygen preparation delivered in this manner rather than through the customary mask.

#### Draping of Animal

Four towels were placed on the animal in such a manner as to leave a rectangular space measuring about three by twelve inches over the abdomen. These were fastened in place with Backhaus towel clamps. A laparotomy sheet with a mid-line slit was then draped over the whole animal. After the skin incision had been made, extending from one inch below the xiphoid process to the pubic region, skin towels were approximated to the skin edges with Backhaus towel clamps.

#### Nephrectomy

An abdominal incision was made, since both kidneys of the dog are readily accessible if this site is used. The cut was made in the mid-line through the linea alba, and extended from just below the xiphoid process to the pubic region.

Balfour self-retaining retractors were used to spread the

incision since this instrument extends to a width of about six inches.

The left kidney was usually chosen for removal because exposure of this organ is easier.

The peritoneal capsule was stripped from the kidney by tearing the peritoneum with a pair of forceps. After this initial break had been made it was possible to peel the peritoneum off the kidney using the gloved fingers. The perirenal and perivascular fat was then stripped away using a gauze swab. Bleeding points were negligible and could always be controlled by slight pressure with a gauze swab. The ureter was isolated and crushed with haemostatic forceps about three inches from the hilus of the kidney. It was then severed proximal to the haemostat with a sharp razor blade held carefully between the fingers. No ligature was found necessary because the peristalsis of the uretral stump and the valve of the bladder prevent regurgitation of urine.

One-half cubic centimetre of heparin solution (1:1000) was now injected, with the aid of a hypodermic syringe, into the renal artery. This was done to prevent the blood from clotting within the kidney or within the vessels. Following the injection, the needle was quickly withdrawn and two Criles haemostatic forceps placed upon the kidney pedicle, leaving as much of the renal artery and vein as possible.

The vessels were severed proximal to the top forcep using a sharp razor blade. The blood was permitted to flow from the kidney.

A new and very sharp razor blade was found to be ideal for cutting vessels because the ends were left clean with no jagged edges and

thus were more suitable for suturing.

The kidney pedicle was ligated using number two chromic catgut.

The abdominal incision was closed in three layers:

1. internal fascia and peritoneum with a simple continuous suture using number two chromic catgut.
2. external fascia with a simple continuous suture using number two chromic catgut.
3. skin with a vertical mattress suture using black silk.

#### Preparation of Donor Kidney

The vessels of the extirpated kidney were prepared by carefully stripping adventitia from the cut ends. This was accomplished by grasping the ends of each vessel with a pair of cilia forceps and gently pulling the adventitia beyond the cut end of the vessel, then cutting it away with a fine pair of scissors (see Fig. 2, number 2). It was found that this procedure was necessary because adventitia in the suture line interfered with a successful anastomosis and caused leakage at the point of union of the two vessels.

#### Preparation of Recipient Animal

The neck of the recipient animal was prepared by cutting the hair with electric clippers. The skin was washed with tincture of green soap and water, dried, then swabbed with seventy per cent alcohol. This was followed by an application of two and one-half per cent tincture of iodine. This procedure was repeated upon the day of operation

after the animal had been anaesthetized and placed upon the operating table.

#### Anaesthesia

Pentobarbital sodium was the anaesthetic used as it did not require any cumbersome apparatus about or near the head and neck area during the operation as when ether was used.

#### Draping of Neck

Four towels were placed about the prepared area on the animal's neck leaving an area of six by two inches of skin exposed. These towels were covered by a laparotomy sheet with a mid-line slit in such a manner as to drape the entire animal. When the skin incision, extending from below the larynx to the suprasternal notch, had been made, skin towels were used to cover the cut edges of the skin.

#### Preparation of Vessels in the Recipient Animal

The vessels on either the right or left side of the neck were used.

The external jugular vein, lying subcutaneously, was stripped of subcutaneous fascia and fat for a distance of two or three inches. (see Fig. 2, numbers 1 and 2).

Scissors were used to separate the sternocleidomastoid. The carotid artery lies along the trachea with the vagus nerve closely adherent. The vagus was stripped from the carotid carefully in order to

avoid injury.

Ligatures of number two chromic catgut were then placed at the cephalic end of the artery and vein respectively. The blood was "milked" down about two inches in each vessel and serrefine clamps applied to the distal ends of these vessels. Using a sharp razor blade, held between the fingers, each vessel was then severed close to the ligature. After the vessels had been cut, a few drops of sterile mineral oil were dropped into the cut ends to prevent the formation of a clot.

The adventitia was stripped from the cut ends by pulling it beyond the ends of the vessels and cutting it off with sharp scissors. As the adventitia contains a portion of the vasa vasorum it was considered unwise to strip it from the vessel for a distance much greater than the site of anastomosis.

Vascular Anastomosis

MATERIALS USED

For the abdominal operation of nephrectomy and to expose the vessels in the neck the usual surgical instruments are needed. The vascular anastomosis requires some special instruments. It has been found that eye instruments are of a size suitable for this delicate surgery. The kit used in these experiments consisted of the following:

- 1 pair iris scissors
- 3 Halstead mosquito forceps
- 1 cilia forcep
- 2 iris forceps
- 6 5-0 black silk eighteen inches in length with straight three-eighths inch swaged-on needle (each operation)
- 1 vial mineral oil
- 1 razor blade
- 1 Pasteur pipette with rubber bulb
- 2 serrefines
- 1 two-inch length of small diameter polyethylene tubing for use as ureteral catheter.

See Fig. 1.

The renal artery was sutured to the carotid artery and the renal vein to the external jugular vein (see Fig. 2 and 3, numbers 1 to 9). It was preferred to perform the venous anastomosis first, then to suture the arteries as the last step.

The cut ends of the vessels are apposed by three guy sutures (see Fig. 2, number 2). These were placed from without the vessel to the inside, and from within the opposing vessel to the outside, and tied with three knots. The suture material used was eighteen inches of five-0 black silk or nylon with a straight swaged-on three-eighths inch needle. The three guy sutures were held by the assistant in such a way as to form an equilateral triangle (see Fig. 3). Two of the guy sutures were held by the assistant, and the third was weighted with a mosquito forcep (see Fig. 2, number 3, and Fig. 4).

The vessels were then sutured in the following manner: Using the needle on the guy suture closest to him the surgeon sewed the vessels with over-and-over sutures about one millimetre apart, to the upper guy suture held by the assistant. When this point was reached a tie was made with the upper guy suture. The vessel was now rotated away from the surgeon and the second side of the triangle sewn. When this had been accomplished the whole vessel was rotated back toward the surgeon through an arc of two hundred and forty degrees so that the third side could be sutured. The guy sutures were cut and the vessel allowed to roll back to its original position (see Fig. 3, numbers 5 to 9). The cut guy sutures were placed nearby where they could serve as emergency sutures later if needed.

The same procedure was carried out for the anastomosis of the two arteries.

When suturing was completed, the serrefine on the vein was released before releasing the serrefine on the artery (see Fig. 5).

If the anastomosis was successful no bleeding would be evident. Sometimes, however, it was necessary to insert one, or at the most two, emergency sutures to control a point of haemorrhage (see Fig. 6).

#### Anchoring the Kidney

The kidney was held in place by undercutting the skin on either side of the neck in order to make a "pocket" in which it could lie (see Fig. 7). This proved to be sufficient anchorage for the vessel. No attempt was made to suture it in place as no practical area in the kidney could be found which would serve to hold suture material. The organ becomes adherent very quickly.

The ureteral stump was exteriorized through a stab incision in the skin, (see Fig. 7). The point for this incision was decided upon by the position of the kidney. It was felt that the ureter hanging straight down would permit easier catheterization, since the catheter could be pushed in a straight line to the pelvis of the kidney without encountering curves or kinks.

It was found that in autotransplants catheterization was necessary for the first few weeks. After this time the ureteral stump would heal permitting the continuous drip of urine without the necessity of a catheter. Homotransplants required catheterization during the entire functioning period.

At first ordinary ureteral catheters were used but it soon became apparent that they became too quickly plugged with urinary sediment. Polyethylene tubing of suitable diameter was substituted and this material permitted a maximum flow of urine with a minimum tendency to obstruction.

EXPERIMENTAL PROCEDURE

Immediate Autotransplants

These cases were used as controls. After nephrectomy, either unilateral or bilateral, the blood vessel anastomosis was completed as quickly as possible. The time in which the kidney was without a blood supply varied between twenty minutes and one hour. The abdominal incision was not closed until after the vascular surgery had been completed. Other than keeping the kidney wrapped in a cotton swab which had been moistened with warm saline, no special precautions were taken nor special procedure carried out.

Williamson (17) in 1926 carried out this type of experiment and found that his cases functioned normally for long periods of time. He reported that hydronephrosis and infection eventually appeared.

The cases reported here all received penicillin as a daily routine. One hundred to two hundred thousand units of crystalline penicillin were sprayed over the site of the vascular anastomosis at the completion of the operation. Three hundred thousand units were injected intramuscularly at the same time. This postoperative procedure was carried out daily in these cases for at least one week and intermittently for varying periods after that time.

These kidneys were not removed from the neck of the animal until they ceased to function or were removed arbitrarily after varying periods of function.

The histology of these kidneys was essentially normal. There was no evidence of hydronephrosis, and although there were small areas of degeneration no gross lesions developed.

Infection was never a problem, and none of the kidneys became grossly infected. However, in some the number of polymorphonuclear cells indicated infection by some pyogenic organism.

CASE NO. I (Immediate Autotransplant)

This was a one-year-old, male, cross-bred collie in excellent physical condition weighing fourteen kilograms at the time of transplantation.

On February 12, 1951, one kidney was removed from the abdominal cavity and immediately transplanted into the cervical region under pentobarbital sodium anaesthesia. The operation lasted for two hours. Aqueous suspension of penicillin G was given in a daily dose of 300,000 international units postoperatively, as a prophylactic measure.

Records of the urinary output of the transplant were kept on this animal as shown in Table I.

TABLE I

DATE	COLOUR	REACTION	SUGAR	ALBUMIN	BLOOD
February 14	pale yellow and slightly cloudy	alkaline	faint trace	++	++
" 15	" " " " "	-	-	+	+
March 14	" " " " "	alkaline	-	trace	++
" 15	" " " " "	alkaline	-	trace	++
" 16	" " " " "	alkaline	-	trace	++
" 17	" " " " "	neutral	-	+	trace
" 18	" " " " "	neutral	-	+	trace
" 19	pale yellow and clear	alkaline	-	trace	trace
" 21	" " " "	acid	-	trace	trace
" 22	pale amber and slightly cloudy	acid	-	-	trace
" 23	" " " " "	acid	-	-	trace
" 24	" " " clear	acid	-	-	-
" 26	" " " "	alkaline	-	trace	-
May and June	light				
July 9				++	trace

On July 9, 1951, the transplanted kidney was still functioning and excreting clear light-coloured urine at a fairly rapid rate. It was decided to remove it for histopathological examination. The kidney was found to have developed a tremendous collateral circulation through its corticular surface. The anastomoses of both artery and vein were good.

This kidney functioned continuously for 148 days after transplantation, during which time the animal showed no ill effects and excreted normal urine by means of its intact kidney (see Fig. 8). The animal's temperature remained normal throughout the entire period.

The histopathology revealed that most of the kidney was essentially normal (see Fig. 9). However, there was one pole in which degenerative changes were advanced.

CASE NO. 2 (Immediate Autotransplant)

On February 12, 1951, one kidney had been removed from the abdominal cavity of this animal, a seven-month-old brown and white mongrel collie. The organ was transplanted immediately into the cervical region. Prophylactic doses of 300,000 international units of penicillin were administered daily postoperatively. Urine secretion seemed normal at first but no suitable apparatus for collection was found. Urine was collected with a great deal of difficulty on March 14. This difficulty was due to a stricture of the ureter at the point where it traversed the skin. Urinalysis of this sample was as follows:

Colour-----pale yellow and slightly cloudy

Reaction-----neutral

Albumin-----positive

Sugar-----positive

Blood-----trace

On March 15, after functioning for a period of thirty-one days, the transplanted kidney was removed for histopathological examination, which was as follows: This section had the appearance of so-called chronic interstitial nephritis in dogs (see Fig. 10). The glomeruli were normal but the tubules were small and reduced in number. The tubules were crowded by an increase in the interstitial fibrous tissue and mononuclear infiltration. The capsule was markedly thickened and apparently fused with the muscle of the host. The kidney was in remarkably good condition.

CASE NO. 3 (Immediate Autotransplant)

On March 7, immediate autotransplantation of a kidney was performed on a black Cocker spaniel. The kidney functioned until March 11 when it became swollen and ceased to excrete urine. From the time of operation until the kidney function stopped, the animal was given 300,000 international units of penicillin daily.

The results of the urinalysis performed on March 8, 9, and 10 are shown in Table II.

TABLE II

DATE	REACTION	SUGAR	ALBUMEN	BLOOD	ACETONE
March 8	Alkaline	Slight trace	+++	+++	-
March 9	Alkaline	-	Trace	Trace	-
March 10	Alkaline	Trace	++	++	-

The transplanted kidney was examined microscopically. A heavy infiltration with neutrophiles and numerous abscesses or young fibrous tissue replaced the kidney tissue in certain areas. The tubules and glomeruli were intact and apparently functioning (see Fig. 11). Albuminous casts were evident however and the interstitium was infiltrated with mononuclear cells. One small vein was thrombosed. The medulla showed severe haemorrhage and oedema with heavy neutrophile infiltration and abscess formation.

The number of neutrophiles in the sections suggested infection due to a pyogenic organism. Infection was probably the agent responsible for the increase in albumen and blood in the urine sample taken on March 10.

CASE NO. 5 (Immediate Autotransplant)

This dog was a three-year-old, male, cross-bred terrier in good physical condition and weighed twenty kilograms at the time of

operation, May 23. The animal was normal in every respect and the rectal temperature was 102°F. prior to transplantation. No preoperative medication was given.

The animal was anaesthetized with pentobarbital sodium intravenously. Both kidneys were removed and one immediately transplanted into the cervical region. The technique was as previously described. The operation required two hours. After anastomosis of the vessels, the kidney functioned immediately, producing a dark urine which persisted for twenty-four hours. Postoperative medication was limited to intramuscular injections of 300,000 units of aqueous suspension of penicillin daily for the first twenty-three days.

Urinalysis and blood urea tests were carried out intermittently as shown in the table below.

Microscopically the excreted urine seemed quite normal throughout the duration of the experiment with the exception of the sample taken on June 1 which contained a small amount of blood.

The rate of excretion from the transplanted kidney remained quite constant during the entire experiment and was determined to be 25 cc. per hour on June 15.

The animal remained quite active and normal clinically (see Fig. 13) only having an increased rectal temperature of 103°F. on June 1. This subsided by June 2. On June 17 the rectal temperature was 104.5°F. whereupon  $\frac{1}{2}$  gm. dihydro-streptomycin was given in addition to the daily dose of penicillin. On June 18 the temperature

had fallen to 102°F. and penicillin medication was withdrawn with daily doses of 0.5 gm. streptomycin replacing penicillin until June 22, when the animal's temperature was 101.5°F. Antibiotic medication was completely withdrawn from June 23. The animal remained normal in appearance and attitude and the urine flow and microscopic appearance of the urine remained unchanged until July 18 when the animal was sacrificed so that histopathological examination of the kidney could be carried out.

TABLE III

DATE	URINE ALBUMIN	BLOOD UREA	MICROSCOPIC EXAMINATION OF URINE
May 25	+		Few red blood cells. Occasional casts.
June 1	+ -		Numerous pus and red blood cells present.
" 2	trace		Numerous pus cells. Few red cells.
" 3	"		Red cells and pus cells present.
" 4	negative		Numerous pus cells. Few red cells. No casts or epithelial cells.
" 6	negative	97.5 mg.	Pus cells. Few epithelial cells.
" 7	negative	157.6 mg.	Few pus cells.
" 11		120.0 mg.	
" 12	+ -	117-120 mg.	Few pus cells and red cells. Calcium oxalate crystals present.
" 13	+ -		"
" 14	negative		Very few red cells and pus cells.
July 1 and 2	negative		Very few red cells and pus cells.

On June 16 a dye excretion test, using 1 cc. phenol-sulfonphthalein injected intravenously, was carried out. Over the one-hour period following injection the kidney excreted three per cent of the dye. This, in addition to the blood urea records of the previous week, showed that the kidney was not excreting all the waste products of the animal's body.

A leucocyte count on July 14 showed the following:

Total leucocytes -----	5800
Neutrophiles (segmented) -----	74
Neutrophiles (band) -----	4
Lymphocytes -----	12
Eosinophiles -----	10

From this count it may be assumed that there was no marked infection but that there was some foreign body reaction, as indicated by the high eosinophile count.

Histopathology of the transplanted kidney revealed degeneration and some necrosis. Some areas showed inflammatory cells usually associated with inflammation of a chronic nature. Some tubules contained casts but generally speaking kidney damage was quite mild (see Fig. 12).

This case of immediate autotransplantation of a kidney after bilateral nephrectomy functioned for a total of fifty-six days. All during this period, the animal was apparently in good health even though the blood urea level rose to 157.6 mg. on the fifteenth day after the operation. Subsequently, the blood urea started on a slow downward trend, the ultimate level of which was not ascertained as the

animal was sacrificed.

CASE NO. 10. (Immediate Autotransplant)

On June 21, 1951, bilateral nephrectomy was performed on an eighteen kilogram, one and one-half year old cross-bred collie bitch in good physical condition. One kidney was immediately transplanted into the cervical region. The operation required two hours and was performed under pentobarbital sodium anaesthesia. Preoperative medication consisted of 600,000 units of penicillin in aqueous suspension given intramuscularly.

The kidney produced clear fluid urine immediately after the anastomosis was completed. The urine produced on June 22 was very bloody and not suitable for urinalysis. The blood urea level on that day was 77.0 mg. One hundred thousand units of crystalline penicillin in aqueous solution were used to irrigate the ureter and the dose of 600,000 units aqueous penicillin suspension repeated. The animal was haemorrhaging through the incision on its neck and a piece of the ureter had become necrotic and had to be removed. In the afternoon the haemorrhage from the incision was so severe that euthanasia was performed.

The histopathological picture of the kidney was that of severe congestion, haemorrhage and nephrosis (see Figs. 14 and 15). Apparently the amount of blood forced into the kidney from the carotid artery was sufficient to rupture the renal capsule, as was evident on post-mortem examination.

CASE NO. 11 (Immediate Autotransplant)

Bilateral nephrectomy was performed on a mongrel Fox-hound three years old, weighing fourteen kilograms. Pentobarbital sodium was the anaesthetic used. The animal received 600,000 units of penicillin preoperatively. During the nephrectomy the renal vessels inadvertently slipped from between the jaws of the clamp. The vessels were soon ligated but not before the animal had lost about 150 cc. of blood.

One kidney was immediately transplanted into the cervical region. The anastomosis and surface of the kidney were bathed with 200,000 units of crystalline penicillin in aqueous solution. The kidney never functioned and the animal died three hours after the operation was completed in spite of the fact that it was given supportive treatment in the form of 125 cc. "Aminosol" (amino acids and dextrose five per cent) and 1 cc. "Metrazol" (pentomethylentetrazol).

Histopathological examination of the kidney was unavoidably delayed. Histological sections showed some degeneration and necrosis. It was felt that the pathology observed in this

kidney was entirely due to post-mortem change (see Fig. 16).

Refrigerated Autotransplants

In these cases nephrectomy, unilateral or bilateral, was performed twenty-four hours prior to transplantation. Aqueous penicillin (300,000 units) was administered parenterally at the conclusion of the operation. The operative procedure was as previously described.

Kidneys to be stored were handled by one of two methods: (1) In sterile physiological saline, or (2) Without being immersed in any fluid.

Prior to extirpation of the kidney two jars were autoclaved -- one large and one small screw-capped jar, one inside the other. Some of the inner jars contained sterile physiological saline; others were empty.

Immediately after extirpation the kidney was placed in the inner jar which was then sealed with the screw-cap. This in turn was put inside the large jar which was sealed with a screw-cap. Then the whole was immediately placed in an ordinary electric refrigerator. The average temperature for this refrigerator was found to be approximately 4°C.

Twenty-four hours later the kidney was removed from the refrigerator and from its container and transplanted into the neck of the animal from which it had been removed.

The site of operation was sprayed with 100 or 200,000 units of crystalline penicillin. Aqueous penicillin was administered intramuscularly in dosage of 300,000 units postoperatively. This

dosage was continued daily until the kidney stopped functioning.

Function in the refrigerated autotransplant was poor in comparison to the immediate autotransplant. Urine was excreted slowly in small amounts, and was usually blood-tinged. These kidneys functioned for varying periods of time up to fourteen days. In no case was the kidney removed from the neck of the animal until it had ceased to function.

On histopathological examination, these kidneys showed almost complete destruction of the parenchyma. Coagulation necrosis was present. The kidney tubules were completely or almost completely destroyed. Nephrosis was evident; however, the glomeruli seemingly were least and lastly affected. The cellular infiltration was about equally divided between lymphocytes and polymorphonuclear cells.

This series of experiments was carried out to determine if the kidney would remain viable after refrigerator storage for twenty-four hours.

CASE NO. 13 (Refrigerated Autotransplant)

Unilateral nephrectomy was performed on a two-year-old, male, cross-bred Cocker spaniel on July 13, 1951. The animal was in good condition and weighed nineteen kilograms at the time of operation. The kidney was stored in double screw-capped glass jars. The inner jar contained the kidney immersed in physiological saline and the whole was placed in an electric refrigerator at 4°C. for twenty-four hours. The kidney was transplanted into the cervical

region of the same animal on July 14, under pentobarbital sodium anaesthesia. In removal of the kidney from the abdominal cavity the renal artery was severed at its bifurcation. The larger branch was anastomosed to the carotid artery and the lesser branch left unligated as it did not bleed. The operation lasted one hour and forty-five minutes.

The animal received no preoperative medication but the kidney and anastomosis were bathed with 200,000 units of crystalline penicillin in aqueous solution. Postoperative medication consisted of 1 cc. metrazol immediately after the operation and 300,000 units of aqueous penicillin suspension administered intramuscularly each day commencing on July 14. Aqueous solution of 100,000 units of crystalline penicillin, 1 cc., was used to irrigate the ureter several times daily on and after July 16.

Urine excretion started immediately after completion of the anastomosis. The urine produced was clear and fluid. Blood was passed with the urine from July 15 to July 23. The rate of urine excretion was approximately one drop per minute from the time of transplantation until July 24, the fourth day after removal of the animal's second kidney, when the output per minute increased to approximately 0.8 cc. per minute.

On July 20, the animal's second kidney was removed and the area around the transplant was irrigated with 500,000 units of crystalline penicillin in aqueous solution.

The urine flow remained slow until July 24 when it increased considerably. On July 24 the animal commenced vomiting. The urine was clear and the rate of excretion increased.

The animal maintained a normal body temperature throughout the duration of the experiment until death on July 26 at 10:20 a.m.

Urinalysis and blood urea determinations were carried out at intervals as shown in table IV. On two occasions the blood urea level was determined by the Lamotte apparatus using the method of P. S. Hench and M. Aldrich of the Mayo Clinic. This method entails titration of a protein-free blood filtrate against a standard solution of mercuric chloride. The Hench-Aldrich method seemingly gave a somewhat higher value than the Van Slyke-Cullen method by which other blood urea determinations were made.

DATE	BLOOD UREA	ALBUMIN	MICROSCOPIC	MEDICATION TO KIDNEY
July 17	20 mg.	++++	Numerous red cells and pus cells	Irrigated with 100,000 units of aqueous penicillin solution
" 18	25 mg.	++++	Many red cells. 8-10 pus cells per field	Irrigated twice with 100,000 units aqueous penicillin solution
" 20	20 mg.	++++	Many red cells and pus cells	Irrigated with 100,000 units aqueous penicillin solution
" 22	(Lamotte) 230 mg.			" " "
" 23	113 mg.	++++	Numerous red cells	Irrigated 5 times with 100,000 units aqueous penicillin solution
" 24	(Lamotte) 290 mg.	++++		Irrigated with 100,000 units aqueous penicillin solution
" 25	170 mg.	++++	Many red cells	

Histopathological examination of the transplanted kidney revealed nephrosis and fibrosis (see Fig. 17).

This transplant functioned for a period of twelve days. At first the urine excreted was mixed with blood but on the tenth day the urine became clear and the rate of excretion increased. The animal lived for five and one-half days after removal of its second kidney. At no time during the experiment did the dog have an abnormally high rectal temperature.

CASE No. 14 (Refrigerated Autotransplant)

The experimental subject was a one-year-old, cross-bred, spayed female collie in fair condition. One kidney was removed from the abdominal cavity under pentobarbital sodium anaesthesia on August 9, 1951. The dog received 300,000 units of aqueous penicillin suspension. The kidney was placed in a dry screw-capped jar and sealed. This jar was placed in a second dry jar which was also sealed with a screw-cap and the whole stored in an electric refrigerator at 4°C. for twenty-four hours. The animal was again anaesthetized with pentobarbital sodium and the kidney transplanted on August 10, 1951. No crystalline penicillin was applied to the anastomosis or the subcutaneous "pocket". Postoperative doses of 300,000 units of aqueous suspension of penicillin were given daily.

The kidney produced a clear viscous urine five minutes after completion of the anastomosis. On August 11, the rate of excretion from the transplanted kidney was approximately 1 cc. every

ten minutes and the urine had become blood tinged. On August 12, the rate of excretion became extremely slow and the ureter showed signs of necrosis. The ureter was irrigated twice with 100,000 units of crystalline penicillin in 1 cc. of sterile water. Also, 200,000 units of crystalline penicillin in sterile water were introduced around the transplant. The rate of excretion did not increase on August 13 and the urine remained quite bloody. The ureter was irrigated once with 100,000 units of crystalline penicillin solution and once with 100,000 units of crystalline penicillin and 1/8 gm. dihydro-streptomycin mixture in aqueous solution. At that time it was evident that the ureter was necrotic and there was the possibility that it was detached from the kidney. On August 15, the ureter was irrigated twice with 100,000 units of crystalline penicillin and 1/8 gm. dihydro-streptomycin in solution. This procedure was repeated once on August 15. The ureter broke at skin level on August 15 and the kidney ceased to function. On August 17 the animal was destroyed and the kidney removed. Thrombosis of the artery and vein had occurred, although the anastomosis was intact.

Histological section of the kidney showed advanced coagulation necrosis with areas of inflammatory cell infiltration and calcification (see Fig. 18). Complete occlusion of the renal vessels apparently occurred on the fifth day after transplantation.

CASE NO. 15 (Refrigerated Autotransplant)

Unilateral nephrectomy was performed on a male Collie, one and one-half years of age, on August 14, 1951. The animal was in perfect health and weighed twenty kilograms at the time of the operation. The dog was given 300,000 units of aqueous penicillin suspension intramuscularly immediately after nephrectomy. The kidney was placed in double screw-capped jars -- the inner jar contained the kidney immersed in normal saline -- and stored in an electric refrigerator at 4°C. for twenty-four hours.

On August 15, the animal again received 300,000 units of penicillin intramuscularly and the kidney was transplanted by vascular anastomosis into the cervical region. The anastomosis was satisfactory after the application of two additional emergency sutures. The anaesthetic used was pentobarbital sodium. The operation lasted one hour and twenty minutes. Postoperative medication consisted of 300,000 units of aqueous penicillin suspension intramuscularly and irrigation of the ureter of the transplant with 100,000 units of crystalline penicillin in aqueous solution twice daily.

Urine was produced by the transplanted organ within five minutes of completion of the anastomosis. The urine was at first clear and viscid but became blood-tinged on the twenty-second hour of function. On August 15, the animal's temperature was 103°F.

Dihydro-streptomycin in  $1/8$  gm. quantities was used along with crystalline penicillin to irrigate the ureter on August 15. The animal's rectal temperature was normal on August 16 and remained within the normal limits, while the urine became bloody and was excreted at a slower rate. On August 19 the urine contained less blood. On August 20, the urine did not contain any red cells or haemoglobin on microscopic observation.

The animal's second kidney was removed on August 20 under pentobarbital sodium anaesthesia. On August 21 the rate of excretion from the transplant increased from an almost negligible amount to approximately 12 cc. per hour. Urinalysis and blood urea estimations were carried out from August 21 to August 24 with results as shown in Table No. V. The rate of excretion of urine increased on August 22 to approximately 20 cc. per hour and remained unchanged until the death of the animal on the night of August 24. The animal commenced vomiting on the afternoon of August 23. The frequency of vomition increased until death.

TABLE V

## BLOOD AND URINE TESTS

DATE	BLOOD UREA	ALBUMIN	SUGAR	MICROSCOPIC FEATURES
August 20	Second kidney removed			
" 21	45 mgs.	Trace	Negative	Numerous red cells -- some pus cells. Many granular casts. Many red blood cells. Rare cast.
" 22	90 mgs.	+		
" 23	135 mgs.	+		
" 24	171.6 mgs.	+	Negative	Few red blood cells. Bacteria present.

Histopathological examination of the kidney revealed small areas in which neutrophiles had replaced the tubules. There was a generalized increase in fibrous tissue and reduction in kidney parenchyma. In many tubules calcium casts had replaced the epithelium and occluded the lumen. In other areas massive neutrophile infiltration had occurred associated with necrosis of tubular epithelium. The glomeruli were very well preserved in nearly all areas (see Fig. 19).

In this instance of a refrigerated autotransplanted kidney the organ functioned for ten days before death occurred. The urine became bloody on the first day, changing to clear on the sixth day. Total nephrectomy was performed on the sixth day after transplantation and the animal died as a result of renal insufficiency on the tenth day.

CASE NO. 16 (Refrigerated Autotransplant)

Unilateral nephrectomy was performed on a cross-bred fox-hound on August 16, 1951. The animal was a one-year-old male which weighed twenty kilograms and was in good physical condition on the date of operation. The anaesthetic was pentobarbital sodium. After nephrectomy, 300,000 units of aqueous suspension of penicillin were given intramuscularly. The kidney was immersed in normal saline in double screw-capped jars and stored at 4°C. in an electric refrigerator for twenty-four hours.

Transplantation was performed by vascular anastomosis on August 17. The renal vein was very thin and one side of the triangle of the venous anastomosis had to be resutured before the anastomosis was considered satisfactory. No crystalline penicillin was applied to the anastomosis or subcutaneous "pocket". After the operation, 300,000 units of aqueous penicillin were administered daily.

Urine flowed from the ureteral catheter within five minutes of completion of the anastomosis. This urine however was mucoid in nature though free of blood. The urine remained clear throughout the following day, August 18. However, the ureter and pelvis of the kidney were irrigated with 100,000 units of crystalline penicillin. The rate of excretion was quite rapid. Urinalysis showed numerous red cells, a few pus cells and four plus albumin. On August 19 the rate of excretion from the transplant became very slow and the urine contained blood cells on microscopic examination. The ureter was again irrigated with 100,000 units of crystalline penicillin. The ureter was observed to be necrotic on August 20 and there was marked swelling of the animal's neck and a blood clot in the ureter. Solutions forced into the ureter found their way into the subcutaneous tissues. There was no urinary excretion at this time. Penicillin irrigation of the ureter was carried out on August 21. On the same day the transplant was removed from the animal's neck and the vessels were found to be thrombosed.

Histopathological examination of the transplant showed massive necrosis and haemorrhage (see Fig. 20).

CASE NO. 17 (Refrigerated Autotransplant)

On August 23, 1951, a unilateral nephrectomy was performed on a two-year-old male, cross-bred Collie which weighed seventeen kilograms. The animal was in good physical condition with a rectal temperature of 101<sup>o</sup>F. on the day of operation. Pentobarbital sodium administered intravenously was the anaesthetic used. The kidney was immersed in sterile normal saline in the inner of double screw-capped jars, and placed in an electric refrigerator at 4<sup>o</sup>C. for twenty-four hours.

The animal was again anaesthetized with pentobarbital sodium on August 24 and the kidney transplanted into the cervical region by vascular anastomosis. The carotid artery was anastomosed to the larger branch of the renal artery. The smaller branch was ligated. The anastomosis required two emergency arterial stitches and venous sutures to control bleeding points. The operation lasted two hours. Crystalline penicillin in aqueous solution in a dose of 100,000 units was applied to the anastomosis and surface of the kidney. Urine was excreted ten minutes after completion of the anastomosis. This urine was clear and viscous. After nephrectomy, 600,000 units of aqueous penicillin suspension was given intramuscularly, and 300,000 units daily after transplantation.

On August 25, eighteen hours after transplantation, the urine excreted by the transplant was blood-tinged and the excretion rate was slow. One hundred thousand units of aqueous solution of crystalline penicillin were used to irrigate the ureter. Irrigation of the ureter and pelvis of the transplant was continued daily. A blood urea determination on August 25 showed a level of 17 mgs. and the animal's rectal temperature had increased to 102.8°F.

Urine from the transplant remained bloody and the rate of excretion was slow until August 30, when no urine was excreted. A blood level of 14.5 mgs. was shown on August 27. At that time the urine showed many red blood cells and an albumin concentration of four plus.

On August 29 a mucopurulent excretion was noticed being discharged from the incision. The animal's neck was very swollen. The animal was sacrificed and the kidney removed on August 30. Massive subcutaneous haemorrhage, and thrombosis of the vessels was evident. Histopathological examination revealed almost complete necrosis of the kidney tissue with haemorrhage and fibrosis of large areas. Only a few glomeruli remained. In some areas, kidney parenchyma of a somewhat degenerate nature was present. The capsule was represented by a thick layer of fibrin, fibrous tissue and infiltrating cells (see Fig. 21).

In this case the refrigerated autotransplanted kidney functioned for five days. Degeneration of this kidney was hastened by thrombosis of the vessels.

CASE NO. 18 (Refrigerated Autotransplant)

The experimental animal in this case was a one-year-old male terrier which weighed twelve kilograms. One kidney was removed and stored in normal saline contained in double screw-capped jars for two and a half hours during which the kidney had no blood supply, one hour was spent in an electric refrigerator at 4°C. The organ was then transplanted into the cervical region of the same animal on the afternoon of the day on which nephrectomy was performed, August 28, 1951. After anastomosis of the larger branch of the renal artery to the carotid artery the kidney was not as turgid as was expected. This was probably due to the fact that sufficient blood was not reaching the kidney. Four additional sutures were required in the arterial anastomosis and two required to control bleeding points in the vein. Moreover the ureter of the transplant was extremely delicate and possessed an inadequate blood supply. Photographs of the operation were taken, thus lengthening the duration of the operation to two and one-half hours. Anaesthesia was produced and maintained by use of pentobarbital sodium. Post-operatively the animal received 300,000 units of aqueous penicillin suspension intramuscularly and 200,000 units of crystalline penicillin solution on the anastomosis and kidney. The parenteral penicillin was repeated daily in 300,000 unit doses and the catheter and ureter irrigated with 100,000 units of crystalline penicillin daily.

Urine was passed from the kidney fifteen minutes after

completion of the anastomosis. The urine was at first clear though excreted slowly. However, within forty-eight hours it became bloody and caused plugging of the catheter. The rate of excretion remained slow and the urine contained blood every day until September 8, the day before function ceased.

On September 3, the cervical incision was gaping between two sutures and a bloody pus was squeezed out. The incision was irrigated with one half gram of dihydro-streptomycin and 200,000 units of crystalline penicillin. By September 5, the subcutaneous area around the transplant was quite distended with fluid. On aspiration, 15 cc. of a mucosanguinous fluid was withdrawn. The area was irrigated with 200,000 units of crystalline penicillin. At this time the exteriorized portion of the ureter was noted to be necrotic. On September 6, the suture holding the catheter into the ureter became detached, with the result that the catheter was removed. This was however, remedied by insertion of a longer catheter into the ureter.

On September 8 the exteriorized portion of the ureter was broken off by the animal. Again the subcutaneous area around the transplanted organ was aspirated and 23 cc. of a mucosanguinous fluid were obtained. Half a gram of dihydro-streptomycin and 200,000 units of crystalline penicillin were injected into the subcutaneous "pocket". On September 9, the kidney ceased to function.

This transplant functioned for eleven days and twenty hours. The animal's second kidney was not removed because of the apparent infection of the transplant thus rendering the possibility of survival

of the animal very slight. Urinalysis was not carried out because at no time did the kidney excrete at a rate fast enough to make collection possible.

Histological section of the kidney showed almost complete coagulation necrosis (see Fig. 22).

CASE NO. 19 (Refrigerated Autotransplant)

Bilateral nephrectomy was performed on September 6, 1951. The subject was a one-year-old female cross-bred Cocker spaniel which weighed twelve kilograms and was in good physical condition. The operation was performed under pentobarbital sodium anaesthesia. One kidney was immersed in normal saline in double screw-capped jars and placed in an electric refrigerator at 4°C for twenty-four hours. The kidney was transplanted by vascular anastomosis into the cervical region of the animal on September 7, 1951. Pentobarbital sodium was used to produce surgical anaesthesia for transplantation. Crystalline penicillin in a dose of 200,000 units was applied in solution to the anastomosis and the transplanted kidney.

Urine was excreted by the transplant twenty minutes after completion of the anastomosis. The urine was clear and viscous at first but became quite bloody on the following day and caused occlusion of the catheter. The ureter was irrigated twice on September 8 with 100,000 units of aqueous solution of crystalline

penicillin and the animal received 300,000 units of aqueous penicillin suspension intramuscularly. The animal commenced showing signs of uraemia on September 8 with vomition increasing in frequency on September 9. The urine excreted on September 9 contained less blood than it did on the previous day and the rate of excretion had increased considerably. However, the urine sample collected clotted within five minutes of collection. The animal received 300,000 units of penicillin parenterally and the ureter was irrigated with 100,000 units of crystalline penicillin. On September 10, the animal was found dead and the kidney removed for histopathological examination.

Histopathological examination revealed degeneration and necrosis of the tubular epithelium. The tubules were filled with hyaline-like material (see Fig. 23).

This kidney functioned for somewhat more than two days before the animal died from renal insufficiency.

CASE NO. 21 (Refrigerated Autotransplant)

The left kidney was removed from a twenty kilogram male cross-bred Collie on September 13. The animal was three years old and in good physical condition. The kidney was immersed in normal saline and stored in an electric refrigerator at 4°C. for twenty-four hours. The organ was then transplanted into the cervical region of the same animal under pentobarbital sodium anaesthesia. The arterial anastomosis required two emergency sutures and the venous anastomosis

seeped after application of three extra sutures. The seepage ceased after a few minutes with a gauze pad applied to the anastomosis. The subcutaneous "pocket" and the anastomosis were bathed with 200,000 units of aqueous solution of crystalline penicillin. The operation lasted three hours. Postoperatively the animal received 300,000 units of penicillin parenterally and 100,000 units of crystalline penicillin in aqueous solution introduced into the catheter and ureter commencing on September 14. This medication was repeated daily.

Clear fluid urine was produced by the transplant five minutes after completion of the anastomosis. The urine became blood-tinged on September 15 at which time the rate of excretion was fairly rapid. The amount of urine excreted decreased on September 16 and remained scant until the morning of September 25 when the rate increased considerably. The blood content of the urine became noticeably less on September 20, and finally disappeared on September 25, as shown by microscopic examination. The dog commenced vomiting on September 22 the day after its second kidney was removed. Vomition increased in frequency until death. The animal was alive and the transplant was producing clear urine at 8:30 p.m. on September 25 but was found dead on September 26 at 8:00 a.m.

Urinalysis and blood urea reports are contained in Table VI.

The transplant functioned for eleven days, at a fair rate for the first twenty-four hours, then very slowly for nine days with

an increased output on the eleventh day. The blood content of the urine followed a like curve -- very little blood at first, increasing to a peak on the third day and declining on the sixth day.

The animal lived for four days after removal of its second kidney. The rate of urine production increased when the animal became entirely dependent on the transplant for its urinary excretion. The peak of the urinary output was on the last day of the animal's life.

TABLE VI

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BLOOD AND URINE TESTS

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DATE	BLOOD UREA	REACTION	ALBUMIN	SUGAR	MICROSCOPIC
September 19		Alkaline	++++	Negative	Full of red cells.
" 21			++++		Numerous red cells.
" 22			++++		Many red cells.
" 24	185 mgs.		++++		Many red cells.
" 25	330 mgs.	Alkaline	+++		Few red cells -- odd pus cell.

---

Histological section of the kidney showed widespread necrosis with almost complete destruction of the tubules. Calcareous infiltration was present to a very marked degree and there were many psammoma bodies (see Fig. 24).

Immediate Homotransplants

Both animals were anaesthetized simultaneously, and the dogs placed side by side on their respective operating tables. The nephrectomy was performed first and the kidney wrapped in cotton swabs moistened with saline while the vessels in the neck of the recipient animal were prepared.

Upon completion of the vascular anastomosis the operative site was sprayed with 200,000 units of crystalline penicillin. The recipient animal was injected intramuscularly with 300,000 units of aqueous penicillin. This was administered daily until the kidney ceased to function.

This series of experiments was carried out to see if penicillin would materially increase the longevity of the homotransplant or alter the process of nephritis in these kidneys.

No increase in the length of functional time of the homotransplant was evidenced when penicillin was administered. The average time of function was four and one-half days. Reports in the literature prior to the advent of the antibiotics claim a much longer functional period for homotransplanted kidneys, according to Carrel (5) in 1905.

As in the previous experiments, the kidney was not removed from the neck until it had ceased to function. The outstanding picture in these kidneys, when sectioned, was nephrosis. The glomeruli were the last parts of the kidney to be affected. The cellular infiltration was about equally divided between lymphocytes and polymorphonuclear cells,

with perhaps a preponderance of lymphocytes.

CASE NO. 6 (Immediate Homotransplant)

The kidney to be transplanted was removed from the donor dog on May 29, 1951, and immediately transplanted into the cervical region of a one-year-old, male mongrel Collie which was in fair physical condition at the time. The recipient animal was given no preoperative medication but the anastomosis and transplanted kidney were bathed with 100,000 units of crystalline penicillin in aqueous solution, and daily postoperative doses of 300,000 units of aqueous suspension of penicillin were given intramuscularly.

The kidney functioned ten minutes after anastomosis of the vessels was completed. The urine excreted was clear and somewhat mucoid in nature. On May 30 the flow of urine was intermittent and the urine contained blood. At that time the animal had a rectal temperature of 105°F. The animal's temperature dropped to 102.2°F. by the following day and the urine flow was constant although slow. The urine produced was quite bloody. On June 1 the rate of excretion was very slow and the urine was very bloody. One hundred thousand units of crystalline penicillin dissolved in 1.5 cc. of sterile water were used to irrigate the ureter and pelvis of the kidney. Microscopic examination of the urine was made, but complete urinalysis was impossible because of the small amount of urine collected. The sample showed very few epithelial cells. Haemolyzed blood was present. The rate of excretion on June 2 was still slow and the urine was bloody. Penicillin (100,000 units in aqueous solution) was again used to irrigate the kidney. On June 3 the animal commenced haemorrhaging from the neck

incision, and had to be sacrificed. Massive perirenal haemorrhage was evident and the vein was found to have developed a leak at the anastomosis. The kidney functioned until the time of death, producing very bloody urine.

Pathology of the kidney revealed a great deal of haemorrhage and blood pigment. Coagulation necrosis had occurred with the infiltration of inflammatory cells, particularly in the upper portion of the cortex (see Fig. 25).

This kidney functioned for five days before the animal was destroyed; however, judging from the type of urine excreted and the histopathological features it is evident that the organ would not have functioned much longer.

CASE NO. 8 (Immediate Homotransplant)

Unilateral nephrectomy was performed on the donor animal on June 7, 1951, and the kidney was immediately transplanted into the cervical region of a female cross-bred Cocker, eight years of age. The recipient animal was in fair condition and weighed fifteen kilograms at the time of the operation. Bilateral nephrectomy was performed on the animal along with the transplantation. The vascular anastomosis was satisfactory after one emergency suture had been applied to the arterial anastomosis. Three hundred thousand units of crystalline penicillin in aqueous solution were applied to the anastomosis and the surface of the transplanted organ. The animal received 300,000 units of aqueous penicillin suspension intramuscularly

each day from June 7 to June 11.

The kidney functioned thirty minutes after completion of the anastomosis and produced a clear mucoid urine. On June 8 the catheter was plugged with mucoid material, necessitating aspiration by means of a syringe and a twenty-four gauge, one and one-half inch needle. The ureter was flushed with 100,000 units of crystalline penicillin in 2 cc. of sterile water. The urine was bloody and excreted at an extremely slow rate. The urine remained bloody and the rate of excretion slow, causing partial occlusion of the catheter on June 9 and June 10. Penicillin irrigation of the ureter was carried out on both days. On June 10 the animal started to vomit and showed no interest in food although extreme thirst was evinced. The animal's rectal temperature decreased from normal to 98.7°F. by the afternoon of June 11. Vomition increased in frequency and the animal showed a disinclination to move about. On the following morning, June 12, the dog was found dead.

The histological picture presented by the kidney was that of coagulation necrosis. The cortex was invaded by inflammatory cells, some lymphocytes, but chiefly polymorphonuclear leucocytes (see Fig. 26).

This kidney functioned for four days and produced a scant amount of bloody urine before death. The cellular infiltration of polymorphonuclear cells in the histological sections suggested infection by a pyogenic organism.

CASE NO. 12 (Immediate Homotransplant)

Unilateral nephrectomy was performed on a four-year-old male Sealyham on July 2, 1951. The kidney was immediately transplanted by vascular anastomosis into the cervical region of a two-year-old, seventeen kilogram hound bitch. The operation lasted one and one half hours and was performed under pentobarbital sodium anaesthesia. The anastomosis was satisfactory after two sutures were applied to bleeding points in the vein and one in the artery, and 200,000 units of crystalline penicillin had been applied to the anastomosis. Postoperative medication consisted of 600,000 units of aqueous penicillin suspension on the day of the operation and 300,000 units daily thereafter. One hundred thousand units of crystalline penicillin in aqueous solution was used to irrigate the ureter daily.

The kidney functioned immediately after completion of the anastomosis and produced a clear fluid urine. On the following day, July 4, the urine excreted was mucoid in nature and caused plugging of the catheter. This necessitated frequent aspiration by means of syringe and needle. On July 5 the rate of excretion was very slow and the urine contained a trace of blood. Urinalysis of a sample collected that day showed pus cells and red blood cells in small numbers on microscopic examination. The urine on July 6 and 7 had an increased blood content and the rate of excretion was very slow. The animal's neck became very swollen on July 7 and the kidney ceased

to function on July 8. The animal was sacrificed and histopathological examination was carried out on the kidney.

Histological section of the kidney revealed large areas of infarction with almost complete destruction of the general architecture in these parts. In other portions coagulation necrosis was advanced (see Fig. 27). The length of time this kidney functioned was four and one-half days. The usual picture of mucoid urine at first, followed by an increasingly bloody urine, then cessation of function at about five days, was again shown.

Urinalyses were not performed frequently as the type and amount of urine excreted did not make this possible. Blood urea determinations were not carried out as the animal retained both kidneys throughout the period of the experiment.

### Refrigerated Homotransplants

In these cases the procedure was essentially that heretofore described. Nephrectomy was performed on the donor animal twenty-four hours prior to transplantation. The kidney was kept in a domestic electric refrigerator for the ensuing time. Some were stored in physiological saline, others in a dry jar.

The following day the vascular anastomosis was performed on the recipient animal. The operative site was sprayed with 100 or 200,000 units of crystalline penicillin, and 300,000 units of aqueous penicillin was delivered parenterally. This parenteral injection was carried out daily until the kidney ceased to function.

This series of experiments was carried out to determine if refrigeration for this length of time and at this temperature would destroy the organismal differential of the donor kidney as had been suggested previously by Markowitz (12) in 1949.

On the whole these refrigerated transplants functioned only for the same length of time as did the immediate homotransplant. The urine was excreted slowly from these kidneys and was almost always blood-tinged or definitely bloody. Those kidneys which functioned for only a few days presented a picture of severe nephrosis. The glomeruli were seemingly least and last to be affected. The cellular infiltration was divided largely between lymphocytes and polymorphonuclear cells, with perhaps a preponderance of lymphocytes. In kidneys which functioned for longer

period of time, coagulation necrosis occurred and the parenchyma was entirely destroyed except for the presence of an occasional glomerulus.

CASE NO. 0 (Refrigerated Homotransplant)

The kidney was removed from the donor dog on May 14, 1951, stored in an electric refrigerator at 4°C. for twenty-four hours, then transplanted by means of vascular anastomosis into the cervical region of the recipient. The recipient was a mongrel Collie, in fair physical condition which weighed 17 kilograms at the time of transplantation. The operation lasted for one hour, at the end of which time very bloody urine was observed to drip from the ureter.

The animal had no preoperative medication, 300,000 units of crystalline penicillin in aqueous solution were used to bathe the anastomosis and the kidney in its subcutaneous pocket. A postoperative dose of 300,000 units of aqueous suspension of penicillin was given intramuscularly.

This kidney functioned for only six hours and urinalysis was not attempted.

The kidney was removed from the dog's neck and histological section showed many tubules to be necrotic while others showed degeneration. Albumen was present in the lumen of most of the tubules. There was marked congestion and haemorrhage present. The cellular infiltration was made up mainly of polymorphonuclear leucocytes (see Fig. 28).

CASE NO. 7 (Refrigerated Homotransplant)

Unilateral nephrectomy was performed on the donor animal on June 7, 1951. The kidney was stored in a dry screw-cap jar at refrigerator temperature for twenty-four hours. On June 8, transplantation into the cervical region by means of vascular anastomosis was performed on a five-month-old, female mongrel Collie in fair condition. The recipient animal had received 100,000 units of penicillin daily for six days preoperatively. Previous to transplantation this animal had been used as a kidney donor. The anaesthetic used was pentobarbital sodium administered intravenously to effect. The operation lasted forty-five minutes. Postoperative medication consisted of 300,000 units of crystalline penicillin in aqueous solution applied to the anastomosis and exterior of the transplant, in addition to 300,000 units of aqueous suspension of penicillin given intramuscularly daily. This animal retained one normal abdominal kidney from June 1.

Immediately after the transplantation clear urine was excreted. Twenty-four hours later the urine was blood-tinged and had a mucoid consistency. By June 10, the urine was quite bloody, forming a clot in the catheter, thus necessitating frequent cleaning. At this time the animal had a rectal temperature of 103.4°F. However, on the following day, June 11, the amount of blood in the urine excreted by the transplant had decreased and irrigation with 100,000 units of penicillin was carried out. The animal's temperature had dropped to 102.2°F. and the blood urea level was found to be 12.7 mg.

On June 12 the urine from the transplant contained blood and the rate of excretion was extremely slow. The animal's temperature was unaltered from the previous day. By 8:30 a.m. on June 13 the ureter had necrosed and was torn from the kidney with a very bloody urine being discharged through the skin at the point where the ureter had been led to the surface. This discharged urine seemingly contained pus. The animal was sacrificed and the transplanted kidney removed from its neck. The vascular anastomoses were still satisfactory.

The histological picture of the kidney showed advanced coagulation necrosis. The glomeruli were the least damaged part of the kidney (see Fig.29).

This kidney functioned for five days. Unfortunately the amount of urine excreted was small and the amount of blood which it contained made urinalysis impracticable.

#### CASE NO. 9 (Refrigerated Homotransplant)

The kidney was removed from the donor, a Collie weighing nineteen kilograms, on June 14, 1951, and kept in a dry jar placed in an electric refrigerator for twenty-four hours. It was then transplanted by vascular anastomosis into the cervical region of a ten kilogram mongrel terrier, four and one-half months old. The recipient was in only fair condition but otherwise normal. The operation lasted one hour under pentobarbital sodium anaesthesia. The anastomosis was satisfactory, only requiring one additional stitch in both artery and vein to control bleeding points.

Preoperative medication was not given, but 300,000 units of crystalline penicillin in aqueous solution were used to bathe the anastomosed vessels and the kidney, and to irrigate the ureter. Postoperative medication consisted of daily doses of 300,000 units of aqueous penicillin suspension intramuscularly.

The kidney excreted clear fluid urine five minutes after the anastomosis was completed. The rate of excretion was slow and the urine blood-tinged. On June 16, 100,000 units of crystalline penicillin were introduced to the subcutaneous area around the transplant. The animal developed a cough and showed a rectal temperature of 103°F. on June 16. These symptoms persisted until June 19. The ureter of the transplant was irrigated with 100,000 units of crystalline penicillin in 1 cc. of sterile water on June 18, but the urine remained bloody. A blood urea determination was attempted that day but the sample coagulated.

The rate of excretion of urine remained slow until June 19 and the urine was very bloody. On that day the ureter had become necrotic and broken off at the skin level. It was decided to remove the transplant in view of the respiratory infection and temperature, as well as necrosis of the ureter, even although small amounts of bloody urine were still being excreted. Daily samples of urine were collected but these were too scant and contaminated with blood to achieve accurate results from urinalysis.

Histological section of this kidney showed advanced coagulation necrosis (see Fig. 30).

This kidney functioned for four days and produced very bloody urine for the last three days.

CASE NO. 20 (Refrigerated Homotransplant)

Transplantation of a kidney from animal No. 19 into the cervical region of a six-month-old male, mongrel Pekinese was effected on September 12, 1951. The recipient was at the time subject to a mild dermatitis and laryngotracheitis but otherwise in good health. Transplantation was performed under pentobarbital sodium twenty-four hours after removal of the kidney from the donor dog. The kidney, meanwhile, was stored at 4°C. in double screw-capped jars containing sterile normal saline into which the organ was immersed. The method of transplantation was as previously described. The anastomosis required two emergency sutures in both the artery and the vein to control bleeding points. In addition the renal artery had two major branches and one minor branch which together with the adjoining major branch was anastomosed to the carotid artery. The second major branch which had been severed at nephrectomy was ligated with #2 chromic gut. Two hundred thousand units of crystalline penicillin solution were applied to the anastomosis.

The transplant produced clear fluid urine five minutes after completion of the anastomosis and the animal was given 300,000 units of aqueous penicillin suspension parenterally immediately after the operation and daily thereafter. On September 13, the rate of excretion was quite rapid but the urine produced contained blood. The ureter was irrigated with 100,000 units of crystalline penicillin in

aqueous solution. The urine became more bloody on September 14 and was shown to have a 4+ albumin concentration. Crystalline penicillin solution of 100,000 units was used to irrigate the ureter of the transplant on that day and thereafter. From September 15, the urine excretion rate decreased and the blood content increased until September 18, when excretion ceased. At that time, the ureter was necrotic and leaking subcutaneously. The transplanted organ was removed from the animal's neck on September 19 and the vessels were found to be thrombosed.

The transplant functioned for five days, producing very bloody urine for the last four days. A series of urinalyses could not be carried out as the amount of urine produced was very small.

Histopathological examination showed that the kidney had been almost completely destroyed and replaced by blood (see Fig. 31).

The Use of ACTH in Immediate and Refrigerated Homotransplants

In these cases the kidney was transplanted into the cervical region either immediately after extirpation or after twenty-four hours of refrigerator storage. An attempt was made to allow sufficient time for the anastomosis to become established. Six days after transplantation a bilateral nephrectomy was performed on the recipient animal. In one case the ACTH was given after the bilateral nephrectomy. The other received it from the time of transplantation. Both cases received routine parenteral injections of penicillin throughout the life of the transplant. The surgical procedure was identical to that previously described and the aftercare and treatment was the same as for other transplants with the exception of the administration of ACTH.

The excretion of urine was slow and this fluid tended to be blood-tinged until after the animal's own kidneys had been removed, after which time the urine volume increased slightly. The animals became dehydrated, drank water copiously, and vomited almost from the time at which the abdominal kidneys were removed.

The histopathological picture in these transplants did not differ materially from that of other homotransplanted kidneys. The kidney parenchyma had undergone necrosis, nephrosis was evident, and the glomeruli were the structures least affected. Cellular infiltration was present, and lymphocytes and polymorphonuclear neutrophils were present in equal numbers.

CASE NO. 4 (Refrigerated Homotransplant and ACTH)

Homotransplantation of a kidney into the cervical region of a one and one-half year old, male, cross-bred cocker spaniel was performed on May 22, 1951. At the time of transplantation the animal was in good health except for a mild dermatitis. The rectal temperature was 102°F. Preoperative medication consisted of 300,000 units of penicillin injected intramuscularly on the morning of the operation.

The kidney to be transplanted was removed from the donor on May 21 and stored in a dry, sterile glass jar at a temperature of approximately 4°C. for twenty-four hours before transplantation. The operative procedure of transplanting the kidney was as previously described, and lasted one hour.

Postoperative medication consisted of the application of 100,000 units of crystalline penicillin to the anastomosis and subcutaneous "pocket" containing the kidney.

On May 23, the urine excreted by the transplanted kidney was a darker yellow colour than that excreted immediately after the operation. Thereafter the urine became clear and lighter in colour, being excreted at the rate of one drop per minute, until May 30 when the consistency became viscous and clotting took place within five minutes.

Bilateral nephrectomy was performed on the animal on May 28

under pentobarbital sodium anaesthesia. Shortly before this operation the animal had forcibly withdrawn the catheter which had been secured in the ureter by means of 5-0 twisted silk suture inserted through the ureter and catheter in such a manner as not to occlude the lumen of the latter. After this operation, one cubic centimetre of metrazol (pentamethylentetrazol, ten per cent solution) was given as a stimulant and antidote to the anaesthetic to aid recovery of the now nephrectomized dog. In addition 12 mg. ACTH was administered at 8:00 p.m., three and one-half hours after nephrectomy, and at midnight 14 mg. ACTH was given. ACTH was continued in 12 mg. doses every four hours, starting at 8:00 a.m. with a 14 mg. dose at midnight and no ACTH at 4:00 a.m., until May 31, the night before the animal died (see Fig. 32).

On May 28 the skin sutures were removed from the neck incision and small amounts of purulent material indicated the possibility of infection around the transplanted kidney. At this time the animal's temperature was 103°F. On the following day, May 29, and daily thereafter 300,000 international units of penicillin were administered intramuscularly. The animal's rectal temperature decreased to 102°F. after the first injection of penicillin and remained normal until the day of death when it again rose to 103°F., forty-five minutes before death.

On May 30, the sedimentation rate was 50 mm. in sixty minutes and the blood urea 140.3 mg. per 100 cc. of blood. Vomition had started; this, however, was infrequent and consisted of gastric

contents. The animal was uninterested in food and became increasingly dehydrated although the water intake was high (see Fig. 32).

Subcutaneous and intravenous injections of a total of 400 cc. of saline with five per cent dextrose were given on May 31 in an effort to prevent dehydration.

By the morning of June 1 the dog was extremely lethargic, dehydrated and vomiting frequently. The vomitus at this time contained bile and blood clots. A subcutaneous injection of 200 cc. saline with five per cent dextrose was given. The dose of ACTH was reduced to 6 mg. given at 8:00 a.m. and at noon, in an attempt to reduce the frequency of vomiting. Vomiting was not markedly reduced in frequency. The animal died at 3:46 p.m. that day.

In this case, the homotransplanted kidney functioned for ten days and excreted blood-tinged urine only at the time of death of the animal. The animal died of renal insufficiency with a blood urea of 233.5 mg. per 100 cc. blood as shown by a blood sample taken two hours before death.

Histopathological examination of the transplant showed that there was almost complete destruction of the architecture of the organ. This was especially true in the cortex just below the capsule -- deeper there were a few fairly normal glomeruli. The changes were those of haemorrhage, degeneration and necrosis of the tubules with calcification, proliferation of the interstitial connective tissue and the infiltration of inflammatory cells -- chiefly round cells (see Fig. 33).

CASE NO. 22. (Immediate Homotransplant and ACTH)

One kidney was removed from a Collie dog, six months of age, on September 15, 1951, and immediately transplanted into the cervical region of the recipient which was a six-month-old male Collie in fair physical condition. One branch of the renal artery was anastomosed to the carotid artery; the other branch was ligated, as the renal artery had been severed proximal to the bifurcation at the time of nephrectomy. After completion of the anastomosis, it appeared that one-half of the kidney, through the median plane was not receiving an adequate blood supply. The subcutaneous "pocket" was irrigated with 200,000 units of crystalline penicillin in aqueous solution. The operation lasted one hour and fifteen minutes.

The transplant began to function half an hour after the anastomosis was completed and produced clear fluid urine. The animal was put on a course of ACTH given intramuscularly in 10 mg. doses every four hours from 8:00 a.m. to 8:00 p.m. and 15 mg. at midnight with no dose at 4:00 a.m. The first dose was given along with 300,000 units of penicillin at 4:00 p.m., on September 15. Penicillin and ACTH were given daily. The urine excretion of the transplant was fairly rapid for the first day, becoming very slow before the death of the animal. A small amount of blood appeared in the urine on September 16. This increased until September 22 when the amount of urine greatly decreased.

On September 17 the ureter was torn off by the animal and a

catheter was inserted through the ureteral opening in the skin. The ureter had to be catheterized by making a skin incision in the neck of the animal to expose the ureteral stump. At this time the ureter was found to be fused to the subcutaneous tissue. The catheter was fixed into the lumen of the ureter by means of a suture through the skin and catheter, and the incision and kidney were infiltrated with 100,000 units of crystalline penicillin solution. At the same time the ureter and pelvis of the kidney were irrigated with 100,000 units of penicillin.

Bilateral nephrectomy was performed on the animal seven days after transplantation of the kidney. On the following day the ureteral catheter became plugged and was irrigated with 100,000 units of crystalline penicillin. Vomition was also evident on September 23 with increased frequency on September 24 and 25. At 8:30 p.m. on September 25 the animal was unable to stand and had a rectal temperature of 98.0°F. The blood urea level was 183.5 mg. on September 24 and 298 mg. on September 25. The animal was found dead on September 26.

Histopathological examination showed the kidney to have suffered coagulation necrosis with generalized inflammatory changes (see Fig. 34).

This homotransplanted kidney functioned for ten days. The urine flow was fairly rapid; however, within twenty-four hours only a small amount of bloody urine was produced.

APPENDIX

This appendix contains the photographs and photomicrographs which illustrate some aspects of the experimental data presented in this thesis. The power used for all photomicrographs was x 250 unless otherwise indicated.

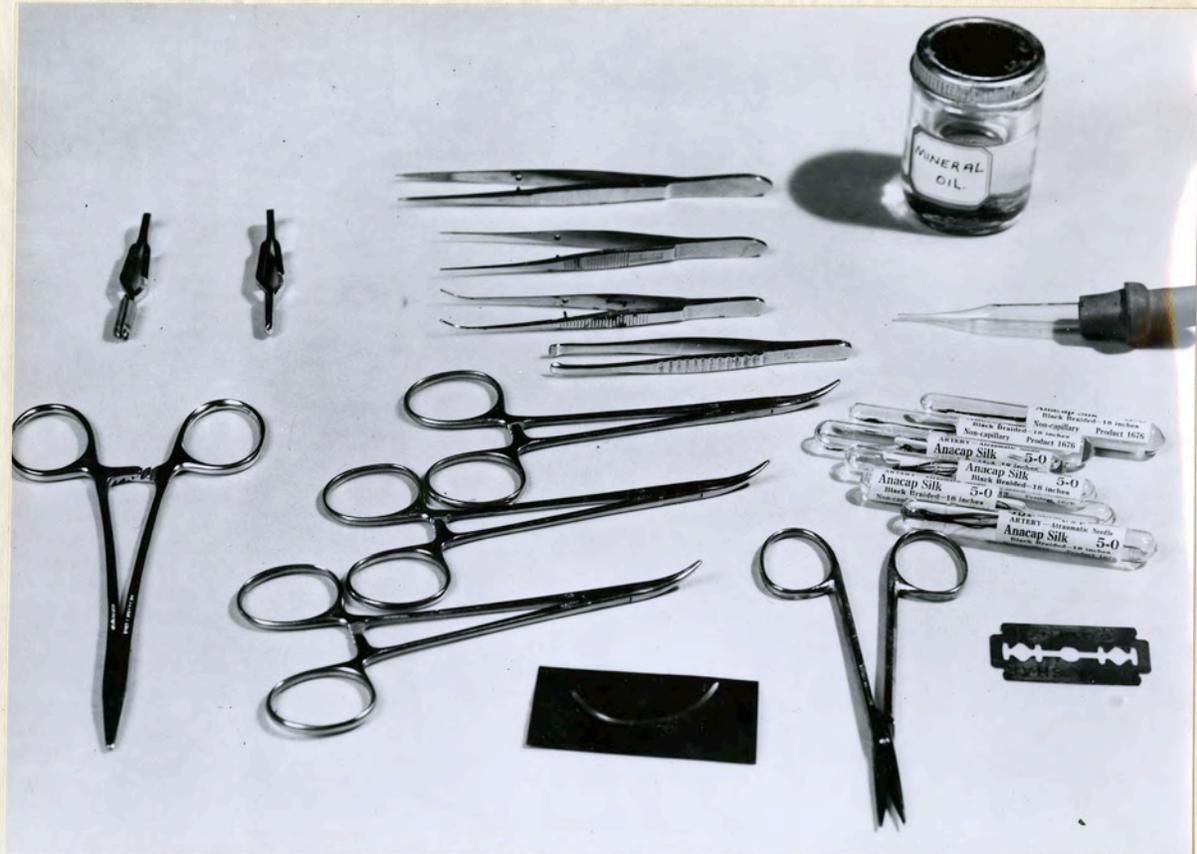


Fig. 1. MATERIALS USED IN BLOOD VESSEL ANASTOMOSIS.

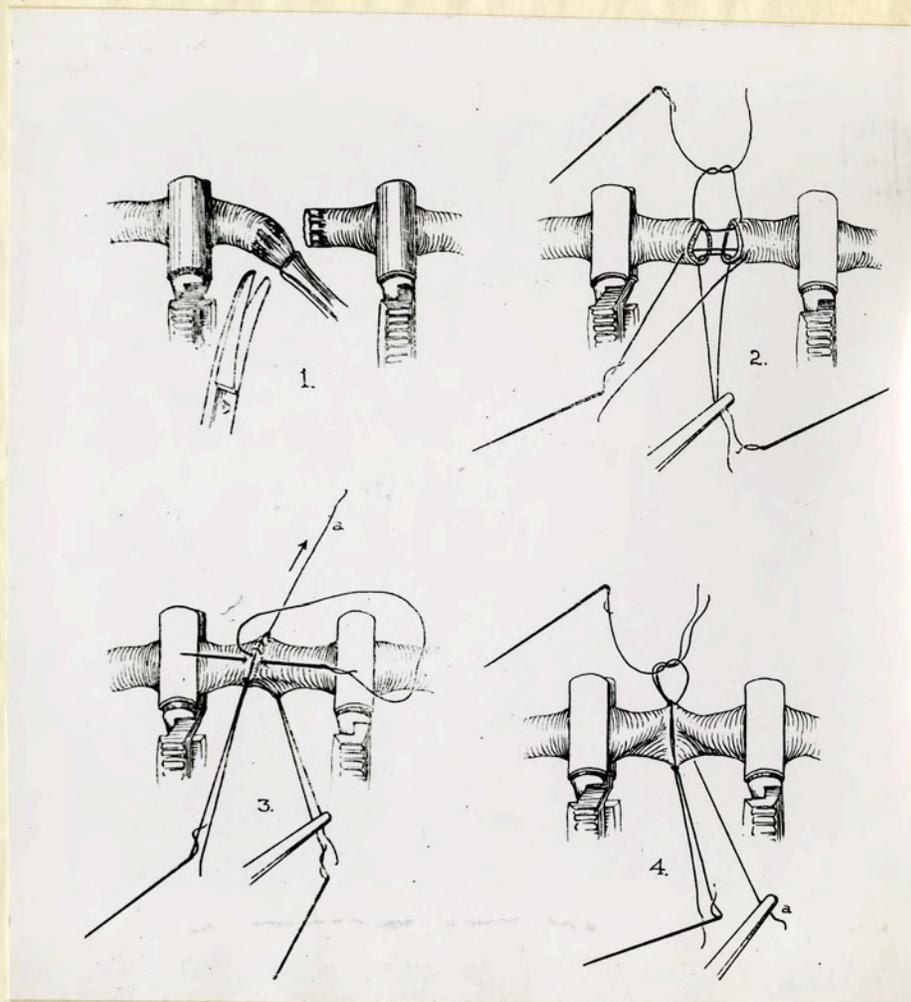


Fig. 2. SHOWING ADVENTITIA BEING STRIPPED FROM VESSEL AND THE PLACING OF GUY SUTURES.

(From Markowitz, Experimental Surgery, 1949)

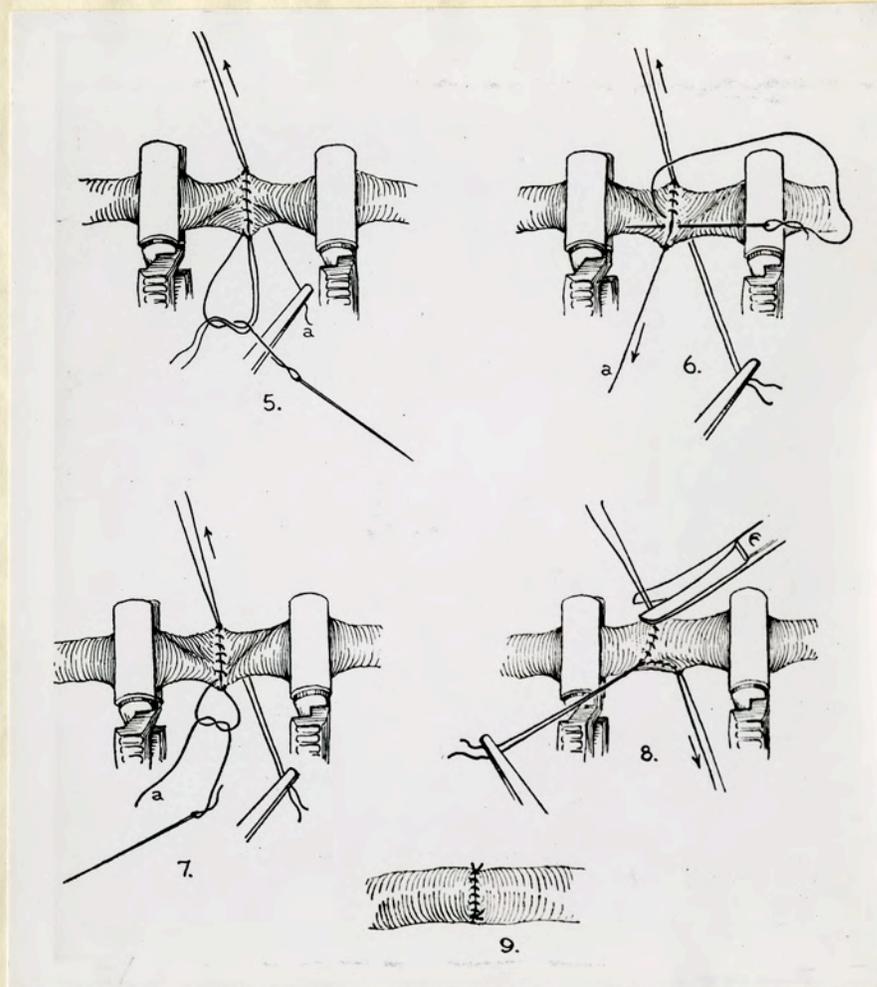


Fig. 3. SHOWING OVER AND OVER SUTURE USED FOR VASCULAR ANASTOMOSIS, AND COMPLETED ANASTOMOSIS OF A BLOOD VESSEL.

(From Markowitz, Experimental Surgery, 1949)



Fig. 4. SHOWING METHOD OF TRIANGULATING BLOOD VESSELS PRIOR TO SUTURING.

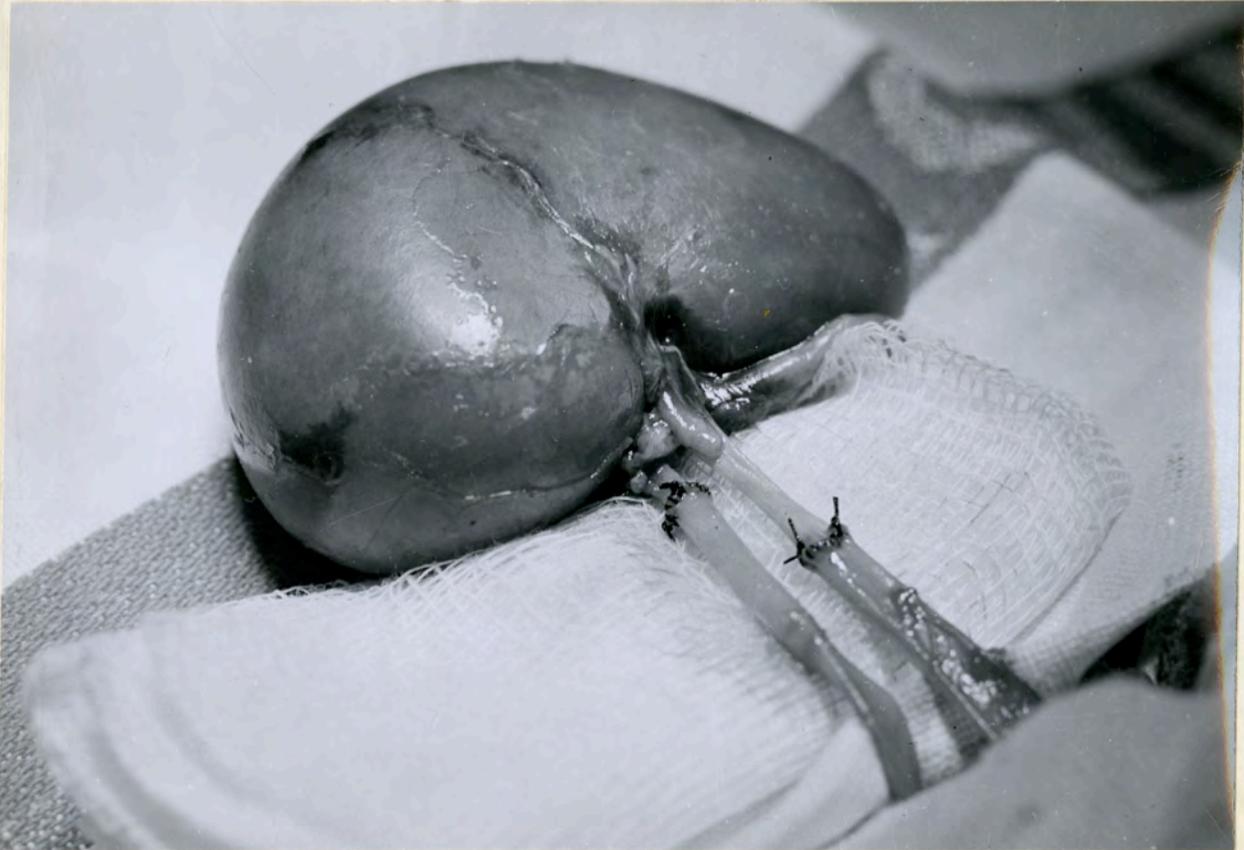


Fig. 5. SHOWING COMPLETED VENOUS AND ARTERIAL ANASTOMOSIS BEFORE SERREFINES ARE RELEASED TO PERMIT BLOOD FLOW THROUGH THE KIDNEY.



Fig. 6. SERREFINES HAVE BEEN REMOVED, AND BLOOD IS FLOWING THROUGH THE KIDNEY.



Fig. 7. AMIMAL NO. I. SHOWING URETERAL STUMP,  
POLYETHELENE CATHETER AND DROP OF URINE  
AT END OF CATHETER.



Fig. 8. SHOWING ANIMAL WITH AUTOTRANSPLANTED KIDNEY  
IN CERVICAL REGION. THIS KIDNEY FUNCTIONED  
FOR 148 DAYS, AT WHICH TIME IT WAS REMOVED FOR  
HISTOPATHOLOGICAL EXAMINATION.

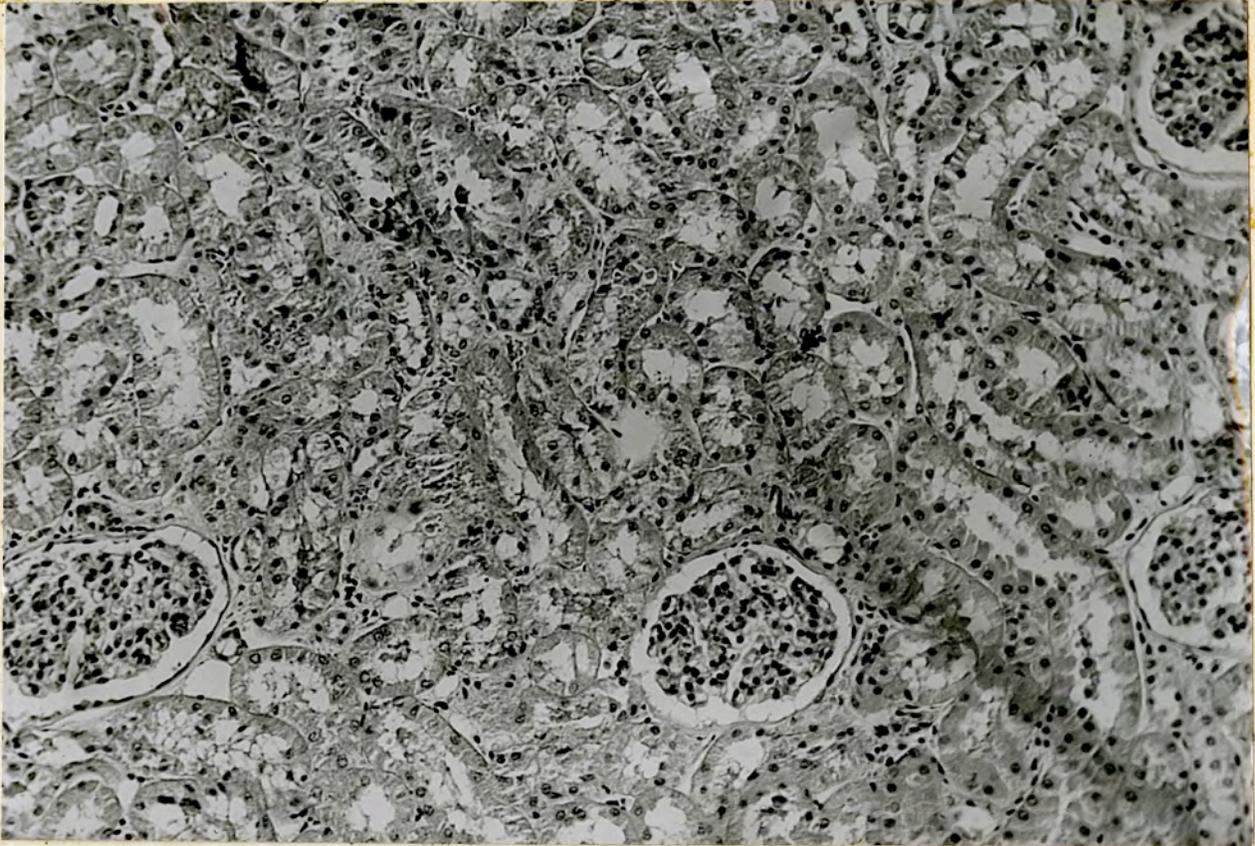


Fig. 9. KIDNEY ESSENTIALLY NORMAL

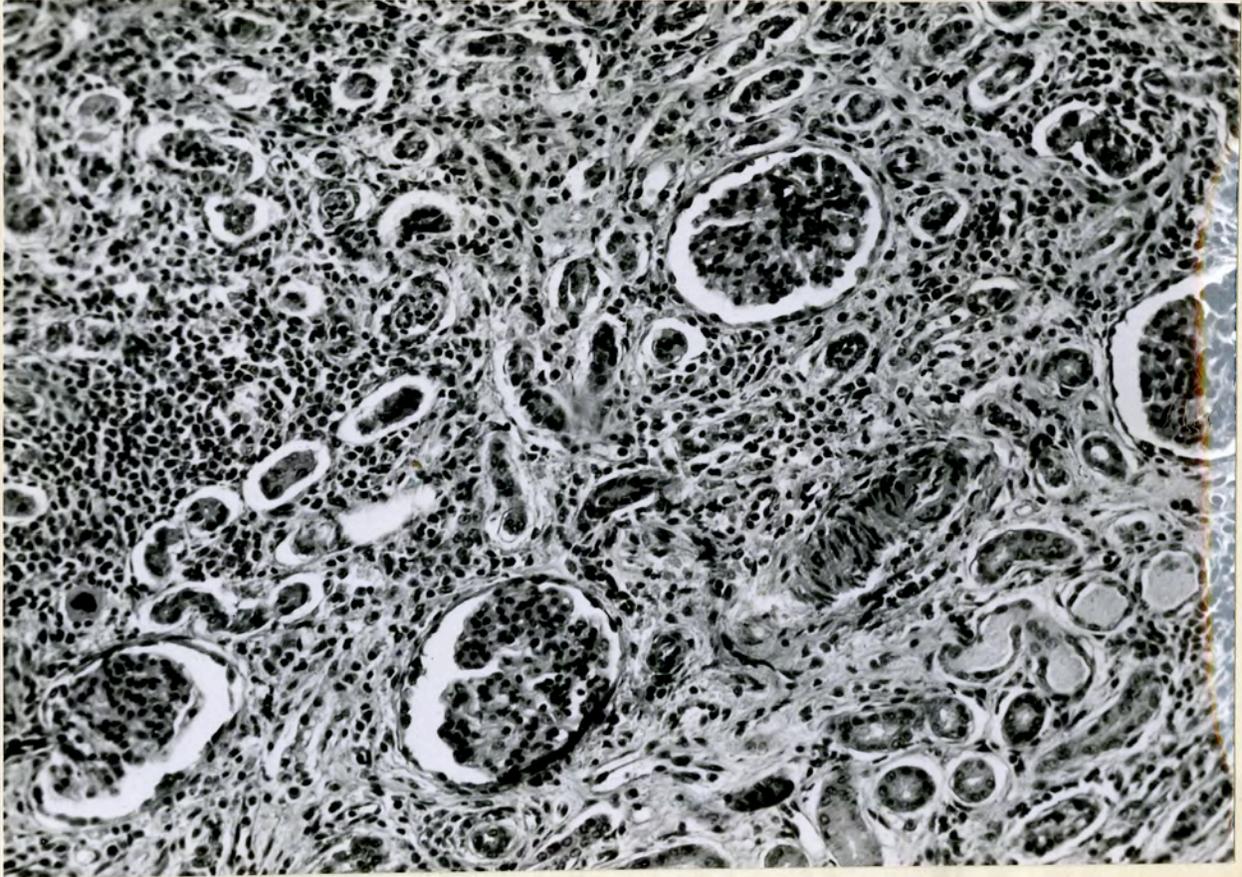


Fig. 10. SECTION OF KIDNEY SHOWING INTERSTITIAL NEPHRITIS. CELLULAR INFILTRATION APPEARS TO BE LYMPHOCYTIC. THE TUBULES ARE SMALL AND REDUCED IN NUMBER.

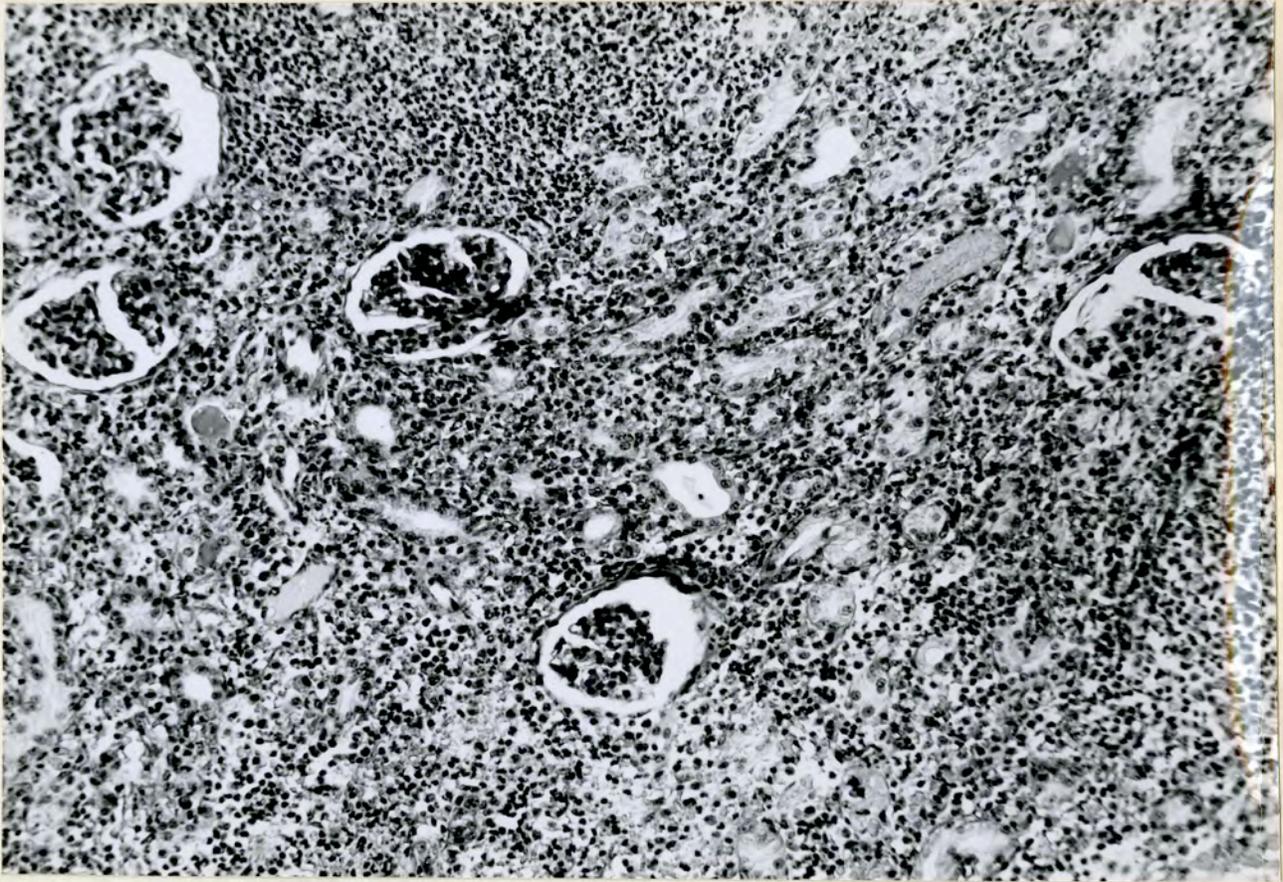


Fig. 11. TUBULES AND GLOMERULI APPEAR INTACT AND APPARENTLY FUNCTIONING. THE HEAVY INFILTRATION OF POLYMORPHONUCLEAR NEUTROPHILES SUGGESTS PYOGENIC INFECTION.

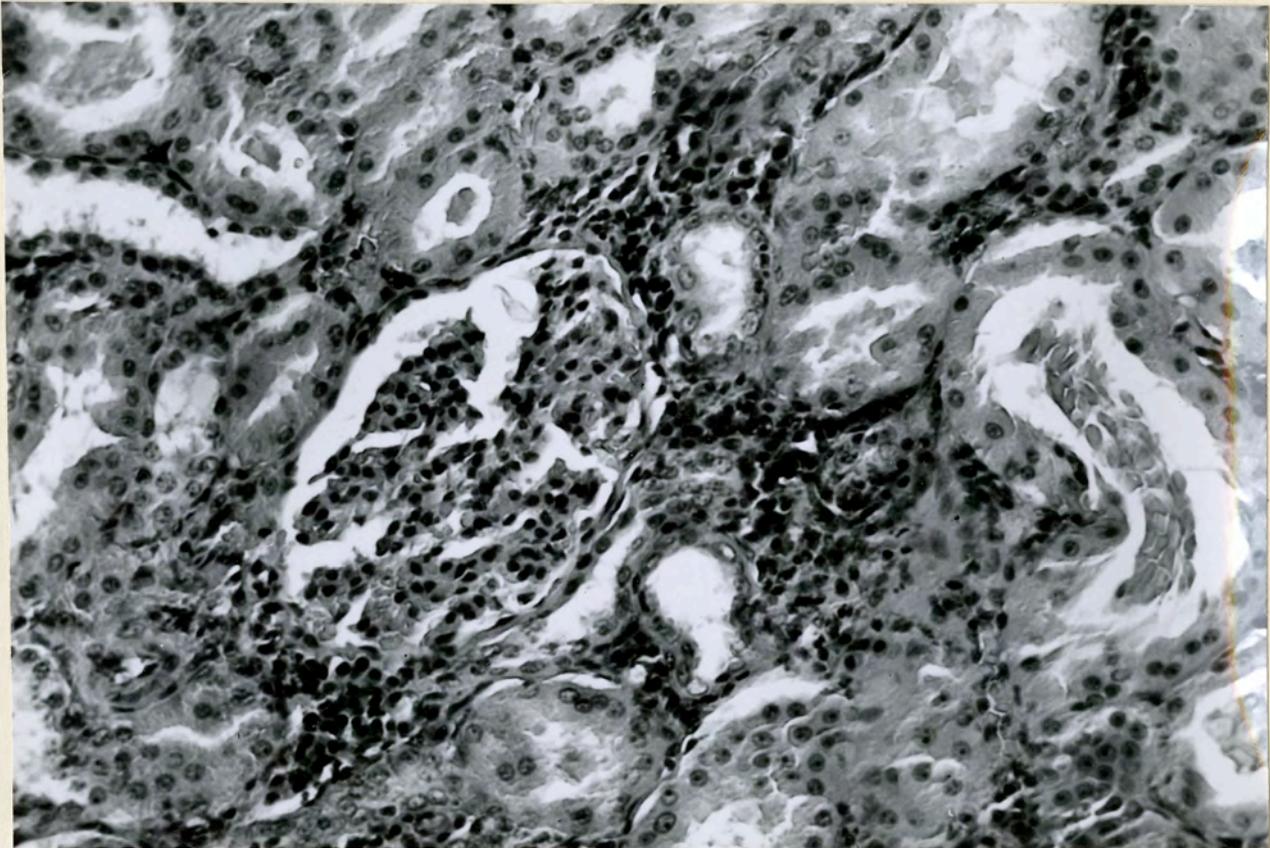


Fig. 12. DEGENERATION AND SOME NECROSIS.  
THERE IS SOME ROUND CELL INFILTRATION.  
DAMAGE IS SLIGHT IN THIS KIDNEY.



Fig. 13. SHOWING ANIMAL NO. 5 ONE MONTH AFTER  
BILATERAL NEPHRECTOMY. THIS WAS AN AUTOTRANSPLANT.  
NOTE STUMP OR URETER IN THIS PICTURE.

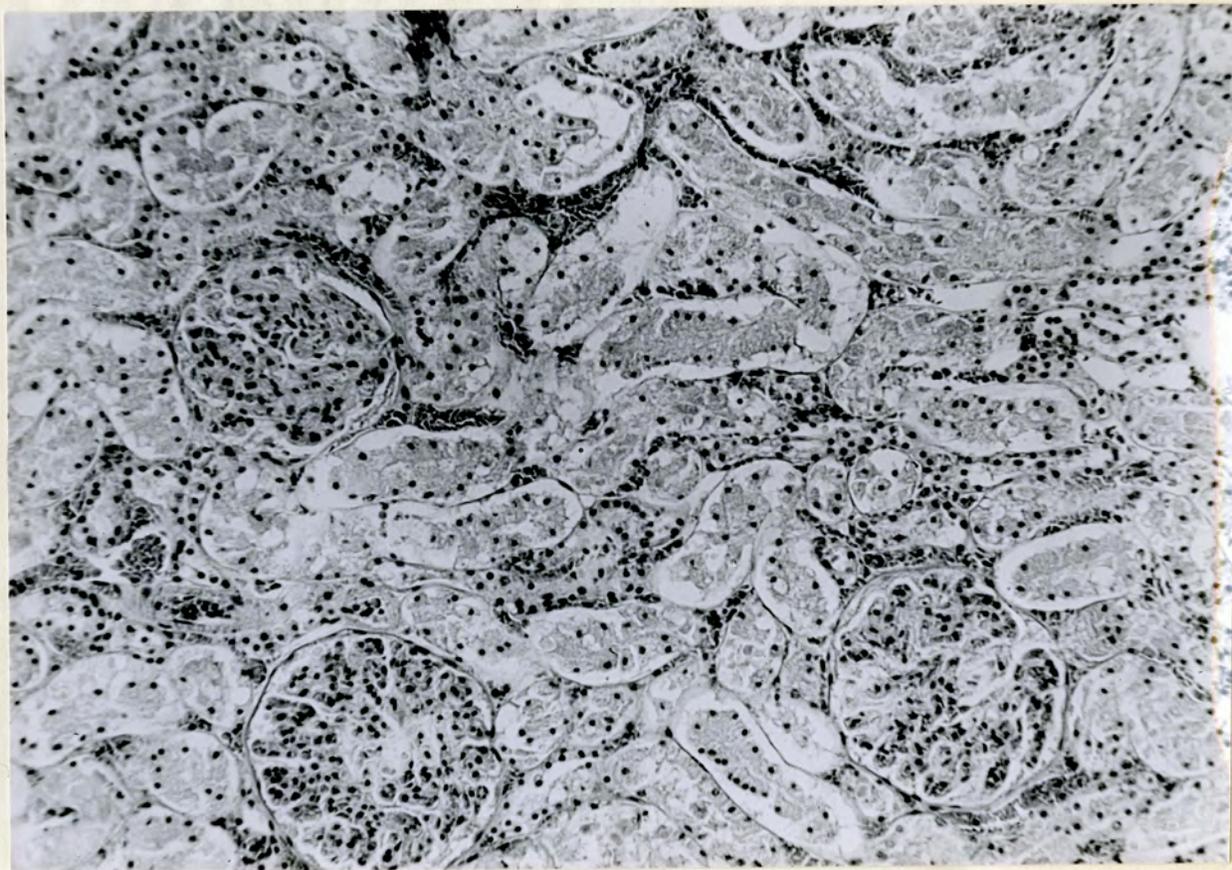


FIG. 14. CONGESTION, HAEMORRHAGE AND NEPHROSIS.

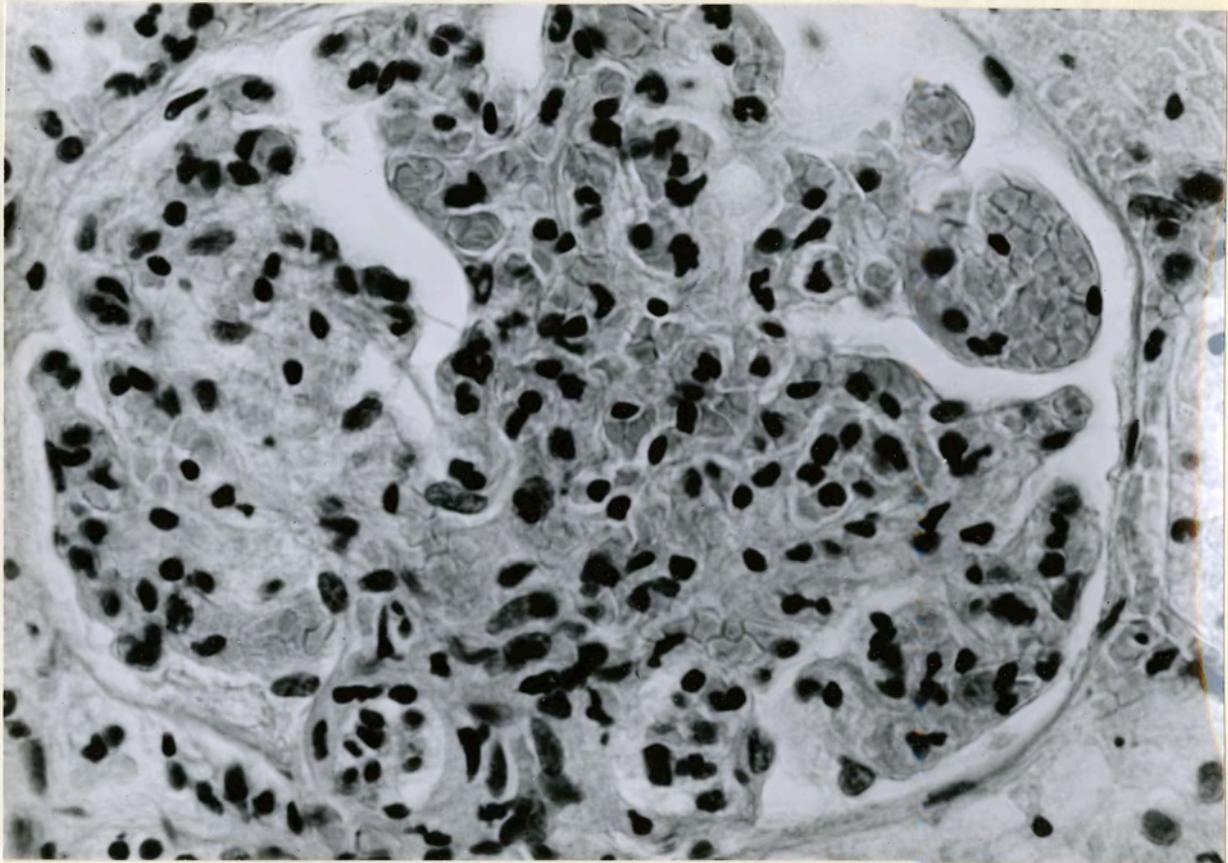


Fig. 15. GLOMERULUS OF FIG. 14.

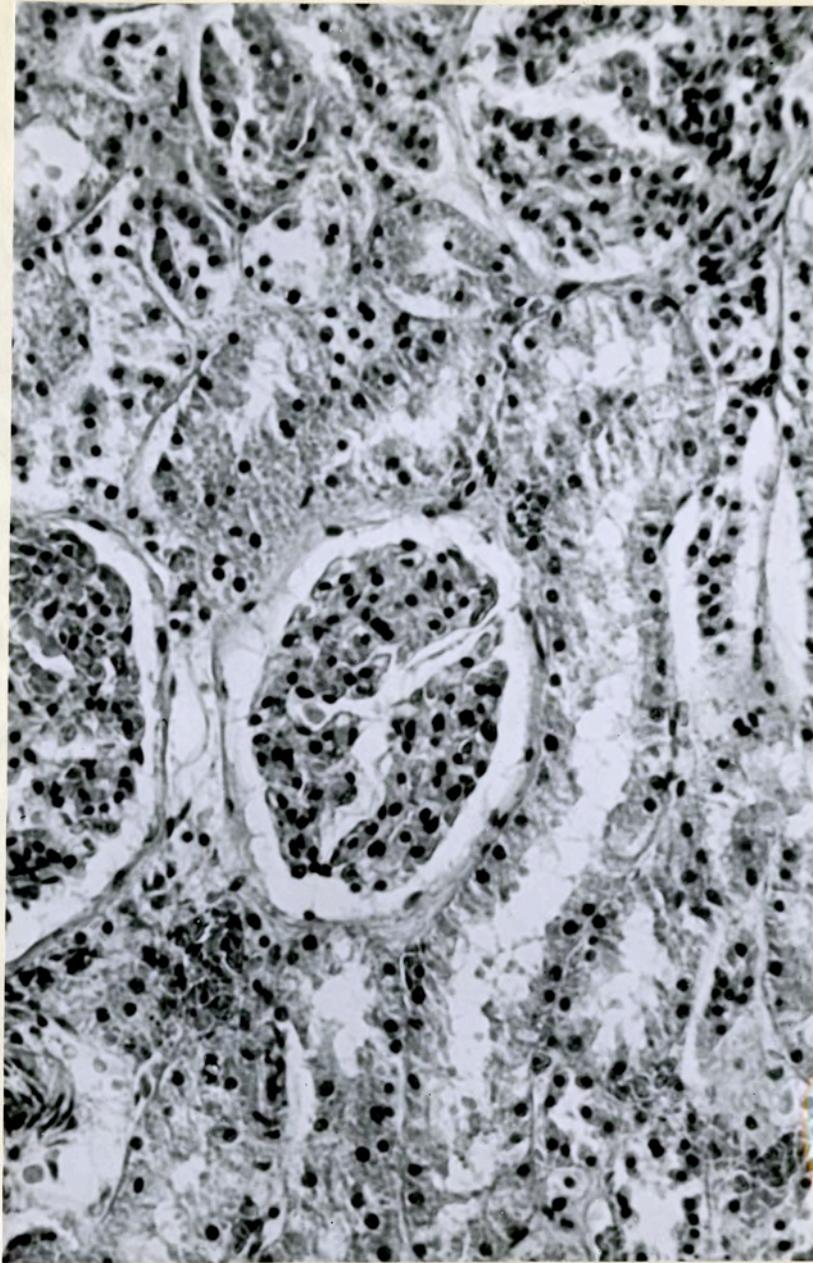


Fig. 16. DEGENERATION AND NECROSIS.

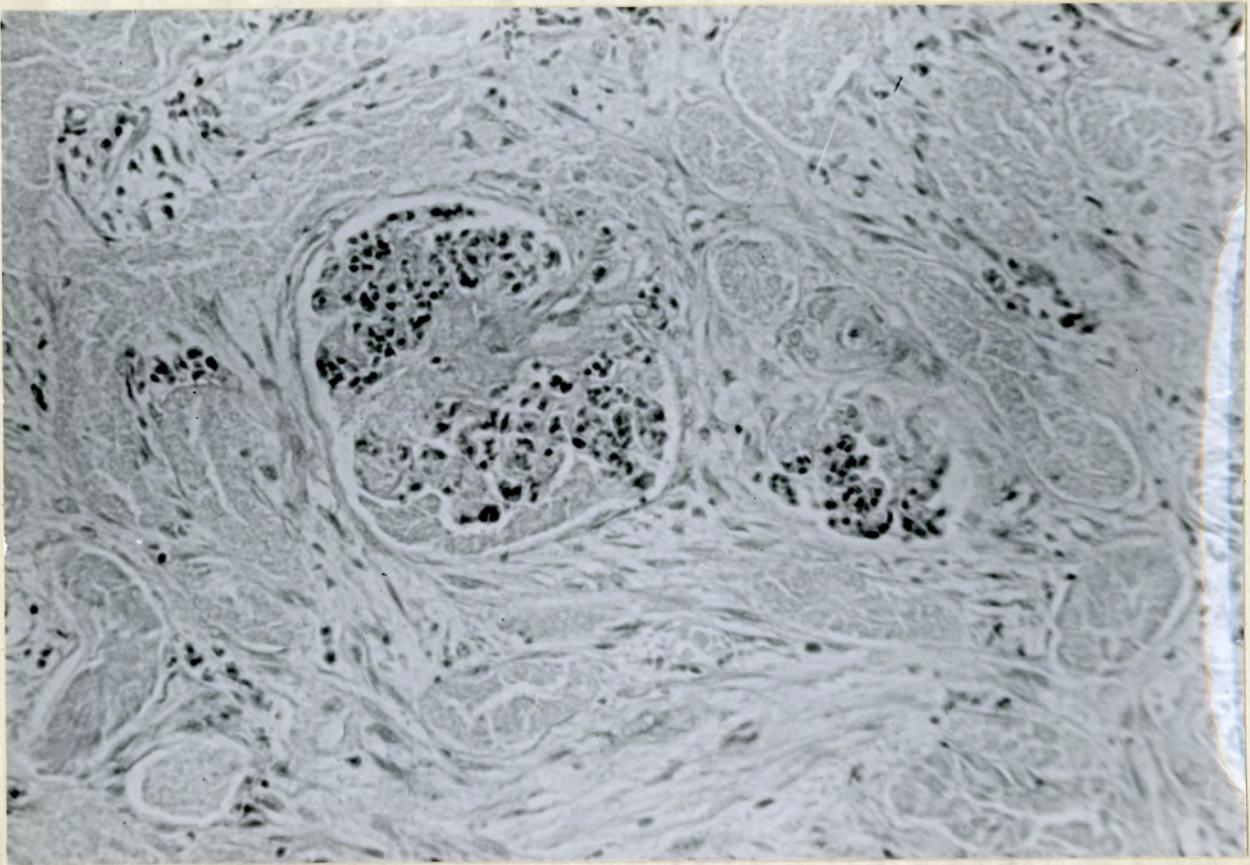


Fig. 17. SHOWING FIBROSIS AND NEPHROSIS.

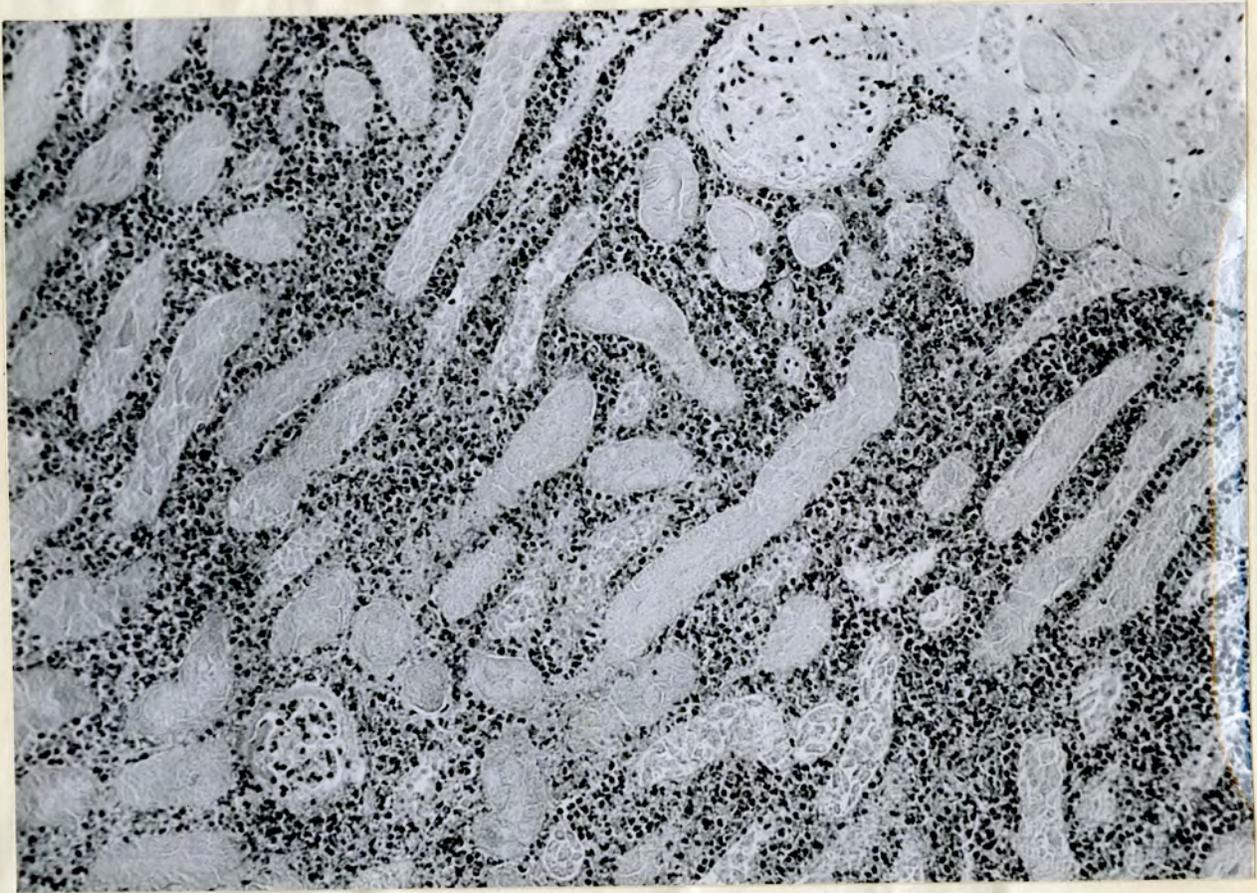


Fig. 18. COAGULATION NECROSIS, CALCIFICATION, AND CELLULAR INFILTRATION.

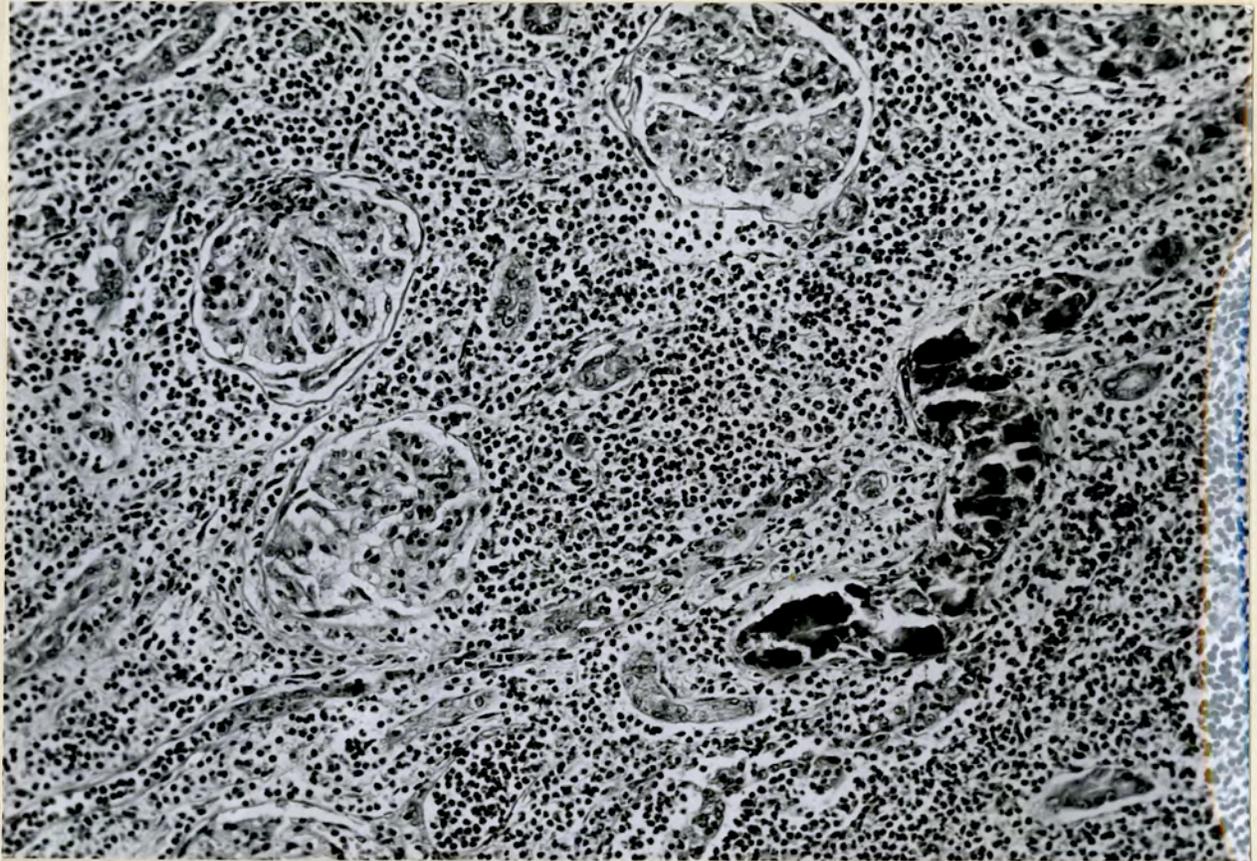


Fig. 19. NEUTROPHIL INFILTRATION OF TUBULES, CALCIUM CASTS IN THE TUBULES, WITH AN OVERALL INCREASE IN FIBROUS TISSUE.

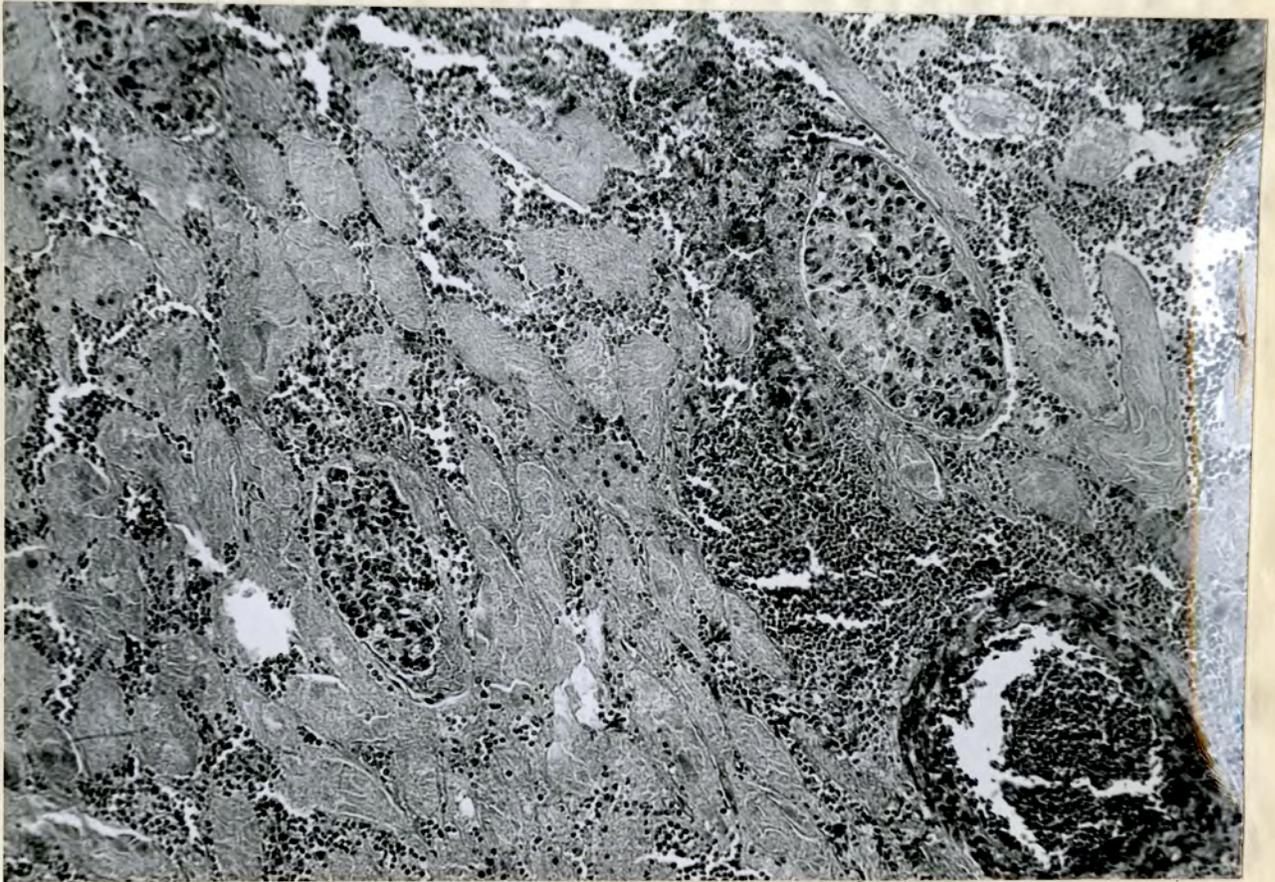


Fig. 20. MASSIVE NECROSIS AND HAEMORRHAGE.

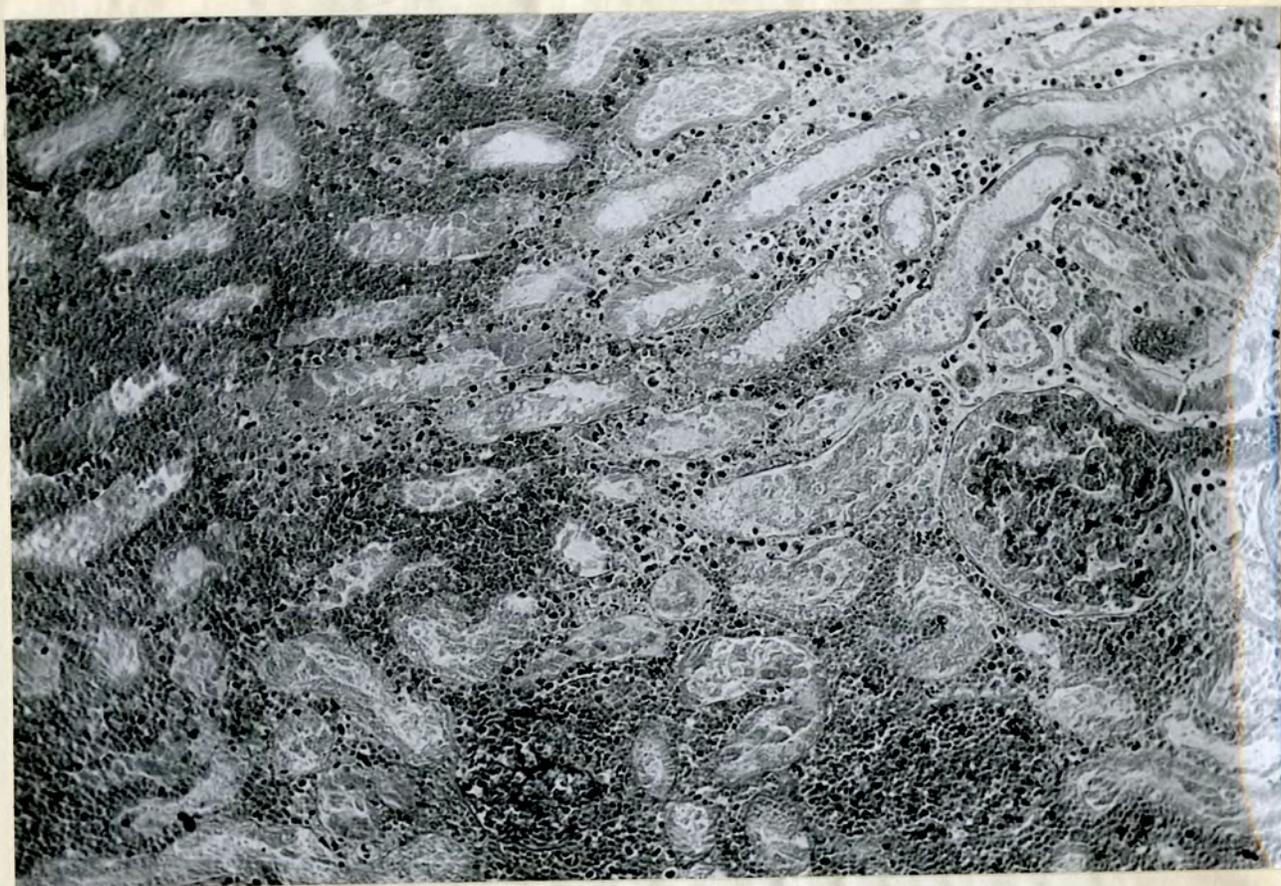


Fig. 21. THE KIDNEY IS ALMOST COMPLETELY NECROTIC.  
HAEMORRHAGE AND FIBROSIS CHARACTERIZE LARGE AREAS.

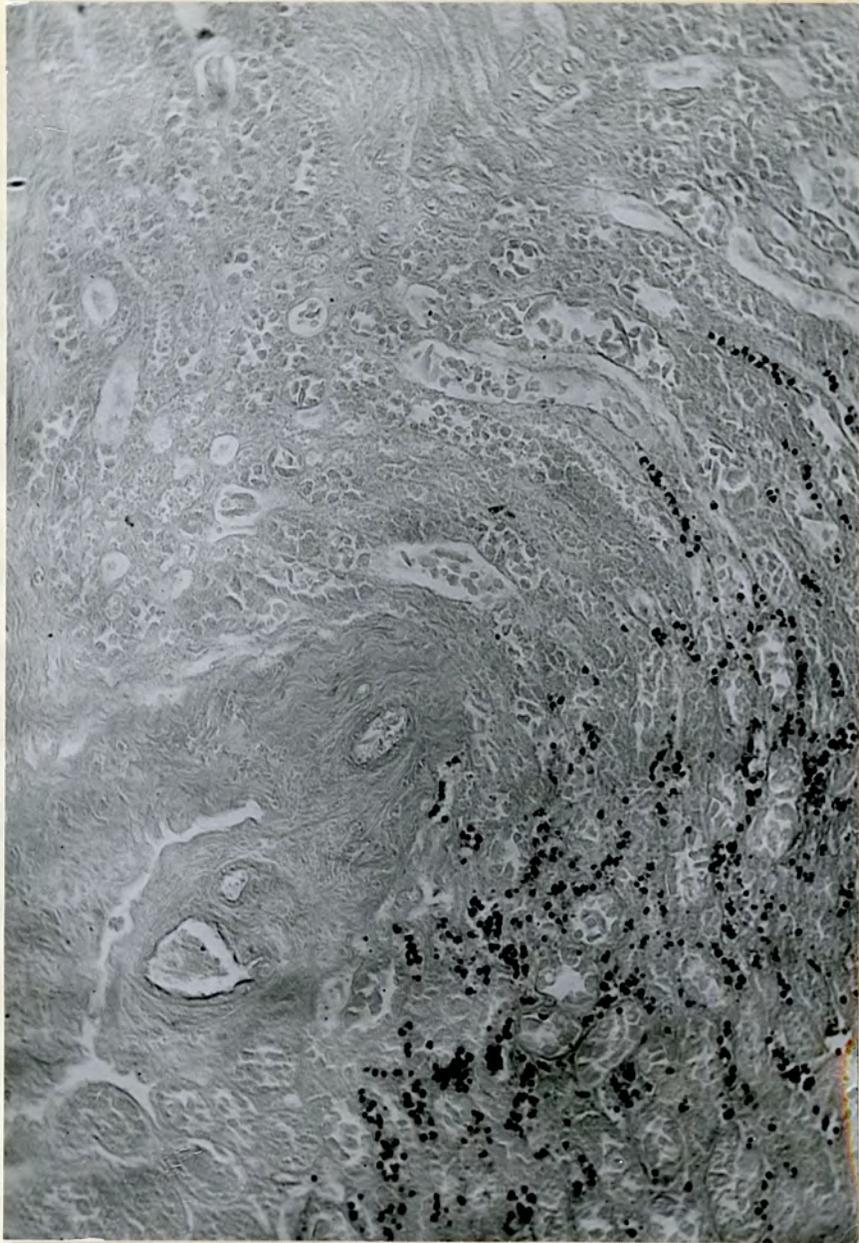


Fig. 22. COAGULATION NECROSIS WITH MARKED INFLAMMATORY CHANGES THROUGHOUT.



Fig. 23. DEGENERATION AND NECROSIS OF THE  
TUBULAR EPITHELIUM.

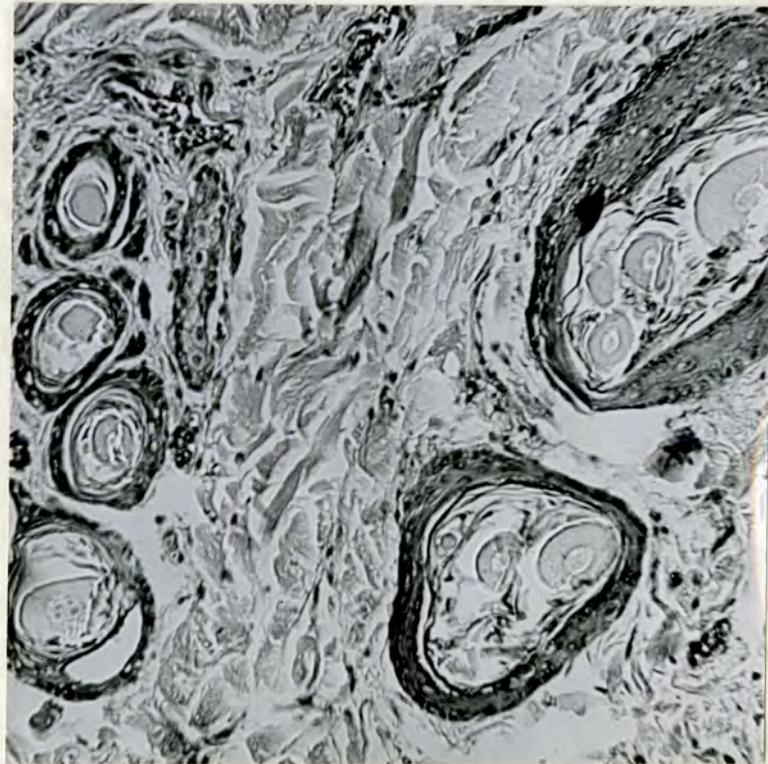
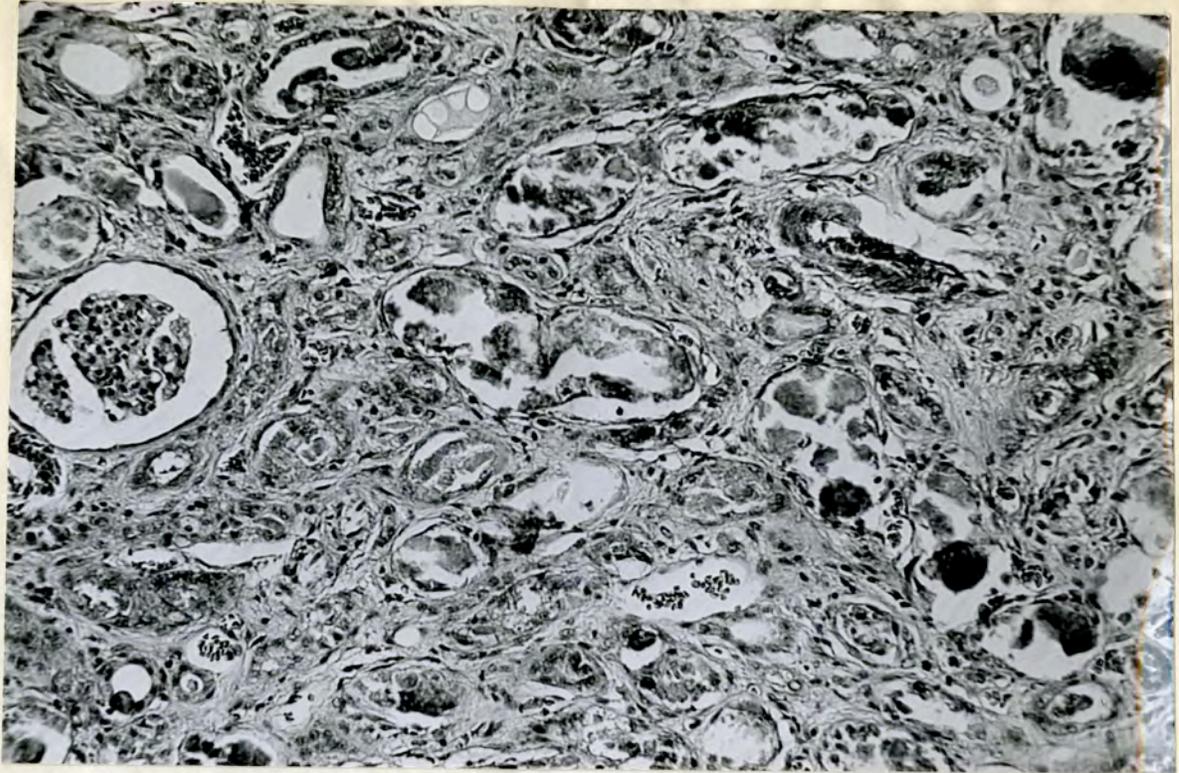


Fig. 24. NECROSIS OF THE KIDNEY TUBULES, CALCAREOUS INFILTRATION AND PSAMMOMA BODIES.



Fig. 25. COAGULATION NECROSIS, HAEMORRHAGE,  
AND INFLAMMATORY CELL INFILTRATION.

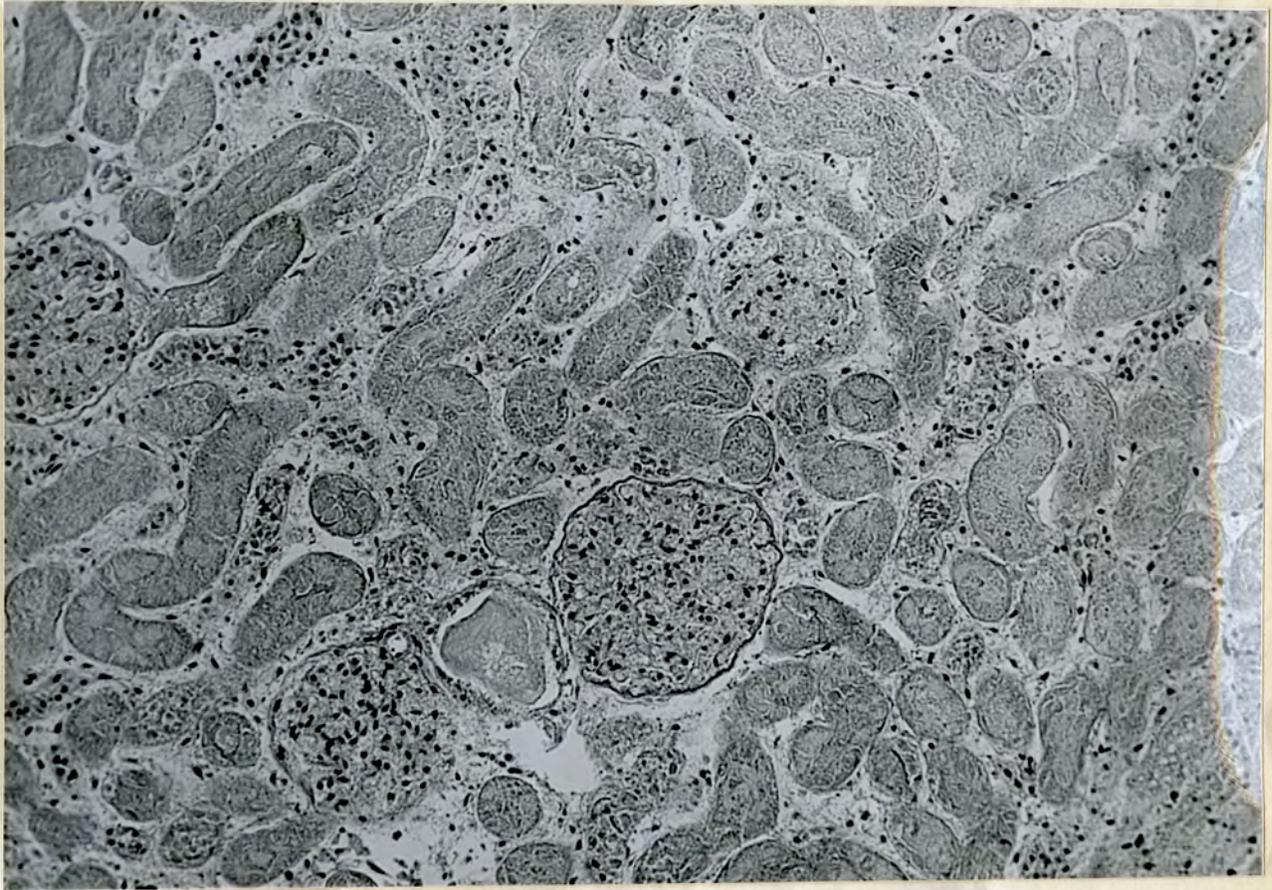


Fig. 26. COAGULATION NECROSIS, CELLULAR INFILTRATION  
OF LYMPHOCYTES AND POLYMORPHONUCLEAR NEUTROPHILES.

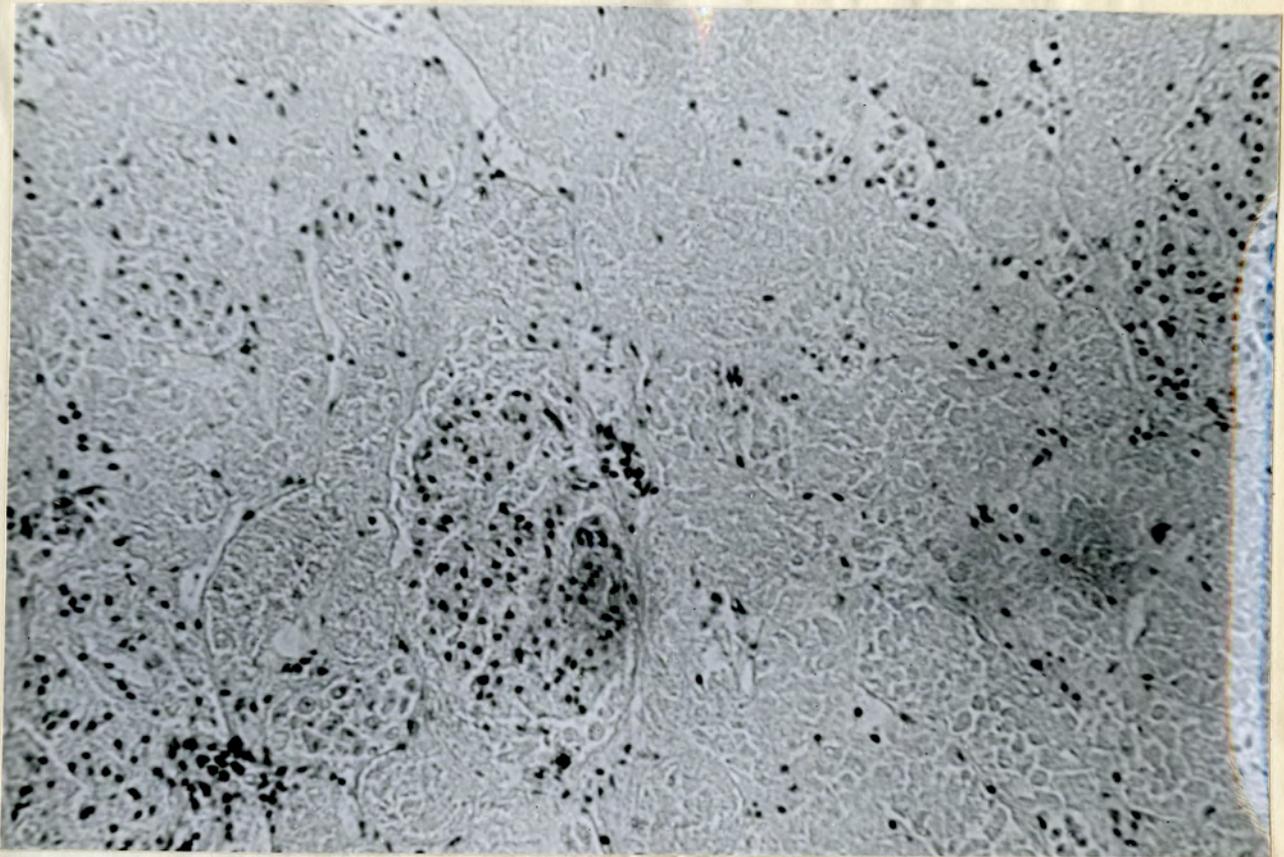


Fig. 27. COAGULATION NECROSIS. NOTE PAUCITY OF CELLULAR INFILTRATION.

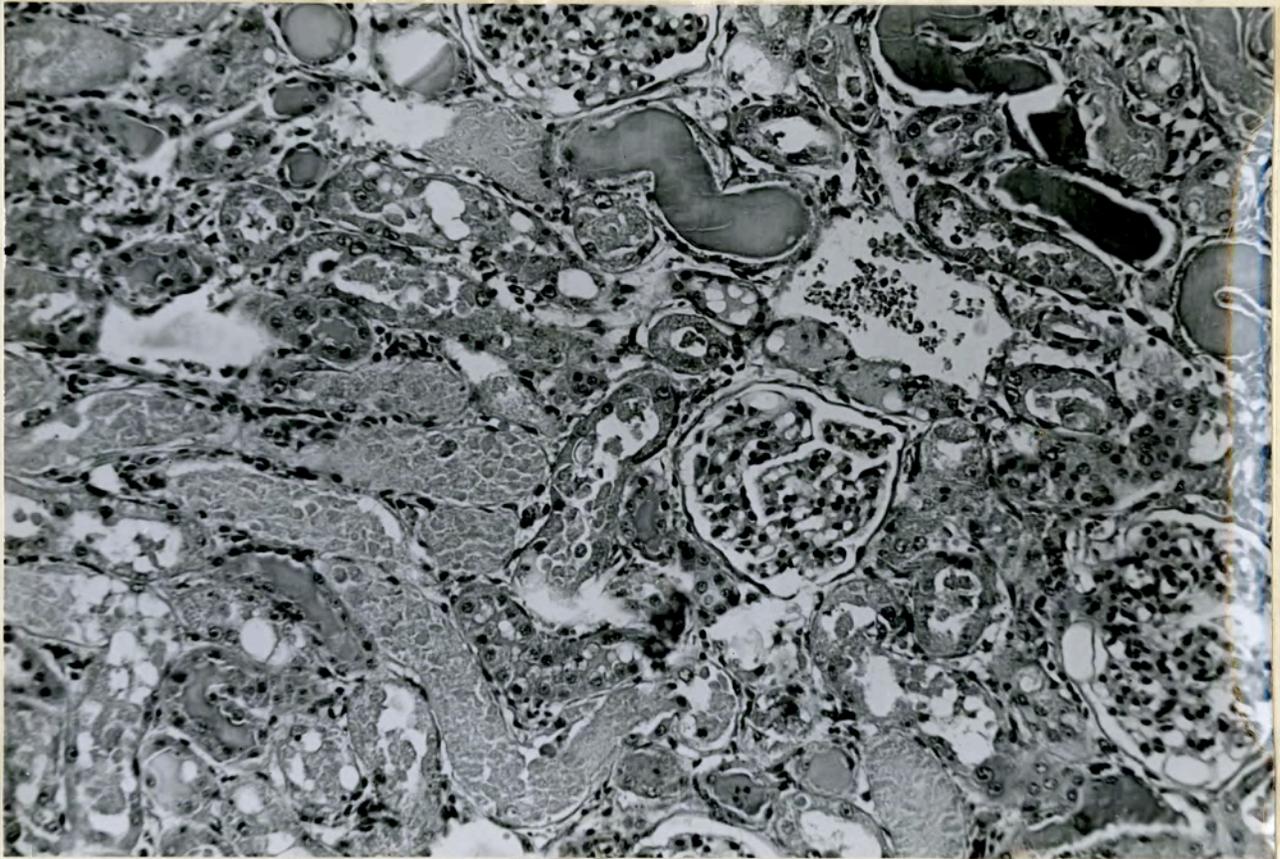


Fig. 28. NECROSIS AND DEGENERATION OF TUBULES. CELLULAR INFILTRATION OF POLYMORPHONUCLEAR NEUTROPHILES, CONGESTION AND HAEMORRHAGE.



Fig. 29. COAGULATION NECROSIS.

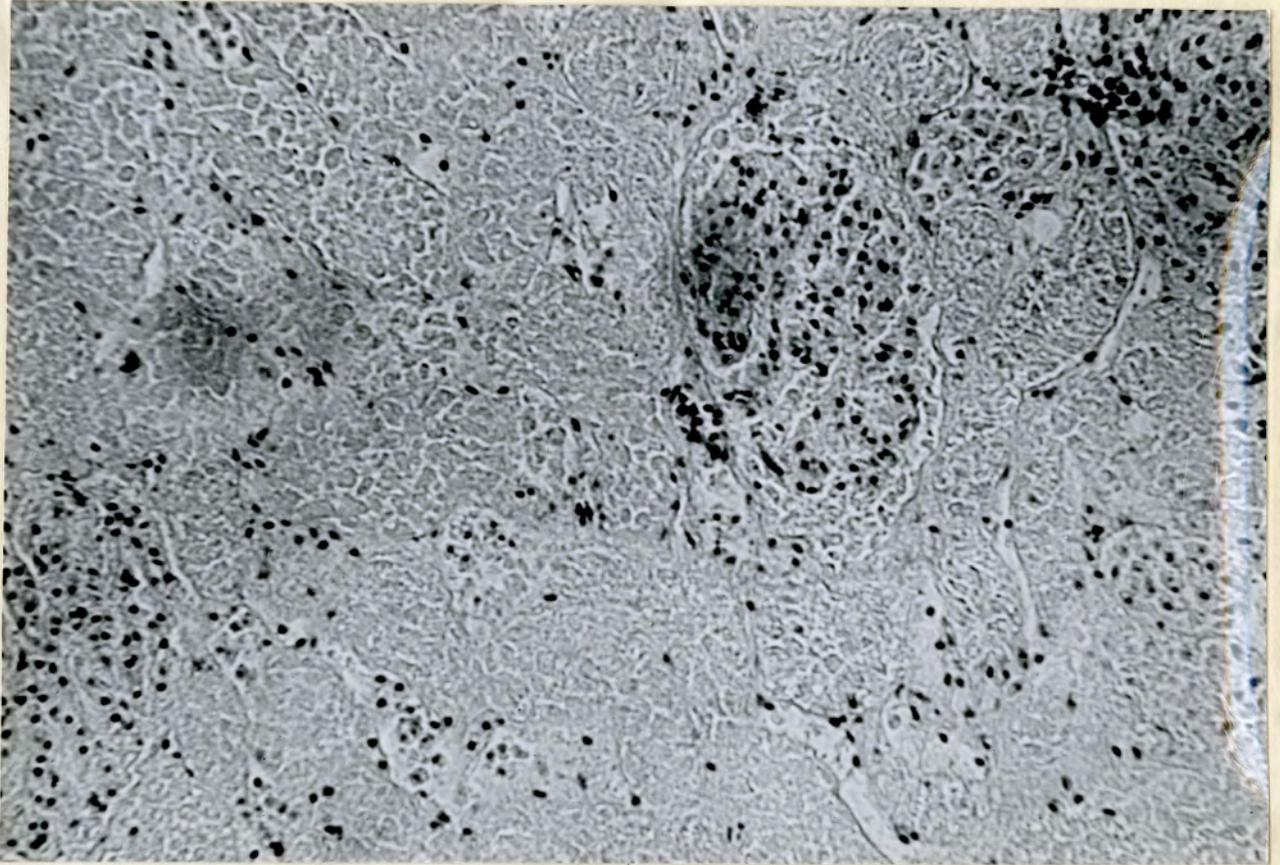


Fig. 30. ADVANCED COAGULATION NECROSIS. GLOMERULUS IS STILL DISCERNIBLE. CELLULAR INFILTRATION IS SLIGHT.



Fig. 31. DESTRUCTION OF KIDNEY PARENCHYMA WITH  
EXCESSIVE HAEMORRHAGE.



Fig. 32. SHOWING ANIMAL NO. 4 EIGHT DAYS  
AFTER TRANSPLANTATION OF KIDNEY INTO  
CERVICAL REGION.

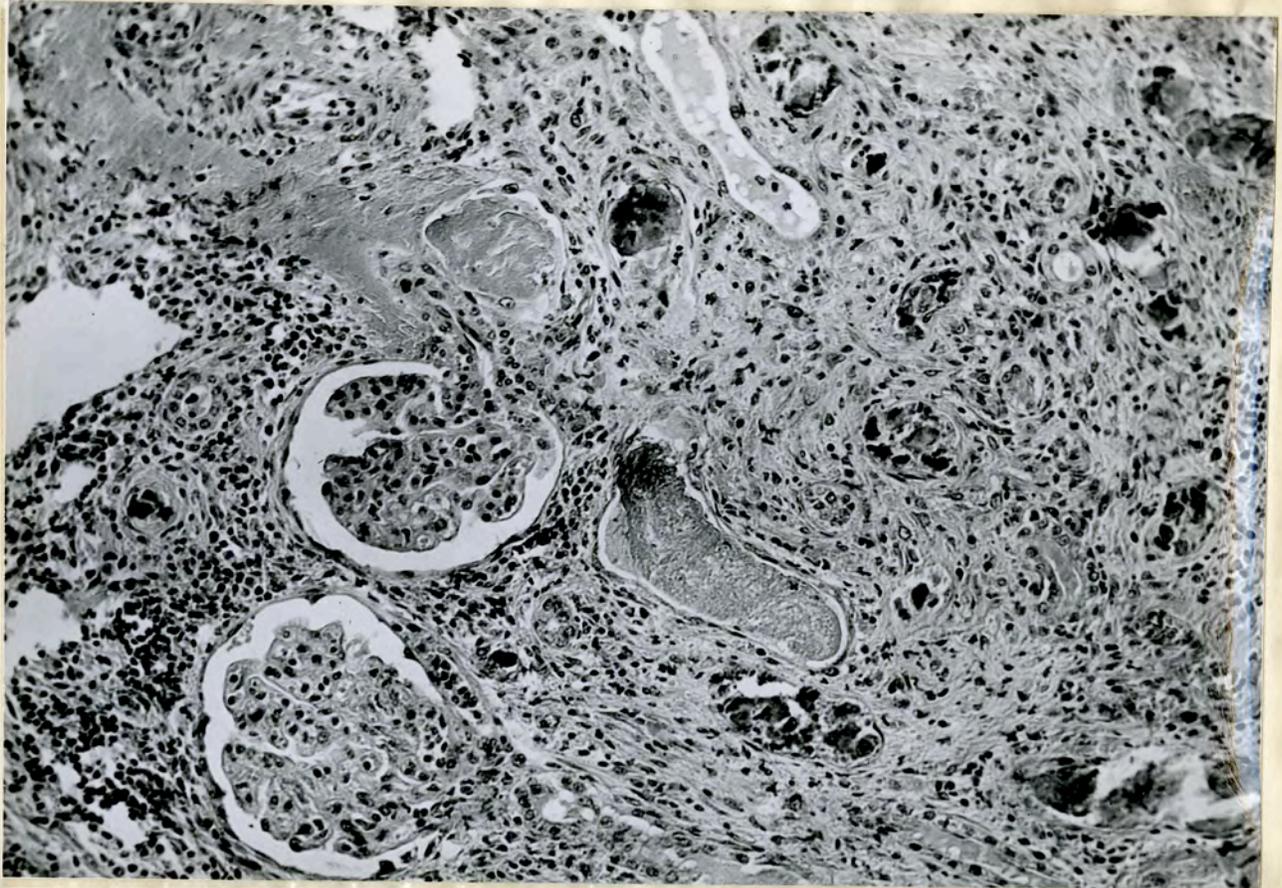


Fig. 33. ALMOST COMPLETE DESTRUCTION OF THE ARCHITECTURE OF THIS KIDNEY. ALSO NECROSIS OF THE TUBULES WITH CALCIFICATION, FIBROSIS, AND ROUND CELL INFILTRATION.

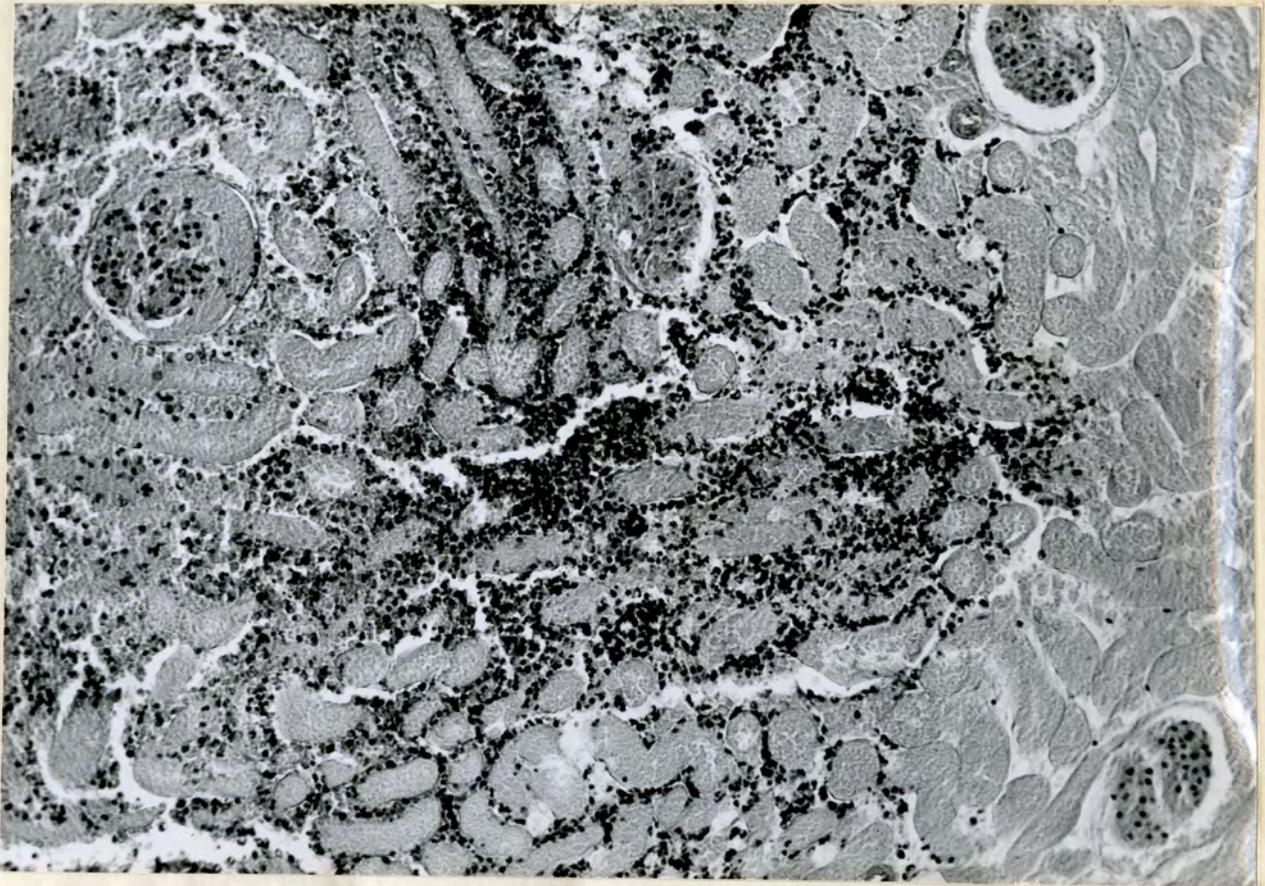


Fig. 34. COAGULATION NECROSIS.

### DISCUSSION

Autotransplanted kidneys implanted in the cervical region, functioned for many months. This site of transplantation subjected the kidney to hazards not encountered in the abdomen. The diameter of the carotid artery, to which the renal artery was anastomosed, is usually greater than the diameter of the renal artery, and therefore the suturing is subjected to a greater strain. The transplants are totally denervated. Still these autotransplants functioned indefinitely. These facts prove that the failure of the homotransplanted kidney to function for long periods of time, was not due to any mechanical failure.

#### The Effect of Penicillin Upon Transplanted Kidneys

During the period of time in which the homotransplanted kidneys functioned, it is obvious that the local and parenteral administration of penicillin did nothing to increase the functional life of the transplant. Williamson (17) in 1926, Carrel (6) in 1905 and other workers reported on kidneys which functioned normally for as long as, or longer than the maximum achieved in this series of experiments, which was fourteen days. The penicillin did, however, serve the purpose of decreasing the number of cases which became infected.

In autotransplanted kidneys penicillin obviously reduced the rate of infection, because the histopathology of these kidneys varies from that reported by Williamson (17) in 1926 insofar as there is no

evidence of hydro-nephrosis or infection after several months. These kidneys did not become enlarged and although there were a few areas of degeneration present, the histological picture was essentially normal.

Williamson (17) in 1926 reports that the typical histological picture in the homotransplant experiment "is that of acute atypical glomerular nephritis, followed by general acute nephritis". This would seem to be contradictory to the general picture in the experiments of this series where the histopathology was essentially that of nephrosis. In cases which had functioned for longer periods of time the picture changed to include a more general interstitial nephritis and the glomeruli were more frequently affected.

As Williamson (17) reported the primary lesions to be glomerular, this is perhaps an indication that penicillin does modify the process of nephritis in the homotransplanted kidney.

The Effect of Refrigeration upon Transplanted Kidneys

During the course of these experiments, it was noted that homotransplanted kidneys which were transplanted immediately into the neck of the recipient animal did not, on the average, function for a longer period of time than did those which were stored in a refrigerator for twenty-four hours. Therefore it was decided to determine if an autogenous transplant would survive as long after refrigerator storage as it would upon immediate transplantation. This did not prove to be the case. Autotransplanted kidneys, after storage in a refrigerator at approximately four degrees centigrade for twenty-four hours, functioned on an average, only as long as did the homotransplanted kidney. The action of these refrigerated kidneys was similar to that of the homotransplant. The urine tended to be bloody, and was excreted slowly. The ureter or catheter tended to become blocked with a clear mucoid fibrinous material and had to be "sucked out" frequently by means of a hypodermic syringe and needle to ensure constant urine flow. The histological picture in these kidneys was the same as that of a homotransplant.

The organismal differential of homotransplants did not seem to be destroyed by refrigeration, at least not by refrigeration, either at this temperature or for this length of time. However, the results obtained were not so conclusive, that this technique should be neglected. The fact that urine was secreted for fourteen days, after the kidney had been refrigerated for twenty-four hours, is remarkable. The use of

blood vessel banks at the present time is proof of the efficacy of tissue storage by means of refrigeration, and might be an indication that further work should be done along the line of refrigeration storage of kidney or other tissue. The work of Parkinson and Woodworth (13) in 1947 would indicate that "deep freezing" of kidneys fails to alter the organismal differential. As the period of twenty-four hour refrigeration was purely an arbitrary one, further work as to how long the kidney will remain viable when stored in a refrigerator should be carried out.

As neither the autotransplant nor the homotransplant functioned for a longer period than fourteen days after refrigeration, it would seem that refrigeration merely served to destroy the viability of the stored kidneys, or at least started an irreversible action of degeneration which quickly went to completion.

#### Halsted's Principle Regarding the Transplantation of Tissues

Halsted, as quoted by Woodruff (18) in 1949, "found that autotransplantation of parathyroid tissue in dogs was successful only if a deficiency of greater than one-half had previously been created in the recipient". He advanced the hypothesis that this deficiency condition was essential for the successful transplantation of endocrine tissue.

This principle would not seem to apply to the auto- or homotransplantation of other tissues. The control autotransplant in this series retained one normal abdominal kidney during the months of functioning of the transplant. Moreover the fact that homotransplants

in totally nephrectomized dogs did not function any longer than homotransplants in dogs with both normal kidneys would be an indication that over half or total deficiency of a given tissue would not materially increase the length of functional time.

ACTH as a Factor in Increasing the Functional Period of Transplants

Woodruff (18), Loeb (11) in 1930, Williamson (17) and other workers have observed that homotransplants become rapidly invaded by lymphocytes. This has been seen in the kidney homotransplants in this series. According to Woodruff (18), "it has been assumed by most workers in this field that the lymphocytes which accumulate around, and eventually permeate, a subcutaneous homograft, are responsible for its destruction, presumably by a process of phagocytosis".

Boyd (3) states that the lymphocyte is an unknown quantity. They do not phagocytose bacteria or other particulate matter. Therefore the likelihood of the kidney homotransplant being destroyed by lymphocytic phagocytosis seems remote. Boyd (3) again cites evidence that lymphocytes may produce antibodies or may store and carry these antibodies.

From the histological evidence presented by homotransplanted kidneys, this latter view would seem more probable. Another explanation for the presence of lymphocytes in homotransplanted kidneys would be that they are attracted to the site as soon as the process of necrosis starts. This theory might be substantiated by the findings of Williamson (17)

who sectioned homotransplanted kidneys at varying periods of time after transplantation. He found that the kidney appears to be essentially normal soon after transplantation. Later sections showed early glomerular changes and lymphocytic infiltration.

Selye (14) has shown that ACTH has a definite effect in decreasing the size of lymph nodes, and that lymphopaenia is produced by ACTH. He has also indicated that ACTH is useful in the treatment of nephrosis. Because nephrosis certainly plays a large part in the failure of homotransplanted kidneys to function and because so many other workers believe that lymphocytes play a part in the destruction of such kidneys, it was decided to administer ACTH to some animals in which transplantation had been carried out. The results of such administration were disappointing. The lymphocyte concentration in the kidneys which received ACTH was as marked as in the former cases as in animals which did not receive treatment.

#### Blood Grouping as a Factor in Increasing the Functional Period of Transplants

In the past it has frequently been hypothesized that if more accurate blood grouping could be established in dogs that homotransplants into an animal of the same blood group would probably be successful. This is doubtful. Lawler et al (9) transplanted a kidney in the human using donor and recipient of the same blood grouping. The kidney failed to function. Dukes (7) has pointed out that the isohaemagglutinins in dogs are so weak that no reaction occurs even if a dog is transfused with incompatible blood. It would therefore

seem that the agent which destroys the homotransplant is blood borne but that it is the individuality of the tissues which prevents successful homotransplantation, rather than incompatible blood groups. It would be interesting to see if an animal which had previously been desensitized to the donor's tissue would accept a homotransplant and permit it to remain functional.

SUMMARY AND CONCLUSIONS

A series of experiments was carried out on kidney auto-transplants and homotransplants, to determine the effect of penicillin, refrigeration, and ACTH on the longevity of the transplant.

1. Penicillin did not increase the functioning time of the auto- or homotransplant. It did, however, decrease the number of infected cases. It is possible that the disease process in homotransplanted kidneys was modified by the use of penicillin from that of glomerulonephritis to that of nephrosis.

2. Refrigeration of auto- and homotransplanted kidneys for twenty-four hours, at approximately four degrees centigrade, did not alter the organismal differential of these kidneys. It served rather to destroy the viability of the kidney.

3. ACTH did not decrease the lymphocytic infiltration of homotransplanted kidneys, nor did it affect the process of nephrosis.

These experiments would indicate that an incompatibility of tissues prevents successful homotransplantation. It would be interesting to determine the possibility of desensitizing the recipient animal to the tissues of the donor, to see what effect this would have on the functional life of a homotransplant.

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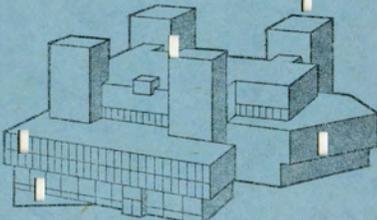


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