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AN ULTRASTRUCTURAL STUDY OF TUBULES, VASCULATURE
AND INTERSTITIUM IN HUMAN RENAL HOMOGRAFTS

by

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B.S. Longwood College, 1963
M.A. Indiana University, 1965

Thesis

submitted in partial fulfillment of the requirements for the
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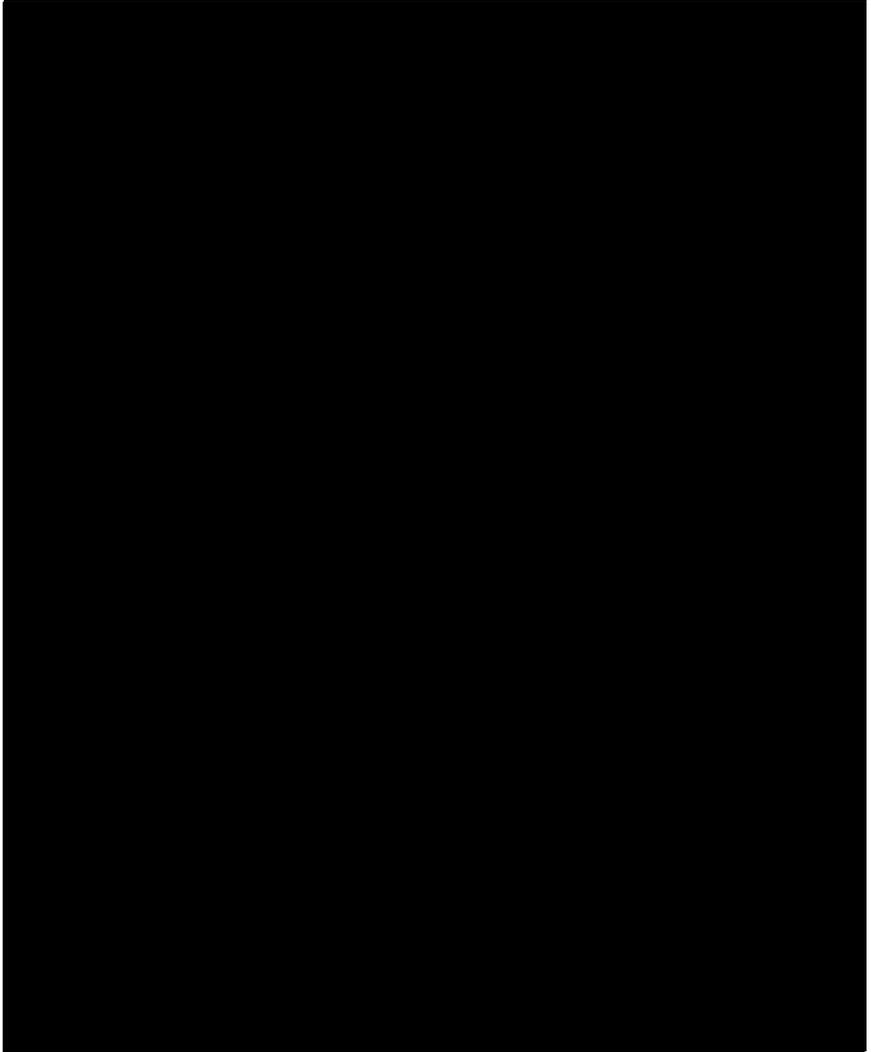
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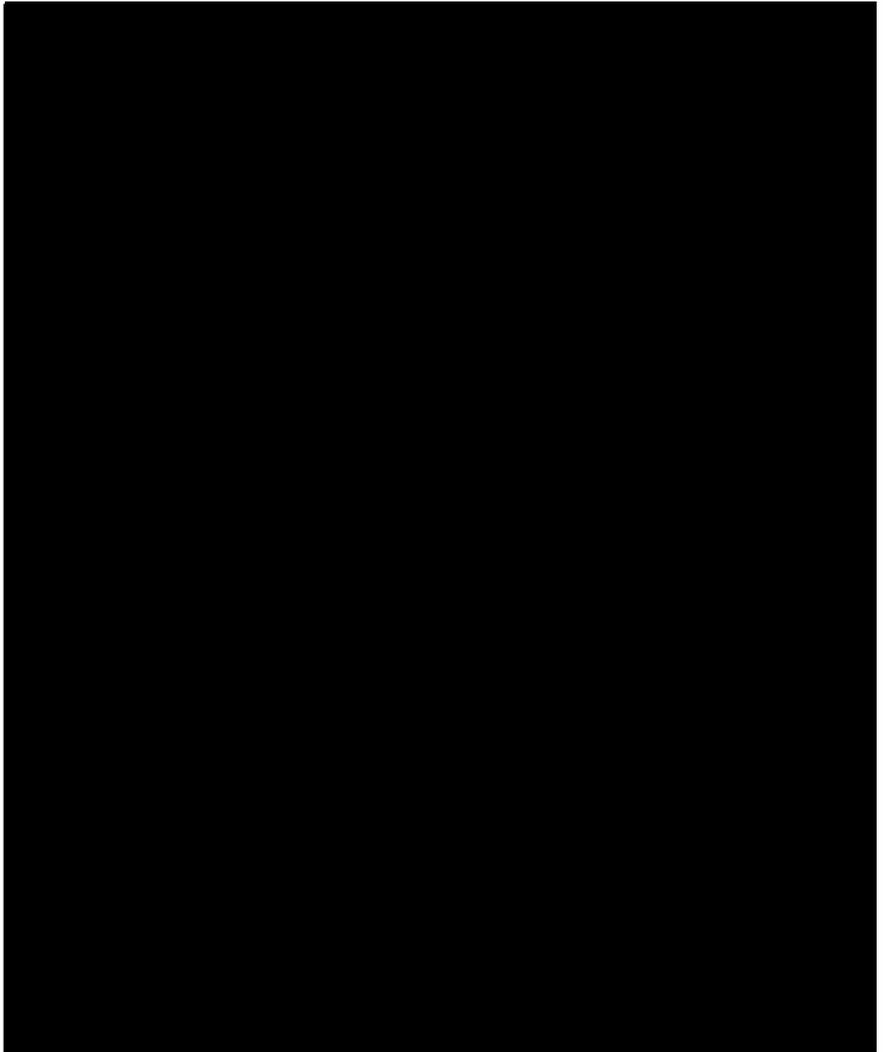
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INTRODUCTION

In recent years renal homotransplantation has become more frequently used in the treatment of terminal renal disease in man. By carefully selecting donors with the aid of tissue compatibility tests and employing immunosuppressive drugs, these transplants have become increasingly more successful. It is true, however, that even with good tissue match-up and drug treatment rejection episodes occur, frequently within the first two postsurgical weeks. The majority of these rejection crises may be reversed by increasing drug dosages and occasionally applying x-irradiation to the area of the transplant. In other cases rejection by the recipient is responsible for cessation of function of the transplanted kidney, terminating in its ultimate removal.

Millard et al. (1970) suggested that light microscopic studies of biopsy tissue obtained from transplanted kidneys at various intervals postoperatively would be useful in early clinical management of the patients. The primary purpose of the present study is to determine if electron microscopic studies of renal tubules in tissue obtained one hour postoperatively would also be useful in indicating

subsequent graft rejection or dysfunction.

Light (Dempster, 1952; Simonsen et al., 1952; Porter et al., 1964; and Shorter et al., 1964) and electron microscopic (Darmady et al., 1955; Kountz et al., 1963; Porter et al., 1964; and Williams et al., 1964) observations of canine homotransplanted kidneys are numerous but none of the biopsies studied were obtained prior to six hours post-transplantation.

Ultrastructure of normal human kidney has been described (Rhodin, 1958; Flume et al., 1963; Brewer, 1965; Myers et al., 1966; Tisher et al., 1966; Bulger et al., 1967; and Tisher et al., 1968) as has the ultrastructure of normal monkey renal tubules (Tisher et al., 1969). Electron microscopic studies of human renal homotransplanted tissue including the glomerulus, tubules and vasculature have also been reported. However, the majority of the biopsies described were obtained one week or more following transplantation (Hamburger et al., 1965; Porter et al., 1966; Shimamura et al., 1966; and Busch et al., 1971).

Hume et al. (1970) included one hour biopsies in their studies of glomerulonephritis. Weymouth et al. (1970) described the ultrastructure of the glomerulus one hour post-transplantation and were able to correlate changes in

the glomerulus with future function of the transplant.

Electron microscopic studies of the tubules, vasculature or interstitium one hour after transplantation have not been previously reported.

Therefore, it seemed pertinent to study these tissues, correlating structure with clinical function in an effort to determine if this information could be used to postulate the future of the individual renal transplant.

LITERATURE REVIEW

There is evidence that animal grafts of various types have been used by scientists and clinicians even as early as the Renaissance. In 1596, Gaspare Tagliacozzi (Beathard, 1969) described a technique in which a flap of tissue from the upper arm of a patient was utilized to restore a lost nose. Two hundred years passed before another significant contribution to the field of tissue grafting was made. Baronio (Beathard, 1969) performed free skin autografts in sheep and demonstrated that these grafts would survive.

In 1823, another nasal restoration operation was reported; Bunker used a free skin graft from the thigh (Beathard, 1969). Bert in 1863 (Beathard, 1969) observed differences in autografts, homografts and heterografts in experiments involving skin transplants in animals of the same and different species. He was one of the first to report that autografts survived longer than either homo- or heterografts. Further advances in the area of tissue grafting were reported by Reverdin in 1869 and Thiersch in 1886 (Beathard, 1969) who described the histological appearance of the successful skin graft.

It was not until the twentieth century that organ transplantation was attempted to any significant degree. The primary problems appeared to be mechanical and involved the reestablishment of the blood supply to the transplanted organs. These difficulties were alleviated when Carrel (1902) working in Chicago, devised some basic techniques for vascular surgery.

Renal Transplantation in Experimental Animals

The first successful organ transplants were reported in 1902 by Ullman in Vienna. His experiments included both renal autotransplants and homotransplants (allotransplants) in dogs. Coinciding with Ullman's work was that of De Castello (1902) concerning homotransplantation in dogs. In 1906, Carrel and Guthrie introduced improved techniques for vascular anastomoses and reports of technically successful transplantation followed. During this period Carrel made many significant contributions to the field of renal transplantation and, as a result, was awarded the Nobel Prize in Physiology and Medicine in 1912.

Carrel and Guthrie (1906b) developed an "en masse" method of renal homotransplantation. Both kidneys, upper ureters, suprarenals, segments of the aorta and inferior vena cava, and surrounding tissues were removed from one

dog and placed in another dog. The kidneys of the recipient were then removed and the animal survived for ten days (Carrel, 1908). The results of "en masse" renal transplantations in 14 cats were reported by Carrel in 1908. Twelve of the cats died within two weeks, five of these died immediately following surgery. The transplanted kidneys of the remaining two cats excreted urine until the cats died at 31 and 36 days, respectively. From these experiments, Carrel concluded that "...an animal which has undergone a double nephrectomy and the grafting of both kidneys from another animal can secrete almost normal urine with his new organs and live in good health at least for a few weeks. This demonstrates that it is possible to reestablish efficiently the function of transplanted kidneys."

During the next 20 years many reports of renal auto-, homo-, and heterotransplantations appeared in the literature (Carrel, 1909 and 1910; Villard and Tavernier, 1910; and Villard and Perrin, 1913). The first long-term survival of a renal transplant was reported by Zaaiker in 1908. He removed the kidney of a dog and placed it in the inguinal region of the same animal. Homotransplants in dogs were described by Unger (1910) and Ingebrigtsen (1914). The survival time in these experiments was no longer than 24 days.

The authors did describe some minor histological changes in the homografts consisting primarily of lymphocytic (round cell) infiltration of the interstitium. Ingebrigtsen concluded that differences between donor and recipient were responsible for homograft failure.

Working at the Mayo Clinic, Dederer (1920) transplanted the kidney of a puppy to the cervical area of its litter mate. The kidney functioned well for 26 days when the animal died of distemper. Williamson (1923 and 1926) performed autogenous and homogenous renal transplants in dogs and goats. He compared the survival times of the two types of transplants and found the autotransplants survived for months whereas the survival times of the homotransplants averaged four days. He concluded that the differences in survival times were due to the degree of similarity in the genetic makeup of the donor and recipient. Williamson also described histological changes in the transplants. Microscopic observations were also described by Ibuka (1926) and Holloway (1926). Ibuka reported lymphocytic and plasma cell infiltration in the interstitium and tubular degeneration. He described the glomeruli as appearing normal. Holloway found that the cellular abnormalities could be observed within the first 24 hours post-transplantation.

In 1924 Avramovici reported results from dog and cat heterotransplants and several canine homotransplants. Two of the heterotransplants were described as surviving 49 and 58 days, respectively. The homotransplants did equally as well with 73 day survival for one unilateral transplant and as long as 60 days for bilateral transplants. Microscopic studies were reported in only one case, a bilateral homotransplant that survived for 56 days. Some inflammation of the interstitium, cellular disruption and necrosis of the tubular epithelium and lesions in the glomeruli were reported. The lack of confirmation of such long survival times for hetero- and homotransplants by other investigators leaves room for doubt as to the validity of some of Avramovici's findings.

Wu and Mann (1934) described the results of five autotransplants and 13 homotransplants in dogs. Biopsies were taken every 24 hours for five days from the time of transplantation. The kidneys were placed in the cervical region with anastomoses to the common carotid artery and external jugular vein. They found that the autotransplants survived for an average of 72 hours and the homotransplants for an average of 31 hours. The function of both types of transplants was identical, however, histologically, the

homotransplants were more pathological than the autotransplants. As had previously been reported, round cell infiltration of the interstitium and degeneration of the tubules were present in the homotransplants. The glomeruli were reported as appearing normal.

In 1947 Parkinson and Woodworth described the results of auto-, hetero- and homotransplantation in goats. After removal from the donor, the organs were placed in cold storage at -40°F for one to two weeks. There were many technical errors in these experiments; but in those that were successful the authors found, as had other investigators, that the autotransplants survived longer than did either the homo- or heterotransplants. Lefebvre (1949) demonstrated that a renal transplant in dogs is capable of normal urea clearance for 19 days after transplantation. In his experiments the recipients underwent bilateral nephrectomy shortly after the donor kidney had been placed in the neck. The longest survival time was 21 days.

Dempster (1953 and 1955), Simonsen et al. (1953) and Darmady et al. (1955) described in detail the histological appearance of canine renal homotransplants beginning one day after transplantation. Plasma cell, lymphocytic and some

monocytic infiltration of the interstitium was present primarily in the vicinity of the glomeruli and blood vessels. Swelling of the endothelium and/or intima of some small arteries and tubular degeneration were also noted. In some cases, coagulation necrosis was present in an entire tubule. The glomeruli were cytologically normal. It was suggested that ischemia due to vascular changes was directly responsible for tubule necrosis and ultimate transplant failure. Simonsen and his group also found that if the first transplant was removed and a second from the same donor replaced the first, the second transplant disintergrated at a faster rate than did the original. If, however, the second transplant was from a different donor, there was no significant change in rate of disintergration from the first.

Medawar and co-workers (Gibson and Medawar, 1943; Medawar, 1944, 1945, 1946a and 1946b) working with skin grafts in rabbits first demonstrated the immunological basis of transplant rejection. In the next few years several reports were published confirming the immunological aspects of rejection in kidney transplantation (Billingham et al., 1953; Egdahl and Hume, 1955 and 1956; and Hume and Egdahl, 1955). In 1959, Schwartz and Dameshek and Meecker

et al. reported the correlation of the immunosuppressive drug, 6-mercaptopurine, and survival of skin grafts in rabbits. The drug was injected into the animals at the time of grafting and at intervals thereafter. Both groups reported increased survival time of the graft with the drug. Calne (1960) and others (Zukoski et al., 1960 and 1962) found that 6-mercaptopurine also increased the survival time of renal homotransplants in dogs. In 1961, Calne and Murray treated dogs with another immunosuppressive drug, Imuran (azothioprine) after renal homotransplantation. They reported that this drug also prolongs transplant survival and is not as likely to cause bone marrow hypoplasia as is 6-mercaptopurine. Zukoski, Callaway and Rhea (1965) reported that prednisone is also capable of prolonging canine homotransplant survival. In one case, prednisone treatment was initiated two days prior to transplant and given in doses of 30 mg per day for 428 days. At the time of their report the dog had been off prednisone for 749 days with normal function of the transplant.

Several investigators (Martin et al., 1964; Lee et al., 1964; Cleveland et al., 1965; and Kauffman et al., 1965) have shown that local x-irradiation to the area of the transplanted kidney will prolong kidney function in dogs although

not as satisfactorily as do Imuran and prednisone. Cleveland and his group (1965) suggested that "...local graft radiation interferes with cells which pick up and transport antigen from the graft to the antibody producing centers of the host."

Ultrastructural studies of transplanted canine kidneys (Shorter et al., 1964; Porter, 1964; and Williams et al., 1964) have added to the morphological description of transplant rejection. As a result of these observations, it has been suggested that the lymphocytes that infiltrate the interstitium give rise to plasma cells which cause immunological destruction of the peritubular capillaries. It is postulated that this disruption of the capillaries results in ischemia which is responsible for tubular necrosis and transplant failure.

Renal Transplantation in Man

During the first half of this century attempts of renal transplantation were confined primarily to experimental animals. There are, however, some early reports of kidney transplant operations performed in an attempt to prolong the survival of patients with chronic renal disease. In 1906, Jaboulay made two unsuccessful attempts to transplant porcine kidneys to two women suffering from terminal renal

disease. The renal vessels were anastomosed to the brachial artery and median vein in the area of the elbow. Neither heterotransplant functioned more than two or three hours after transplantation. Two decades later, Voronoy (1936) performed a homotransplant operation placing the kidney in the groin. The patient survived only 48 hours post-operatively with no histological studies described. Hume et al. (1955) reported that in 1945 Landsteiner and Hufnagel removed the kidney from a cadaver and transplanted it to a patient who suffered from acute renal failure. The kidney was transplanted to the arm via the brachial artery and cephalic vein. The transplant never secreted an appreciable amount of urine and was removed after 48 hours. The patient's own kidney resumed functioning soon after transplantation.

In 1950 Lawler et al. transplanted a cadaver kidney to a patient of the same ABO blood type whose left kidney had been removed as a result of polycystic kidney disease. The renal vessels of the transplanted kidney were anastomosed to the renal vessels of the recipient and the donor ureter was anastomosed to the host ureter. The transplant functioned well post-operatively and the patient left the hospital 30 days following surgery. On the 62nd day indigo carmine dye

was injected and the presence of a slight stricture in the left ureter was observed. The area was cleaned during an operation on the 63rd day but the kidney was not removed until nine and a half months post-transplantation. At this time it was discovered that the kidney had been completely destroyed (Lawler et al., 1951).

Hume et al. (1955) reported that Servelle and co-workers (1951) performed a human homotransplantation in which the kidney was placed in the pelvis and the renal vessels anastomosed to the iliac vessels. By the 19th post-operative day a urine output of 600 cc per day was observed. Unfortunately, the patient died unexpectedly on the same day. Dubost et al. (1951) performed two human homotransplantations, placing the kidneys in the pelvis with the renal vessels anastomosed to the right iliac vessels of the hosts. In one case, the kidney excreted 230 cc of urine in a 24 hour period before the patient died on the 16th post-operative day. The second kidney excreted an insignificant amount of urine.

Kuss, Tienturier and Milliez (1951) described the results of five human homotransplantations in which cadaver kidneys were placed in the pelvis with anastomoses of the renal vessels to the hypogastric artery and external iliac vein. The ureter was sutured externally to the skin.

One of the patients died on the operating table and in another patient the transplanted kidney was removed at 48 hours post-transplantation. The ureter became necrotic in the three remaining cases. The third kidney excreted small amounts of urine for 18 days and the patient died on the 20th day. In the fourth transplant maximum urine output for 24 hours was 72 cc on the 10th day. The kidney was removed after three and a half months. The fifth patient died 35 days post-operatively. The maximum urine output for this patient was 45 cc in one day. At autopsy, the kidney was found to be extensively infarcted.

One of the first human renal homotransplants with a live donor was reported in 1953 by Michon et al. The recipient was a 16 year old boy who underwent a nephrectomy of what was soon discovered to be his only kidney. His mother donated a kidney which functioned well for three weeks and was then rejected. There was no period of acute tubular necrosis as had been found in cadaver transplants (Hume, et al., 1955). Lymphocytic infiltration of the interstitium was reported.

In 1955, Hume et al. published the results of nine renal homotransplantations performed from 1951 to 1953. Cadaver donors were used in each case. Only four of the nine functioned well for as long as 180 days. All four

kidneys were placed in the thigh with anastomoses to the femoral vessels. The ureters were brought through the skin. A period of acute tubular necrosis occurred before the four kidneys functioned and later biopsies revealed some tubular degeneration. Round cell infiltration of the interstitium, especially in the cortical regions, was also reported. In the latter stages of rejection thrombosis was present in the smaller renal vessels with an increase in the thickness of the intima in a few cases. The glomeruli were relatively normal. The authors suggested that renal cell death and subsequent kidney failure were due to ischemia.

Murray et al. (1955) and Merrill et al. (1956) reported results of renal homotransplantations involving identical twins. The kidneys were placed in the iliac fossa and functioned well from the outset. Following these first reports several more accounts of homotransplantation in identical twins appeared in the literature (Murray et al., 1958; Menville et al., 1961; Goodwin et al., 1962; Hodges et al., 1963; and Hopewell et al., 1964). No rejection episodes were encountered in any of the cases reported.

Renal homotransplantation between nonidentical twins was reported by Hamburger et al. (1959) in Paris and by Merrill et al. (1960) in Boston. Both groups administered

sublethal whole body x-irradiation to the recipient prior to transplantation. The French team reported a rejection crisis in the third week which they were able to reverse with the use of immunosuppressive drugs. The transplant was still functioning when the report was written four months later. The group in Boston reported normal function of the transplant 14 months post-operatively although histological examination revealed evidence of rejection at eight months.

From the results of renal transplantations with unrelated living donors, Kuss et al. (1962) concluded that "A human being can live a long time with a transplanted kidney from an unrelated donor (17 months in one case)", and suggested that tests to select the best donors should be devised. They also stated that technical conditions should be improved and more effective immunosuppressive drugs should be developed.

Since 1962 several reports of human renal homotransplantations have been published (Hamburger et al., 1962a and b; Hamburger et al., 1963; Hume et al., 1963; Kuss et al., 1963; Murray and Harrison, 1963; Shackman et al., 1963; and Hamburger et al., 1965a, b and c) in which whole body x-irradiation was administered to the recipient

before transplantation. The transplant survived only a few days in most cases and occasionally the patient died from marrow aplasia.

In recent years the use of immunosuppressive drugs has increased and at the present time treatment with these drugs is the primary method of inhibiting transplant rejection. In 1963 Murray et al. reported that the results of drug therapy with Imuran, azaserine, actinomycin C, and prednisone were very encouraging. Since then, several accounts have been published in which Imuran, actinomycin C and/or prednisone were administered with good results. In some cases, local graft x-irradiation was used in combination with drugs during rejection episodes (Hume et al., 1963; Mowbray et al., 1965; Hume et al., 1966; Pletka et al., 1969; Woodruff et al., 1969; Hume et al., 1970; and Weymouth et al., 1970).

Light and electron microscopic studies of human transplanted kidney tissue from biopsies and necropsies (Galle and Montera, 1962; Dempster et al., 1964; Kincaid-Smith, 1964; Porter, 1964; Starzl et al., 1965 and 1966; Porter, 1955; Porter et al., 1966; Shimamura et al., 1966; Hume, 1967; Glasscock et al., 1968; Beathard, 1969; Millard et al., 1970; and Busch et al., 1971) have confirmed the results of

studies of transplanted canine tissue (Kountz et al., 1963; Dempster and Williams, 1963; Porter, 1964; Shorter et al., 1964; Williams et al., 1964; and Porter, 1965). The outstanding features of both human and canine homotransplanted kidneys include:

1) round cell (lymphocytic) and to a lesser extent polymorphonuclear cell infiltration of the interstitium within a few hours of transplantation. This infiltration was greater in tissue that was being actively rejected and was present especially in the vicinity of damaged tubules. Busch et al. found PMN's present in the peritubular capillaries and stated the amount of infiltration varied directly with the degree of arterial and glomerular injury. Some hemorrhage in the interstitium was also reported by Busch and his co-workers.

2) thickening of the intima of small arteries due to cytoplasmic swelling of the endothelial cells and often accompanied by the separation of these cells from the underlying basement membrane. Vacuolation of the sarcoplasm of the smooth muscle cells surrounding the arteries often appeared within three hours after transplantation (Busch et al. 1971).

3) polymorphonuclear leukocytes and platelets clinging to the endothelial lining of the peritubular capillaries resulting in a breakdown of the capillary wall and escape of fluid into the interstitium.

4) varying degrees of tubular damage leading up to necrosis of the tubular cells due to ischemia. The cells of the proximal tubules were more affected than those of the distal and collecting tubules. Intense vacuolation was evident in some cells while other cells of the same tubule the number of vacuoles was minimal. In the necrotic appearing cells coalescing vacuoles are often present (Williams et al. 1964).

Weymouth et al. (1970) described ultrastructural changes in the glomeruli of human homotransplanted kidneys as early as one hour after transplantation. These alterations included hypertrophy and hyperplasia of the endothelial cells, variability in the thickness of the basement membrane, increase in the number of polymorphonuclear leukocytes, and in some cases, a large amount of cellular debris in Bowman's space. The authors found that "The severity of glomerular changes correlated roughly with the future renal function of the graft...".

Occasionally, rejection of the homotransplant occurs within a few minutes after vascular anastomosis is completed.

Histological studies of this "hyperacute rejection" revealed polymorphonuclear leukocytic infiltration of the glomeruli and peritubular capillaries and a lack of mononuclear cells. Fibrin "plugs" were found in peritubular capillaries of some one-hour biopsies. Progressive thrombosis and fibrin accumulation were followed by cortical necrosis (Williams et al., 1967; Starzl et al., 1968; Beathard, 1969; and Myburgh et al., 1969). Williams et al. (1968) concluded that "...humoral antibodies to histocompatibility antigens may be responsible for hyperacute graft rejection."

MATERIALS AND METHODS

Biopsies from 17 renal homotransplant patients at the Clinical Transplant Center at the Medical College of Virginia were studied. All patients had experienced terminal renal disease and required hemodialysis for survival. In most cases, bilateral nephrectomies were performed either prior to or at the time of transplantation. In two cases (S.G. and C.J.) nephrectomy occurred after transplantation (Table I).

Donor kidneys removed from cadavers (E.B., W.D., S.G., C.J., E.Mc., and C.S.) were without a blood supply for approximately 40 to 50 minutes while the time of renal ischemia for those from living related donors varied from 15 to 30 minutes. After removal from the donor, the kidneys were perfused at 10°C with 500 cc of Ringer-Lactate solution to which heparin and human albumin were added (Weymouth et al, 1970). The donor kidney was placed in either iliac fossa of the recipient in a retroperitoneal position. The common, internal or external iliac vessels were used for anastomosis with the renal vessels. Usually, the ureter of the transplanted kidney was inserted into the recipient's

Table I. Clinical Course¹

Patient	Sex	Age	Disease	Donor	Tissue Typing ²	Date of Transplantation	Follow-Up
Excellent Clinical Course							
E.B.	M	28	C.N.	Cad	A	10-67	Discharged - Good function
F.C.	F	29	C.P.	RLD	A	9-68	Discharged - Good function
W.C.	M	16	C.G.	RLD	B	6-67	Discharged - Good function
R.C.	F	40	C.P.	RLD	C	9-67	Discharged - Good function
C.J.	F	39	Tox. of Preg.	Cad	A	10-67	Discharged - Good function
V.M.	F	12	C.P.	RLD	B	7-67	Discharged - Good function
R.M.	M	26	C.G.	RLD	A	1-68	Discharged - Good function
P.W.	M	26	C.G.	RLD	-	8-66	Discharged - Good function

Abbreviations: C.N., chronic nephritis; C.P., chronic pyelonephritis; C.G., chronic glomerulonephritis; P.K.D., polycystic kidney disease; Cad, cadaver; RLD, related living donor.

¹ All patients received four to six treatments of 150r doses of x-irradiation each, post-operatively. Imuran and prednisone were also administered post-operatively.

² A, less than 5% major mismatches; B, more than 5% major mismatches but no definite units of major group mismatches; C, 1 unit major group mismatch with less than 25% major mismatches; D, 2 units major group mismatches or more than 25% major mismatches (Weymouth et al., 1970).

Table I. continued on page 24

Table I. (con't)

Patient	Sex	Age	Disease	Donor	Tissue Typing	Date of Transplantation	Follow-Up
<u>Transplant Rejected</u>							
W.D.	M	25	C.P.	Cad	-	7-66	Discharged - Good function Rejection episode and BUN remained above 25
S.G.	F	35	C.P.	Cad	C	8-66	Hyperacute rejection 8-66; transplant removed 8-66.
W.J.	M	31	C.P.	RLD	D	7-67	Moderate rejection 8-67; transplant removed 9-67.
N.K.	F	30	C.P.	RLD	-	12-66	Transplant removed 2-67; sepsis around transplant.
J.L.	M	29	C.P.	RLD	C	3-67	Clinical course erratic; died 3-68 of renal failure.
E.M.	F	50	P.K.D.	Cad	-	12-66	Chronic rejection 3-67; died of subdural hemorrhage 1-70.
C.R.	M	14	C.P.	RLD	-	7-66	Chronic rejection 10-66; transplant removed 1-69.
S.S.	M	22	C.G.	RLD	C	7-66	Chronic rejection 10-66; transplant removed 3-67.
C.S.	M	28	C.P.	Cad	-	7-66	Chronic rejection 11-67; discharged - good function.

bladder. Occasionally, the host ureter was anastomosed to the donor ureter or renal pelvis (Hume, 1967).

All patients were treated postoperatively with Imuran, prednisone and local x-irradiation (given in four to six treatments of 150 r doses each). Drug treatment was increased if rejection was experienced.

Of the 17 patients involved in this study, one (S.G.) experienced hyperacute rejection and underwent transplant nephrectomy three days following surgery. Eight other patients (W.D., W.J., N.K., J.L., E.MC., C.R., S.S., and C.S.) had no rejection crises within the first two weeks but did experience mild, chronic rejection subsequently (Table I). The remaining eight patients (E.B., F.C., W.C., R.C., C.J., V.M., R.M., and P.W.) had a good clinical course with no rejection episodes (Table I).

In five cases (E.B., W.C., R.C., J.L., and V.M.) biopsies were taken from the donor kidney just prior to transplantation (control kidneys) and used for comparison with post-transplanted tissue. Wedge biopsies were taken from the 17 transplanted kidneys one hour postanastomosis. The tissue was fixed immediately in cold (4°C) phosphate-buffered 3% glutaraldehyde (Sabatini et al., 1963) (appendix) and post-fixed in 2% phosphate-buffered osmium tetroxide

(Millonig, 1962) (appendix). It was embedded in D.E.R. 332 (Lockwood, 1964) (appendix) after dehydration in an ethyl alcohol series (appendix). A Porter-Blum MT-2 ultramicrotome was employed in cutting both thick and thin sections. The 1 μ thick sections were placed on glass microscope slides and stained for approximately one minute with a 0.1% toluidine blue and 0.1% methylene blue stain (Lynn et al., 1966) (appendix). After a water rinse, they were air dried, rinsed with xylene and mounted in Permount. Thin sections of 600\AA° - 1000\AA° thickness were obtained with a diamond knife and placed on 200 and 400 hole uncoated copper grids. They were stained for four minutes with lead citrate (Reynolds, 1963) (appendix).

Thin sections were studied with an RCA EMU-3G electron microscope. Light photomicrographs of the thick sections were taken with a Leica camera mounted on a Leitz microscope with a Leitz 1/3x adapter.

RESULTS

Only the tubules, vasculature, and interstitium of the cortex kidney were studied in this investigation. These tubules include, in the order of sequence as found in the kidney, the proximal convoluted tubule, the thick descending limb of the thin loop of Henle (also referred to as the straight portion of the proximal tubule), the thin loop of Henle, the ascending thick limb of the loop of Henle (also referred to as the straight portion of the distal tubule), the distal convoluted tubule and the collecting tubule. The relationship of these tubules to each other is shown in Figure 1.

Light Microscopy

Donor (Control) Biopsy

Proximal Tubules. The epithelial cells of the proximal convoluted tubules were simple low columnar in shape (Fig. 2) and contained a spherical nucleus which was usually located in the basilar position although in some instances it was observed in the center of the cell. A nucleolus was evident as a dense, spherical structure in most nuclei. Lateral cell membranes were indistinct in four cases. The tissue of the fifth case (J.L.) contained

large extracellular spaces between adjacent tubule cells with thin cytoplasmic bridges connecting adjoining cells (Fig. 3). The nuclei of these cells were identical to those found in the proximal tubule cells previously described. In this case the cytoplasm stained darkly, making any organelles in the cytoplasm indistinguishable. The cytoplasm of the proximal tubule cells in the biopsies of the other four patients did not stain as darkly and the presence of mitochondria was indicated by small, dense localizations throughout the cells. Vacuoles and, occasionally, large darkly staining granules were also noted. Other organelles and inclusions such as Golgi apparatus, endoplasmic reticulum and lipid droplets could not be distinguished. In many areas of the proximal tubules, a brush border was observed lining the lumen (Fig. 2). This border was not distinguishable in J.L.'s biopsy. Cellular debris was found in the lumina of all proximal tubules observed. The amount of debris varied with the case and with individual tubules within the same case. Very little luminal debris was present in the tissue of R.C. (Fig. 2) where as the lumina in the tissue of E.B. were packed with debris (Fig. 4). The debris appeared to consist of cytoplasmic fragments containing

mitochondria, vacuoles, dark granules and, occasionally, nuclei. The presence of microvilli in the debris as shown by electron microscopy indicated that the debris is composed of pieces of proximal tubular cells. In some areas, large cytoplasmic projections into the lumina from the apical surface of the tubule cells were readily observed (Fig. 2). The apical membrane covering these projections was void of a brush border as described by Tisher et al. (1966). In some instances the cell membrane appeared to be ruptured.

Loop of Henle. The tissue used in this study was obtained from the cortical region of the human kidney and therefore did not include samples of the thin loop of Henle which is located in the medullary portion of the human kidney. Occasionally sections from the thick limbs of the loop of Henle were observed. In the case of the descending thick limb (pars recta of the proximal tubule of Tisher et al., 1966), the cells were simple cuboidal in shape with a centrally placed nucleus. The lateral cell membranes were not obvious and the brush border was not as prominent as in the proximal convoluted tubules. In many instances, it was impossible to distinguish between the two parts of the proximal tubule by cytological differences only. Serial

sections are necessary for positive identification of the tubules.

The ascending thick limb of the loop of Henle (pars recta of the distal tubule of Tisher et al. 1968) was composed of a layer of low cuboidal cells (Fig. 6). In some areas it was possible to distinguish the lateral cell membranes whereas in other areas these membranes were observed. The cytoplasm apical to the nucleus tended to bulge into the lumen. There appeared to be few organelles in the cytoplasm and little debris in the tubule lumina.

Distal Tubules.

Simple cuboidal cells

lined the lumina of the distal tubules. The nuclei were identical in shape and chromatin pattern to those observed in the proximal tubules but were located at various levels in different cells of the same tubule. A single nucleolus was usually seen in each nucleus. The presence of mitochondria, especially in the basilar two-thirds of the cells was indicated by moderately dense areas in the cytoplasm. The low magnification made it impossible to ascertain the shape of these organelles. In some cells, darkly staining granules, larger than mitochondria, were observed especially in the apical region of the cells. Lateral cell membranes were observed in some instances (Fig. 5). Some

extracellular spaces between the distal tubule cells of one case (J.L.) were noted, but these spaces were not as prominent as those found in the proximal tubule of the same case. In addition, the cytoplasm of these cells did not appear to be as condensed as that of the proximal tubule cells. A brush border was not observed in any of the distal tubules studied.

Collecting Tubules. The shape of the epithelial cells of the collecting tubules ranged from simple cuboidal (Fig. 6) to simple columnar (Fig. 7), however, the majority of sections studied contained low columnar cells. The nuclei were spherical and in most cases, contained one nucleolus. The nuclei were variably located in the cytoplasm but was usually centrally placed. Few organelles were present in the cytoplasm although in some tubules the presence of mitochondria was indicated (Fig. 7). As in the distal tubules, some contained large, darkly staining granules which were usually located in the apical cytoplasm (Fig. 6). Lateral cell membranes were distinguishable in most tubules. A few of these epithelial cells stained darker than others (Myers et al., 1966). These cells occurred singly, separated by two or more lighter staining

cells (Fig. 7). It was noted that the cytoplasm of the dark cells contained more small densities (probably mitochondria) than that of the lighter cells. Very little, and in some cases no, cellular debris was found in the lumina of the collecting tubules. There was some bulging of the apical cytoplasm into the lumina but no cytoplasmic blebs were observed. A brush border was not present.

Interstitium and Vasculature. The interstitium consisted of lightly staining, heterogeneous appearing material located between the individual tubules and between the tubules and peritubular vasculature including capillaries and some larger vessels. This interstitial material was more abundant in some areas of the tissue than in others. It was composed of irregularly-shaped cells which contained elongated nuclei and were located between the epithelial and vascular basement membranes as described by Bulger et al. (1967). These cells were present only in moderate numbers and appeared to be very similar morphologically to fibroblasts found in connective tissue in other areas of the body. Leukocytes, plasma cells, macrophages and erythrocytes were not observed in the interstitium. It was noted that the interstitial tissue in the biopsies from E.B. and J.L. appeared to be more abundant than in the other donor

tissue studied.

The peritubular capillaries were numerous in each of the five biopsies observed. The morphology of these vessels was as described by Bloom and Fawcett (1966) and consisted of a lumen surrounded by the cytoplasm of a single layer of endothelial cells. One endothelial nucleus was usually observed jutting into the lumen in transverse sections of the vessels whereas two or, rarely three nuclei could be seen in oblique sections. This endothelial cell layer was surrounded by a thin basement membrane which was, in turn, surrounded by the interstitial tissue previously mentioned. Erythrocytes were occasionally present in the lumina of these vessels but no debris was seen.

Arterioles and venules were observed in some tissue sections and their morphological appearance also agreed with that presented by Bloom and Fawcett. The lumina of these vessels were larger than those of the capillaries and more endothelial nuclei lined the lumina of the arterioles and venules. These larger vessels were also composed of one to three layers of smooth muscle cells which were bordered by interstitial tissue. The walls of the arterioles contained more layers of smooth muscle than those of the venules. The lumina of

obliterated with cellular debris than in the donor tissue (Fig. 9). Large vacuoles were present in the epithelial cells of the proximal tubules in which the lumina contained the greatest amount of debris. Most of the vacuoles were located apically. The one hour tissue of J.L. was similar to the J.L. donor tissue previously described. Darkly staining cytoplasm and enlarged extracellular spaces were clearly evident in both biopsies.

Loop of Henle. Very few examples of the thick limbs of the loop of Henle were observed. Those that were present resembled the corresponding convoluted tubule with the exception of cell height and cytoplasmic density. The cells of the descending thick limb were cuboidal with lightly staining cytoplasm. The brush border was not as obvious as in the pars convoluta. Some cellular debris was present in the tubule lumina and darkly staining granules were seen in the apical cytoplasm. The ascending thick limbs were identical to those found in the donor tissue previously described.

Distal Tubules. Simple cuboidal cells formed the epithelium of the distal tubules. Abundant mitochondria were present in the cytoplasm in the basilar two-thirds of

the cells (Fig. 10). The nuclei were located primarily in the apical portion of the cells and the cytoplasm on the luminal side of the nuclei bulged into the tubule lumina. Cellular debris was present within some lumina; however, very few, with the exception of those in the tissue of J. L., were filled with debris. As in the cells of the proximal tubules, dark granules were observed in the apical region of many of the distal tubule cells. Lateral cell membranes were indistinct in all sections studied, but enlarged extracellular spaces between the cells in the J. L. biopsy were present. A brush border was not observed in any of the distal tubules studied.

Collecting Tubules. The cytoplasm of the simple low columnar or cuboidal cells lining the lumina of the collecting tubules contained fewer organelles than either the proximal or distal tubule cells of the same biopsies. Some cells contained large, dark granules primarily in the apical region and some mitochondria were randomly distributed throughout the cytoplasm. As in the donor tissue, the variation in concentration of organelles and granules in the cytoplasm of these collecting tubule cells resulted in the presence of light and dark cells (Figs. 11 and 12). The light cells far outnumbered the dark cells which were

present singly between groups of light cells. In a few instances, the nuclei of the dark cells appeared to be pyknotic (irregularly shaped and very darkly stained) (Fig. 11) whereas the nuclei of the majority of dark cells were indistinguishable from those of the light cells (Fig. 12). The lateral cell membranes were distinct in most tubules. Occasional cellular debris was observed in the tubule lumina but no brush borders were seen.

Interstitium and Vasculature. The appearance and abundance of interstitial tissue in the one hour biopsies did not vary significantly from that observed in the donor biopsies. In some cases (E.B., F.C., C.J., and J.L.) it was slightly thicker than in others; however, this variation in interstitial thickness was also noted in the donor tissue. The nuclei of the interstitial cells were identical to and were present in approximately the same concentrations as those observed in the donor tissue. Cellular infiltration was not seen in this tissue.

Peritubular vasculature was basically identical to that described for the donor tissue (Figs. 8 and 13); however, in some arterioles the tunica media was slightly hypertrophied. Debris was not observed in the lumina of the vessels.

Electron Microscopy

Donor (Control) Biopsy

Proximal Tubules. The proximal tubule cells studied were simple low columnar (Fig. 14) or cuboidal in shape. The latter may have been cells of the pars recta (Fig. 1) described by Tisher et al. (1966) but without serial sections of the tubules it is impossible to determine with any degree of certainty. For this reason and because of the similarity between the internal structure of the columnar and cuboidal cells, they will be described together as the proximal tubule cells.

Microvilli were numerous on the apical surface of the tubule cells and were of a uniform length (approximately 1.5μ). The microvilli formed the brush border of the proximal tubule cells as seen in light microscopy. Occasionally a microvillus appeared to branch resulting in two projections from a common base. The apical membrane between the microvilli invaginated into the underlying cytoplasm to form small tubules and spherical vesicles ranging in size from 0.2μ to 0.5μ . Larger vacuoles (0.8μ) were also present in the apical cytoplasm but were not as numerous as either the tubules or vesicles (Fig. 14). Coating the microvilli and lining the tubules,

vesicles and vacuoles was a fuzzy-appearing substance; probably a polysaccharide glycocalyx as described by Gennett (1963). The apical membrane of several cells formed blebs which projected into the tubule lumina (Fig. 14). Microvilli were absent in these areas as were apical tubules and vesicles.

Some interdigitations of the lateral cell membranes of adjoining cells was noted. These interdigitations increased in number toward the base of the cells with the apical portion of the membrane remaining relatively straight. In a few instances, the entire length of the lateral border appeared to be free of interdigitations. In all cells studied, junctional complexes were observed (Fig. 14) whose appearances adhered to the description of those present in other types of epithelia (Bloom and Fawcett, 1968). A short (500 \AA), tight junction (zona occludens) was observed at the luminal end of the lateral cell membrane followed immediately basilar by the intermediate junction (zona adherens). In this region, which was approximately 0.3μ in length, the intercellular space was wider (400 \AA) than that in the zona occludens. The third component of the junctional complex, the macula adherens or desmosome, was located at various points on the membrane between the zona adherens and the base of the

cell (Fig. 14). The desmosomes were approximately 0.2μ in length and the membranes appeared to be the same distance apart as in the zona adherens (400 \AA). Extracellular spaces usually no wider than 0.2μ were observed between adjacent lateral membranes of the proximal tubule cells in all cases studied; however, as was mentioned in the light microscopic portion of this study, the biopsy obtained from one case (J.L.) revealed grossly enlarged extracellular spaces (up to 1μ in width) both lateral to and at the basal portion of the cells (Fig. 15).

Extensive folding of the basal membrane was present in these cells of the proximal tubules especially in the lateral areas. Some of the folds or interdigitations extended apically almost to the level of the nucleus. Portions of adjacent tubule cells containing mitochondria were located within these basilar membrane infoldings (Rhodin, 1958).

A single, spherical nucleus was centrally located in the majority of cells observed (Figs. 14, 15 and 16). The nucleus contained a basically homogeneous chromatin matrix with only occasional clumping, usually located peripherally (Figs. 14 and 15). This condensation of chromatin was most likely due to the glutaraldehyde fixation of this tissue (Fawcett, 1966). The nuclear envelope was composed of an

outer and an inner limiting membrane in which pores (800 Å in diameter) could frequently be observed (Fig. 16). Ribosomes appeared to be attached to the cytoplasmic surface of the outer membrane in many cells. The perinuclear space located between the two limiting membranes was often obliterated but when distinguishable, appeared to be approximately 200 Å wide (Fig. 15). One or two nucleoli were present in most nuclei and appeared as dense, spherical or oval structures containing a meshwork of interwoven threads.

Mitochondria were randomly distributed throughout the cytoplasm of the proximal tubule cells with the majority of these organelles appearing to be spherical in shape and measuring from 0.5 μ to 1 μ in diameter (Figs. 14 and 15). Elongated forms were observed primarily in the basal portion of the cells. These structures were approximately 0.5 μ in width and 1.5 μ in length. In a few instances branched mitochondria were observed. The cristae were oriented at right angles to the long axis of the organelle in the elongated mitochondria and at various angles in the spherical mitochondria. The mitochondrial matrix consisted of a dense, homogeneous material in most cases (Fig. 14); in the biopsy from E.B. the matrix appeared to be slightly heterogeneous with

condensations of matrical substance (Fig. 16). Mitochondrial swelling and blebbing of the limiting membranes were also noted in this biopsy and only occasionally in biopsies from other patients. Matrical granules were present only rarely in the mitochondria of the proximal tubule cells. In the tissue of J.L., however, these granules were numerous (Fig. 15). It should be noted that enlarged extracellular spaces and condensed cytoplasm were also present in the J.L. tissue but not in the other biopsies.

The Golgi apparatus consisted of a complex of small vesicles (usually 0.2μ to 0.5μ in diameter) and elongated cisternae located either lateral or apical to the nucleus and usually juxtannuclear. The appearance of these complexes was identical to that of the Golgi complexes observed in the distal tubules (Fig. 17). The components of the complex were swollen in some cells while in other cells the vesicles and cisternae were present in lesser degrees of dilation.

Granular and to a lesser extent agranular endoplasmic reticulum were present throughout the cytoplasm of the cells of the proximal tubules. In many instances both types appeared to be more concentrated in the basilar portion of the cells. It was also noted that the agranular endo-

plasmic reticulum was frequently located near the lateral cell membranes. The granular endoplasmic reticulum, in many cases located in the immediate vicinity of mitochondria, was often swollen and appeared as large vesicles (Fig. 16) rather than as a complex of cisternae and small vesicles as described by Bloom and Fawcett (1968).

Free ribosomes were observed throughout the cytoplasm of the proximal tubule cells. They were frequently present in small clusters or rosettes of approximately six particles each (Fig. 14). In most tubule cells, the greatest concentration of ribosomes was in the basilar two-thirds of the cells.

Microbodies were observed in the cytoplasm of cells of the proximal tubules as illustrated by Figure 14. These single membrane limited inclusion bodies as described by Tisher et al. (1966) were usually located adjacent to granular endoplasmic reticulum. The microbodies were smaller (0.5μ) than the mitochondria and were usually spherical although some elongated forms were observed. The matrix of these bodies was homogeneous and appeared to be more dense than that of the mitochondria. Linear densities mentioned by Tisher et al. (1966) were not observed in the microbodies.

Another type of single membrane limited inclusion body found primarily in the apical portion of the proximal tubule cells was the cytosome (Figs. 14, 16 and 20) also described by Tisher et al. (1966). The cytosome has also been referred to as a lysosome body (Fawcett, 1966). These bodies were spherical but the size varied from that of a small mitochondrion (0.8μ) to larger than that of the largest mitochondrion observed in this tissue (2.5μ). Many cytosomes contained a light matrix within which dense granules were present in varying concentrations (Fig. 14). Others consisted of a dense matrix containing only one or two granular densities (Fig. 20). The proximal tubule cells in the biopsy from one case (E.B.) contained large cytosomes which contained a light matrix and numerous densities usually peripherally placed which upon close observation were found to consist of many small vesicles grouped together (Fig. 16). All of the proximal tubules studied in the tissue of this case included cells which contained these large cytosomes.

Multivesicular bodies were not as prevalent as the cytosomes but were observed in occasional cells (Figs. 15 and 16). These inclusions, also limited by a single membrane, were smaller (0.3μ) than most microbodies and contained several minute vesicles (300 \AA). The matrix

was usually composed of a lightly staining homogeneous material. Multivesicular bodies were not localized to any one specific area of the cell but were found throughout the cytoplasm. Cytosegresomes as described by Tisher et al. (1966) and Trump and Bulger (1968) were not observed in the tissue obtained for this study.

Electron microscopy confirmed the light microscopic observations of the unfailing presence of cellular debris in the lumina of the proximal tubules (Fig. 14). The debris consisted of large membrane-bound sections of cytoplasm containing mitochondria, vesicles, cytosomes, microbodies, endoplasmic reticulum, and free ribosomes. Microvilli were often observed on the surface of these cytoplasmic segments. Luminal debris also consisted of individual organelles and inclusions found free in the lumina. The limiting membrane of many of these structures was disrupted.

The width of the basal lamina (basement membrane) of the proximal tubules ranged from approximately 0.4μ in some areas (Fig. 14) to 1μ in others (Figs. 15 and 16). The basal lamina was electron dense and homogeneous in most cases. In one case (J.L.), however, it had the appearance of a fibrous network (Fig. 15). In all cases studied collagen fibers and other components of intersti-

tial tissue blended with the material of the basal lamina forming an irregular border away from the epithelial border of the basal lamina.

The biopsy of J.L. also contained dark spiralled, lamellated bodies located in the enlarged extracellular spaces previously described (Fig. 15). The structures, which were of various sizes (0.3μ to 0.6μ), were not enclosed in a membrane and appeared to be composed of fibrous lamellae. They were not seen in any other donor tissue studied.

Loop of Henle. As was described in the light microscopic results, the tissue used in this study was obtained from the cortex of the kidney and, therefore, did not include sections of the thin loop of Henle (Fig. 1).

Distal Tubules. The cells of the distal tubules were simple cuboidal or low columnar in shape and as was true of the proximal tubule cells, the smaller cuboidal cells may be cells of the pars recta of the distal tubules (Tisher et al., 1968). Both cell types, however, will be described under one heading as was the case with the proximal tubules. Cells of the macula densa (Fig. 1) of the distal tubules did not appear to be present in the sections that were studied.

Unlike the cells of the proximal tubules, the apical membranes of the distal tubule cells were free of microvilli and with the exception of one case, contained very few projections into the tubule lumina. In the one case (J.L.) large, somewhat pointed projections were observed on the apical surface of the cells. These projections did not resemble the microvilli of the proximal tubule cells. In another case (E.B.) the apical membrane was disrupted and cellular contents were present in the lumina in the disrupted area (Fig. 18). Apical vesicles were observed in most cells of the distal tubules but these vesicles were smaller ($500 \text{ \AA} - 1000 \text{ \AA}$) and not as numerous as those present in the proximal tubule cells. The vesicles of the distal tubule cells were also lacking the fuzzy coating seen in the vesicles of the proximal tubule cells.

Junctional complexes of the distal tubule cells (Figs. 17 and 18) including the zona occludens, zona adherens and the desmosomes appeared to be identical in structure to those present in the proximal tubules. The desmosomes, however, were not as numerous in the distal tubules. The complexity of the interdigitations in the lateral cell membranes was inconsistent. In some tubules

the lateral cell membranes were extremely tortuous especially in the basilar portion (Fig. 18) while in others the interdigitations were minimal (Fig. 17). The interdigitations or infoldings of the basal cell membrane (adjacent to the basal lamina) were more extensive than those of the proximal tubule cells, often enclosing mitochondria within the folds (Fig. 17). Extracellular spaces between the lateral cell membranes as observed in the proximal tubules were not seen in the distal tubules.

The nucleus in the majority of distal tubule cells was apically placed (Figs. 17 and 18) and were similar in appearance to those present in the cells of the proximal tubules. In several cells, the outer limiting membrane of the nuclear envelope extended out from the inner limiting membranes forming enlarged areas in the perinuclear space (Fig. 18). As in the proximal tubule cells, ribosomes were observed on the cytoplasmic surface of the outer limiting membrane but were not seen in the perinuclear space between the two limiting membranes. The chromatin was frequently clumped especially peripherally along the inner limiting membrane. Most of the nuclei contained one nucleolus which had an heterogeneous, threadlike structure identical to that observed in the proximal tubule cells (Figs. 17 and 18).

It was noted that the mitochondria were generally more numerous in the columnar cells of the distal tubules (Fig. 17) than in the cuboidal cells. Based on the descriptions of Rhodin (1958) and Tisher et al. (1968), the taller cells containing the abundance of mitochondria were located in the distal convoluted tubules while the shorter cells with fewer mitochondria were cells of the pars recta portion of the distal tubule which is continuous with the thin loop of Henle (Fig. 1). In both types of cells most of the mitochondria were located in the basilar portion of the cell and were often elongated in shape although ovoid mitochondrial profiles were observed. The usual orientation of the elongated organelles was for the long axis of the mitochondria to be parallel to the surfaces of the lateral cell membranes. The few mitochondria in the apical portion of the cells were primarily ovoid or spherical structures. The cristae were oriented perpendicular to the long axis of the elongated mitochondria and radiated from the central area to the periphery in the spherical mitochondria. The cristae appeared to be more numerous in the mitochondria of the distal tubules than in those cells of the proximal tubules. The matrix of these organelles was densely homogeneous with

the exception of those in the biopsy of E.B. in which the matrical material of some mitochondria was clumped resulting in a lighter overall appearance (Fig. 18). The limiting membranes of these heterogeneous mitochondria were frequently disrupted or irregular. Dense matrical granules were observed in the mitochondria of the cells of the distal tubules in only one donor biopsy (R.C.). The average size of the mitochondria in the distal tubule cells was approximately 0.6μ which was smaller than those of the proximal tubule cells (0.8μ).

The Golgi apparatus was usually located lateral to the nucleus (Fig. 18) although in a few instances it was found between the nucleus and the base of the cell (Fig. 17). The structure of this complex was similar to that of the Golgi complexes located in the proximal tubule cells. The cisternae and many of the vesicles were often swollen. The vesicles ranged in size from 0.1μ to 0.9μ in diameter.

As was also noted in the proximal tubule cells, the granular endoplasmic reticulum of the distal tubule cells frequently appeared as dilated vesicles with ribosomes bordering the cytoplasmic surface of the single membrane (Fig. 17 and 18). These endoplasmic reticular vesicles (0.2μ to 0.5μ in diameter) were located among the

mitochondria usually in the basilar portion of the cells. Agranular endoplasmic reticulum was observed only occasionally as was also the case in the proximal tubule cells. Small, smooth-surfaced vesicles (0.1μ) were scattered throughout the cytoplasm; however, their number was much less than that of the granular endoplasmic reticular vesicles. Free ribosomes were present singly and in clusters randomly distributed throughout the cytoplasm of most distal tubule cells. In one case (E.B.) large areas of cytoplasm lacked both free ribosomes and mitochondria (Fig. 18).

Neither microbodies nor cytosegresomes were observed in the cells of the distal tubules. Cytosomes were occasionally noted in some of the distal tubule cells especially in those of the J.L. biopsy. In this tissue they were frequently located in the apical portion of the cells and contained a dense matrix. These structures were approximately 0.6μ in diameter. Multivesicular bodies were present in some distal tubule cells but their number was even less than that of the cytosomes previously mentioned.

Large, membrane limited deposits of lipofuscin pigment similar to those described by Tisher et al. (1968) were observed in most distal tubule cells (Figs. 17 and

18). These deposits were composed of complexes of lipid and pigment droplets which varied in number (12 to 15), size (0.05μ to 2μ), and density. Each complex usually contained two or three large, lightly staining droplets surrounded by numerous, small darkly stained spheres. These inclusions were often irregular in shape although occasional spherical structures were observed (Fig. 17). These lipofuscin droplets were present in both the apical and basilar cytoplasm of the cells of the distal tubules.

Some luminal debris was observed in the distal tubules but in far lesser amount than was present in the lumina of the proximal tubules. The debris in the distal tubules consisted primarily of single cellular inclusions and organelles, usually mitochondria (Fig. 17). Occasionally portions of membrane limited cytoplasm containing cellular structures such as nuclei and vesicles were observed.

The appearance of the basal lamina of the distal tubules was similar to that of the proximal tubules. The portion of the basal lamina bordering the epithelial cells of the tubules was dense and homogeneous while that portion adjacent to the interstitium was irregular and blended with the fibers of the interstitium. The width of the basal

lamina of the distal tubules ranged from 0.05μ to 0.4μ (that of the proximal tubules averaged 0.4μ).

Collecting Tubules. The majority of cells of the cortical tubules were simple, low columnar although some cuboidal cells were observed. Light and dark cells as described at the light microscopic level were again distinguishable with the electron microscope (Fig. 19). The basic structure of the two cell types (light and dark) was essentially the same with the exception that the dark cells appeared to contain greater numbers of organelles and inclusions. This difference was also described by Myers et al. (1966). The apical membranes of both cell types were slightly convex over the area of the nucleus and contained few or no microvilli. Those that were present were only in the dark cells (Fig. 19) and were short (0.2μ), blunt structures. Coated apical vesicles (0.1μ) were frequently observed close to the base of the microvilli. Apical vesicles were also present in the cells without microvilli and were identical to those observed in the cytoplasm of the cells with microvilli. In many cases, these vesicles were quite numerous. Occasionally sections through a cilium were present in the apical portion of the cells of the collecting tubules.

The lateral cell membranes of both light and dark cells of the collecting tubules contained some basilar interdigitations but were usually free of extensive infoldings in the apical portions (Fig. 19). Tight junctional complexes were observed between the luminal portions of the lateral membranes of adjacent cells. The zona occludens and zona adherens, however, did not extend as far basally in the collecting tubules as in either the proximal or the distal tubules. As was the case in the distal tubules, desmosomes were not abundant in the collecting tubules. The lateral cell membranes of adjoining cells of the collecting tubules were in close proximity and extracellular spaces were not evident in the tissue studied. The basal membrane was infolded to varying degrees. In some, very few infoldings were observed (Fig. 19) and those that were present were usually located laterally rather than directly basal to the nucleus. In other tubules, the infoldings were more extensive and resembled those present in the cells of the distal tubules with the larger interdigitations located laterally, including mitochondria within the folds.

The nuclei of all cells of the collecting tubules were spherical and were centrally placed in the cytoplasm. The chromatin was homogeneous with very little clumping

as was observed in the proximal and distal tubule cells. One or two nucleoli with an appearance of interwoven threads were present (Fig. 19). The nuclear envelope and an outer limiting membrane which in some instances was separated from the inner membrane by an enlarged, vacuolar-like perinuclear space (Fig. 19) rather than the narrow (400 \AA) slit-like space were usually observed. This enlargement of the perinuclear space was also noted in the cells of the distal tubules. Nuclear pores were observed in the nuclear envelope and occasional ribosomes were seen adjacent to the cytoplasmic surface of the outer limiting membrane.

In most cases, the mitochondria of the collecting tubule cells were less abundant and smaller (0.4μ) than those of either the proximal (0.8μ) or the distal tubule cells (0.6μ). The mitochondria of the collecting tubule cells were usually distributed throughout the cytoplasm; less frequently there was a concentration in the basilar portion of the cells. It was also noted that the dark cells of the collecting tubules contained more mitochondria than did the light cells (Fig. 19). Spherical and elongated mitochondria were present but the majority were ovoid with cristae oriented perpendicular to the long axis of the organelle. The dense matrix was usually homogeneous

and lacked matrical granules.

The Golgi complexes were located lateral to the nuclei and were identical in structure to those observed in the cells of both the proximal and distal tubules. Endoplasmic reticulum was poorly developed in the cells of the collecting tubules but occasionally dilated vesicles of granular endoplasmic reticulum were observed (Fig. 19).

Cytosomes, multivesicular bodies and lipofuscin droplets were present in the cytoplasm of the collecting tubule cells. The cytosomes were spherical and varied in size from 0.2μ to 0.8μ . There were more cytosomes present in the dark cells than in the light cells, but in both cell types the cytosomes were located either apical or lateral to the nucleus (Fig. 19). Some of these structures contained a light matrix with small, dark granules while others were almost completely filled with dark granules of varying size. Multivesicular bodies were also observed more frequently in the dark cells than in the light cells. These inclusions were usually located on the basilar side of the nucleus and were approximately 0.3μ in diameter. They were identical in structure to those present in the proximal and distal tubule cells with several small vesicles contained within a single membrane.

The lipofuscin droplets observed in the cells of the collecting tubules were not as large nor as plentiful as those found in the distal tubules; averaging less than 1μ in diameter. These inclusions of the collecting tubule cells also contained fewer spherical granules than those of the distal tubules; the granules that were present varied in density. Microbodies were not observed in the cells of the collecting tubules.

Free ribosomes were present in the cytoplasm of these cells both singly and in clusters of six to ten granules (Fig. 19). The number of ribosomes in the cytoplasm of the dark cells was greater than in the light cells with the largest concentration directly peripheral to the nucleus and apical to it.

The basal lamina of the collecting tubules did not differ in appearance from those observed in the proximal and distal tubules. The average thickness of the basal lamina was 0.3μ and as in the proximal and distal tubules, the portion directly bordering the epithelial cells was dense and primarily homogeneous while the opposing side blended in with the collagenous and elastic fibers of the interstitial tissue (Fig. 19).

The electron microscopic study confirmed the light microscopic observations of a lack of debris in the lumina of the collecting tubules. Some debris was occasionally seen, but it was present in small amounts and consisted of individual cellular organelles rather than organelle-containing portions of cytoplasm as were frequently observed in the lumina of the proximal and distal tubules.

Interstitialium and Vasculature. The interstitium of the donor tissue consisted of a low density material which contained longitudinal (Figs. 14 and 21) and transverse (Figs. 14, 20 and 21) sections of banded collagen fibers, iridescent-appearing areas which may represent the presence of elastic fibers (Figs. 17 and 19), and fragments of the interstitial cells which were present throughout the interstitium (Figs. 20 and 21). These interstitial cells appear similar to fibroblasts and most likely are responsible for the production of the collagenous fibers located in the interstitium. As was stated in the light microscopic description of the donor tissue, the interstitial components were located between the basal laminas of the capillaries and those of the renal tubules.

The interstitial material of one patient (J.L.) appeared to be more abundant than that of the remaining

four patients from which donor tissue was obtained. This electron microscopic observation confirmed the light microscopic findings described previously.

The cellular fragments mentioned in a previous paragraph were actually sections through the pseudopodia-like interstitial cells (Figs. 20 and 21). The cytoplasm of these cells contained organelles and inclusions similar to those observed in the cells of the tubule epithelium. Small vesicles (0.1μ) were present in many cells as were both single and grouped free ribosomes. Agranular endoplasmic reticulum was not observed whereas granular endoplasmic reticulum was quite prevalent in the form of grossly dilated vesicles and cisternae (Fig. 20). A Golgi apparatus consisting of three or four parallel cisternae with several small vesicles adjoining the cisternae was frequently seen near the nucleus. The mitochondria of these interstitial cells were either ovoid or elongate in shape and were approximately the same size as those present in the distal tubule cells (0.5μ to 0.6μ) (Figs. 20 and 21). The matrix of the mitochondria was heterogeneous and the cristae were frequently obscure. Cytosomes containing granules and matrixes of varying densities were also present in the cytoplasm of the interstitial cells. The average size

of these inclusions (0.4μ) was smaller than that of the cytosomes observed in the cells of the proximal and distal tubules. No microbodies, multivesicular bodies, nor cytosegresomes were seen in the tissue studied. The nuclei were frequently irregular in shape and the chromatin clumped peripherally (Fig. 21). Nucleoli were occasionally observed. A basal lamina (basement membrane) surrounding the interstitial cells as reported by Bulger et al. (1967) was not observed in this study.

In the tissue of one case (R.C.) erythrocyte infiltration of the interstitium was clearly evident (Fig. 21). This infiltration was present in an area of capillary abundance. The lumina of the capillaries in this tissue contained cellular debris consisting of both individual organelles and inclusions and cytoplasmic fragments containing mitochondria, endoplasmic reticulum, vesicles, cytosomes, and ribosomes. The endothelium of these capillaries appeared to be interrupted in several areas (Fig. 21).

The peritubular capillaries of the donor tissue were of the fenestrated variety in which the thin (0.1μ) areas of the endothelial cytoplasm lining the capillary lumina were pierced by small pores approximately 800 \AA in diameter (Fig. 20). These pores were traversed by a thin

membrane or diaphragm which was clearly seen in most sections. Occasionally, the thin, fenestrated portion of the endothelial cytoplasm was separated from the basal lamina surrounding the capillary. This phenomenon was observed in tissue from all donor cases studied.

The endothelial nuclei of the perinuclear capillaries were visible in many sections and in each case the nucleus, covered by a thin layer of cytoplasm, projected into the capillary lumen (Figs. 18, 20 and 21). These nuclei were usually elongate with homogeneous chromatin although occasional clumping was observed peripherally. Lateral to the nucleus the cytoplasm tapered to a thin, fenestrated endothelial lining mentioned previously. Vesicles and cisternae of granular endoplasmic reticulum present in various states of dilation were observed in this cytoplasm as were occasional mitochondria smaller (0.5μ) than those of the renal tubule cells. A Golgi apparatus composed primarily of cisternae, free ribosomes present both singly and in clusters, and small vesicles (400 \AA) were also observed in the endothelial cytoplasm (Fig. 20). Small cytosomes (0.3μ) containing dense matrical material were only rarely present in the cytoplasm. Multivesicular bodies, microbodies, and cytosegresomes were not observed

in this tissue. Tight junctions were present between the membranes of adjoining endothelial cells (Fig. 20). These area appeared to be composed of a zona occludens adjacent to the capillary lumen and a zona adherens which extended to the basal lamina in some instances. Desmosomes were not observed. The thickness of the basal lamina surrounding each capillary was less (0.3μ) than that of the basal lamina surrounding the renal tubules (0.5μ). The structural appearance and density of both types of basal lamina were identical.

Larger blood vessels were also observed in areas of the interstitium surrounding the renal tubules. These vessels included primarily small arteries although occasional small veins were identified. The endothelium of the arteries projected in a scallop-like manner into the vessel lumina (Fig. 22). The endothelial nuclei were irregular in shape and often displayed peripheral clumping of the chromatin. Small vesicles were observed in the cytoplasm usually apical to the nucleus. Scattered throughout the cytoplasm were small cytosomes (0.3μ) containing granules of various densities and structures that were similar to the multivesicular bodies observed in the cells of the renal tubules. The mitochondria present in the

endothelial tissue were also small (0.4μ) in comparison to those seen in the renal tubule cells and contained a dense, homogeneous matrix which lacked matrical granules. Cisternae of granular endoplasmic reticulum were present throughout the endothelial cytoplasm as were free ribosomes. Golgi complexes were occasionally observed in some endothelial cells of the arteries in which case their appearance was identical to the Golgi complexes present in the capillaries. This similarity in capillary and arterial structures was also true for the tight junctions. The basal lamina of the endothelial cells of the arteries and veins was undulated with the cytoplasm of the endothelial cells extending between the undulations (Fig. 22). The average thickness of this basal lamina was 0.7μ while that of the renal tubules was 0.4μ . The tunica media of the small arteries consisted of one to three layers of smooth muscle cells arranged concentrically around the endothelial cell layer. Each of the smooth muscle cells was surrounded by a thin (0.1μ) layer of material which was identical in appearance to that of the endothelial basal lamina. This material was frequently continuous with the endothelial basal lamina or with the same substance surrounding adjacent smooth muscle cells. In regions in which the continuity of these layers was absent less dense

areas containing fibrous tissues or many vesicles of various sizes were observed between the layers (Fig. 22). The cellular organelles of the smooth muscle cells were usually concentrated around a centrally placed nucleus which was irregular in shape and displayed a basically homogeneous chromatin with some peripheral clumping. This clumping has been attributed to glutaraldehyde fixation by Fawcett (1966). Oval and elongated mitochondria (0.4μ), granular endoplasmic reticulum, free ribosomes, and occasional vesicles were observed in all smooth muscle cells studied. Occasionally, cytosomes containing a dense matrix and averaging 0.3μ in diameter were present in the cytoplasm of these cells; multivesicular bodies, microbodies and cytosegresomes were not observed. Large lipofuscin deposits (1.3μ) identical to those present in the distal tubule cells were present in close association with the nuclei of some smooth muscle cells. Also observed within the cytoplasm were concentrations of electron dense particles which appeared to be larger than ribosomes (Fig. 22). These particles were in all probability glycogen particles although positive identification was impossible at this magnification.

Collagenous fibers and fragments of interstitial cells were observed peripheral to the smooth muscle cells of the tunica media. This area represents the tunica adventitia which was adjacent to the basal lamina of the renal tubules. The amount of smooth muscle tissue surrounding the endothelium of the small veins was reduced in comparison to that present in the small arteries while the thickness of the tunica adventitia of the veins remained approximately the same as that of the small arteries. Sections through nerve fibers were not observed in the tunica adventitia of any of the vessels studied.

One Hour Biopsy

Tissue from 17 renal transplant cases was obtained for this study; however, in one case (C.S.) the tissue was found to be poorly prepared and unusable for electron microscopic examination. The description of the tissue from the remaining 16 cases follows.

Proximal Tubules. In the majority of cases the proximal tubule cells of the one hour biopsies were essentially identical in appearance to those of the donor tissue. The simple, low columnar cells displayed a brush border of microvilli which were approximately 1.5μ in length (Fig. 23). As was reported in the description of the cells of the donor tissue, the apical membrane covering areas of the cytoplasm which projected into the lumina as blebs were void of microvilli. The lateral cell membranes and tight junctions of the one hour tissue were identical to those of the donor or control tissue as were the appearance and location of most cytoplasmic organelles and inclusions. In the majority of one hour biopsies the basal lamina of the proximal tubules averaged 1μ in width which was $0.3 - 0.4 \mu$ more than lamina of the donor tissue. The structure of the one hour basal lamina was, however, identical to that of the proximal tubules of the donor biopsies.

The proximal tubule cells of the one hour tissue of two cases (J.L. and E.B.) varied in appearance from the

other 14 cases studied but were identical to the donor biopsies from the respective cases (J.L. and E.B.) In both the donor and the one hour tissue of J.L. the cytoplasm of the proximal tubule cells was condensed and contained large (1.5μ) cytosomes with a dense, homogeneous matrix (Figs. 15 and 24). Dilated extracellular spaces, occasionally containing structures composed of lamellated membranes, were prominent in both biopsies. The basal lamina of the proximal tubules of one hour tissue of J.L. was not as thick as that of the other one hour biopsies but was the same width as that of the J.L. donor biopsy. The one hour biopsies of C.J. resembled the J.L. biopsies in that enlarged extracellular spaces containing occasional lamellated bodies were observed in the proximal tubules. The C.J. tissue was the only tissue other than that of J.L. in which such extracellular spaces were present.

As previously mentioned, the one hour biopsy of E.B. also varied in appearance from the other one hour tissue studied. However, the one hour tissue of E.B. was identical to the E.B. donor or control tissue. Both

biopsies displayed large cytosomes containing a light, homogeneous matrix with clusters of dense vesicles in the matrix.

The one hour biopsy of one other case (R.M.) also exhibited differences in appearance from those of the other cases. The cells of the proximal tubules of the R.M. tissue were more cuboidal in shape and the cytoplasm more compact and electron dense than the cells of the other biopsies. This tissue contained many mitochondria, the majority of which were oval or spherical in shape and averaged approximately 0.8μ in diameter. Large cytosomes (1.5μ) usually containing a lightly staining, heterogeneous matrix and numerous large electron dense granules were present in all cells of these tubules. The tubular lumina in the R.M. tissue were completely obliterated by cellular debris which included cytoplasmic fragments containing organelles and inclusions as well as isolated structures such as mitochondria and vacuoles. Especially prominent in this debris were giant cytosomes (2.5μ) identical in appearance to those observed in the cytoplasm of the proximal tubule cells (Fig. 25).

The presence of large amounts of cellular debris was noted not only in the lumina of proximal tubules of the R.M. tissue but in the lumina of all one hour biopsies studied (Figs. 23 and 24). This debris was identical to that observed in the R.M. tissue with one exception. The giant cytosomes present in the biopsy of R.M. were not observed in any of the other biopsies although smaller cytosomes were observed.

Distal Tubules. As was true of the proximal tubule cells, the majority of cells of the distal tubules in the one hour biopsies were identical in structure to those of the donor or control tissue. Numerous, deep basilar infoldings of the simple, low columnar and cuboidal cells were evident (Fig. 26). The apical cell membranes of these cells were void of microvilli; and were often convex in shape in the region directly apical to the nucleus. The apical half of the lateral cell membranes were relatively straight while extensive interdigitations were noted in the basilar portion of the membrane. Numerous oval and elongate mitochondria, similar to those of the distal tubule cells of the control biopsies, were observed in the cytoplasm, especially basal to the spherical nucleus which was

also similar in appearance to those present in the control tissue. The mitochondria of the one hour biopsy averaged 0.5μ in diameter and contained many cristae in a dense, homogeneous matrix. Other cytoplasmic structures of the distal tubule cells of the one hour biopsies which were identical in appearance to those of the control tissue were granular endoplasmic reticulum, free ribosomes usually in small clusters, and a Golgi apparatus often located basal to the nucleus (Fig. 26). Cytosomes averaging the same size (0.6μ) and of the same structure as those observed in the control biopsies were present in the one hour tissue. These single membrane limited bodies were usually present in the apical portion of the distal tubule cells and contained granules of varying densities in a darkly stained, homogeneous matrix. One or two non-staining areas which appeared to be surrounded by a single membrane were present in some of the cytosomes of the cells of the one hour tissue but were not observed in the cells of the control tissue. Multivesicular bodies and lipofuscin deposits were present in the cytoplasm of most distal tubule cells of the one hour biopsies and were identical in appearance to the same structures observed in the donor biopsies. The basal

lamina of this tissue (one hour) was also identical in appearance to that of the distal tubules of the donor biopsies. Luminal debris, primarily in the form of individual cell organelles such as mitochondria and vesicles, was occasionally observed (Fig. 26). This debris was never present in the excessive amounts which were observed in the lumina of the proximal tubules of the one hour tissue.

The one hour tissue from seven of the cases studied (C.J., W.J., N.K., J.L., E.Mc., R.M., and S.S.) contained tubule cells, both individually and in large groups sometimes involving an entire tubular cross-section, which were shorter than tubule cells previously described. These cells also contained dense, vacuolated cytoplasm and irregularly shaped nuclei. The general appearance of these cells was one of cells undergoing necrosis. As a result, it was difficult in cases in which entire tubules were involved to determine whether these necrotic appearing cells were located in the distal tubules or in the collecting tubules. The presence of deep basilar infoldings in many of these cells would indicate that these are distal tubule cells (Fig. 27). However, individual necrotic cells were observed in tubules that

were identified as collecting tubules (Fig. 28). The observation of similar necrotic appearing cells in the collecting tubules of normal human renal tissue was reported by Myers et al. in 1966.

The appearance of the mitochondria in the "necrotic" cells of the one hour tissue observed in the present study was not altered from that of the mitochondria in the cells of other one hour biopsies. The cristae were distinct and the matrix was homogeneous. The cytoplasm of these cells was, however, more electron dense than that of the "normal" cells and seemed to be compressed so that in many cases, the overall shape was of a thickened squamous cell (Fig. 27). Numerous vacuoles were present in the cytoplasm of the majority of these cells and were frequently identified as dilated areas of granular endoplasmic reticulum. The Golgi apparatus was obliterated in most necrotic appearing cells and dilated areas of the perinuclear space surrounding the irregularly shaped nucleus were evident. The chromatin of these nuclei was clumped to a greater degree than that in the nuclei of the non-necrotic appearing cells. Cytosomes and lipofuscin droplets were observed in most cells but multivesicular bodies were absent. The apical cell

membranes of some "necrotic" cells were disrupted allowing cytoplasmic contents to flow into the tubule lumina (Fig. 27). The basal lamina was unremarkable in both appearance and thickness.

Collecting Tubules. The majority of these cells in the one hour biopsies were identical in structure to those observed in the donor biopsies. The cells were simple, low columnar in shape with centrally placed, spherical nuclei and an apical membrane which lacked microvilli. Both light and dark cells were observed containing essentially the same cytoplasmic organelles and inclusions that were present in the corresponding cells of the donor tissue. As was previously mentioned, a few sections of collecting tubules were observed in which some cells appeared to be undergoing necrosis (Fig. 28). These cells should not be confused with the "normal" appearing dark cells of the collecting tubules. In addition to containing a cell which appears to be undergoing necrosis, Figure 28 also illustrates a light cell adjacent to the necrotic appearing cell. In the latter cell the apical membrane is disrupted and cytoplasmic contents including mitochondria, vesicles and granular endoplasmic reticulum can be seen entering

the tubule lumen. As was mentioned in the description of the cells of the collecting tubules of the donor tissue the cytoplasm of the light cells did not contain as many organelles and inclusions as the cytoplasm of the dark cells. This was especially true in the case of free ribosomes which were plentiful in the dark cells and relatively scarce in the light cells.

With the exception of the presence of the cellular organelles and inclusions in the areas of apical membrane disruption, the lumina of the collecting tubules of the one hour tissue were free of cellular debris. The basal lamina of the collecting tubules of the one hour biopsies were identical in structure to those of the donor or control biopsies.

Interstitialium and Vasculature. Although the interstitium in the majority of sections of one hour biopsies was identical in appearance to that of the donor tissue, there were some areas in the interstitium of the one hour biopsies that contained cells differing from the interstitial cells previously described in donor tissue. Normal appearing interstitial cells with elongated nuclei and irregularly shaped cell processes were also observed in these interstitial areas of the

One hour biopsies

One type of non-interstitial cell which was observed in the tissue of one case (C.J.) (Fig. 29) resembled a macrophage (Bloom and Fawcett, 1966). The nucleus was not visible in this section; however, a large cytoplasmic process was observed which contained several large (3μ) debris-filled membrane limited bodies. These large vacuole-like structures were probably lysosomes containing the products of cellular digestion. Two large electron dense bodies were also present in the cytoplasm of this cell but these structures did not contain debris. The cytoplasm also contained elongated mitochondria, cisternae of both granular and agranular endoplasmic reticulum, and free ribosomes. This cell type was not observed in any of the other renal tissue studied.

Another type of cell was observed in the interstitium of the one hour biopsy in the interstitium of the one hour biopsy from case R.M. This cell type contained numerous vacuoles averaging 0.4μ in diameter (Fig. 30). In some vacuoles the matrix was homogeneous while others contained small granules or densities and resembled the cytosomes observed in the renal tubule cells. Figure 30 illustrates two adjacent sections through this cellular

tissue in which it is impossible to determine whether these are sections through one irregularly shaped cell or whether they are sections through two individual spherical shaped cells. The author favors the second possibility because of the similarity of the internal structure of these sections to that of spherical cells. Each of the sections observed in figure 30 was approximately 9μ in diameter. No nuclear material was present in one section while the other contained a nucleus with homogeneous chromatin and a nuclear envelope which included a prominent perinuclear space. The cytoplasm of the nucleated section appeared to evaginate in a small area into the surrounding interstitium; the cell membrane was in tact in this area. Both mitochondria and endoplasmic reticulum were lacking in the cytoplasm of the sections in figure 30. However, numerous small, dense particles, probably ribosomes, were randomly distributed throughout the cytoplasm of both sections. The renal tubule epithelium adjacent to the interstitium surrounding these sections was abnormal in appearance with electron dense cytoplasm containing many vacuolated areas. The nuclei of these cells were irregularly-shaped and combined with the general cytoplasmic

appearance suggested a necrotic process. In many regions the basal lamina between the renal tubule cells and the interstitium was 3 to 4 μ thick whereas in the donor tissue the basal lamina averaged only 0.4 μ .

The appearance of the vasculature of the one hour biopsies differed in the various cases. In most, the capillaries and small arteries and veins were identical to those described in the donor tissue. However, in a few instances (Figs. 24, 29, 31, 32, 33 and 34) abnormalities were observed. The lumina of many of the capillaries present in the tissue of J.L. were filled with a flocculent appearing substance (Fig. 24). In some of the capillaries in this biopsy cellular debris consisting primarily of mitochondria was also observed. The endothelium was normal in appearance with continuous fenestrated endothelial cytoplasm lining the lumina.

Cellular debris was also present in the capillary lumina of the C.J. biopsy. In this case, however, the endothelium was disrupted in some areas and interstitial material appeared to be entering the lumen (Fig. 29). Numerous, small vesicles were observed in the endothelial cytoplasm forming many of the capillaries in the V.M. tissue (Fig. 31). These vesicles (0.04 μ) were

especially plentiful in the thinner areas of the cytoplasm and were often in parallel rows which were parallel to the basal lamina. The endothelial cytoplasm also contained dilated granular endoplasmic reticulum, clusters of free ribosomes, and multivesicular bodies (0.4μ). In addition, irregularly shaped mitochondria (0.6μ) and vacuoles (0.3μ) containing either a nonstaining matrix or a homogeneous moderately electron dense matrix were present. The nuclei of these cells contained clumped chromatin and were surrounded by a perinuclear space which was dilated in several regions. Discontinuity of the endothelial cytoplasm was observed in some sections while cellular debris was noted in the lumina of all vessels studied.

In three cases (W.C., S.G., and C.J.) large cells containing numerous vacuoles were observed in the capillary lumina. In the first case (W.C.) the cell (Fig. 32) was similar in appearance to those observed in the interstitium of C.J. (Fig. 30). In both cases the cells were 9.0μ in diameter and contained numerous vacuoles (0.4μ) with matrices of varying densities. These vacuoles were surrounded by free ribosomes scattered throughout the cytoplasm. There were some

differences in the cells; occasional mitochondria and granular endoplasmic reticulum were observed in the W.C. cell. Also observed in the cell of the W.C. biopsy were two sections through nuclear material indicating the possibility of a multilobed nucleus in these cells. In addition, the plasma membrane of the cell appeared to be serrated in some regions. The general appearance of the cells suggested that it was a polymorphonuclear leukocyte even though specific granules were lacking (Bloom and Fawcett, 1966). The endothelium of the capillary in which this cell was located did not differ from the description of capillaries in the donor tissue of the same case (W.C.). The fenestrated endothelium was not disrupted and contained mitochondria, granular endoplasmic reticulum, free ribosomes and small vesicles. Some flocculent appearing material was present in the capillary lumen and occasional cellular organelles were observed.

The cell observed in the S.G. tissue was identical to the cell just described in the W.C. tissue with two exceptions. The S.G. cell was only 6 μ in diameter and the cell margins did not appear to be serrated. Sections through two nuclear segments were present in both cells

as were numerous vacuoles in the cytoplasm. The endothelium of the vessel containing the large cell in the S.G. tissue did differ from that of the W.C. biopsy. In the S.G. biopsy the endothelium was disrupted in several areas and cellular debris within the vessel lumen was evident.

The third case in which a large cell was observed in a vessel lumen was C.J. (Fig. 33). This cell was approximately 7.5μ in diameter and had an irregular profile which included several, small pseudopodia-like evaginations. That this cell was a polymorphonuclear leukocyte was suggested by the presence of three segments of nuclear material in the cytoplasm. Numerous lightly staining vacuoles ranging in size from 0.08μ to 0.4μ were located throughout the cytoplasm as were a lesser number of electron densely vacuoles of the same size range. Granular endoplasmic reticulum and free ribosomes were also present in the cytoplasm while mitochondria were not observed. The cytoplasm of this cell also contained an electron dense lamellated whorl similar to those observed in the extracellular spaces between the proximal tubule cells of the J.L. biopsies (donor and one hour). This lamellated body was 0.4μ in diameter and appeared

to be located in a vacuole containing a moderately dense matrix. The appearance of the endothelium of the capillary in which this large cell was located did not differ significantly from that of the capillaries previously described in the donor tissue. Mitochondria (0.4μ), granular endoplasmic reticulum, small vesicles (0.08μ), and free ribosomes were observed in the cytoplasm. In some areas it was noted that the fenestrated endothelium protruded into the capillary lumen and was separated from the underlying basal lamina (Fig. 33). This phenomenon was also observed in some capillaries of donor biopsies (Fig. 20).

The majority of small arteries and veins present in the one hour biopsies were identical in appearance to those studied in the donor or control tissue. However, occasional sections through abnormal appearing vessels were observed (E.B., W.D., S.G., R.M., and P.W.). Disruption of the endothelium and extensive cellular debris in the lumina were present in most of the vessels. An abundance of lipofuscin deposits in the cytoplasm of the smooth muscle cells was frequently observed in the small arteries (Fig. 34). Enlarged extracellular spaces between adjacent smooth muscle cells were also seen in these one hour biopsies. The thickness of the basal

lamina of individual vessels varied in different regions and in several instances it was as much as one micron thick. In one case (P.W.) it appeared that the basal lamina was hypertrophied to a thickness of 5 microns (Fig. 34). This abnormally thick appearance may have been due to an oblique section through the arterial wall rather than the result of a pathological process.

DISCUSSION

An attempt to correlate early renal tubular, vascular, and interstitial ultrastructure of human transplants with the future function of the transplant has not been previously reported. Such a study was made by Weymouth et al. (1970) involving the glomerular tissue of renal homotransplants. The authors concluded that there was a correlation between the ultrastructural morphology of the glomeruli and the clinical course of the transplants. In the present study, which includes the tubules, vasculature and interstitium, such a correlation was not indicated. The structural characteristics of the donor tissue did not vary significantly from those of the one hour biopsies with the exception of one case in which erythrocytes were observed in the interstitium of the donor tissue of R.C. Of the 16 one hour biopsies studied only one (S.G.) showed ultrastructural alterations which could be correlated with the future function of the homograft. In this patient the homograft functioned normally for only six hours and was subsequently removed.

One ultrastructural characteristic present in all of the one hour biopsies was an increase in the amount of both tubular and vascular luminal debris as compared to that present in the lumina of the tubules and vasculature of the donor tissue. This increase in debris was most likely the result of trauma directly related to the mechanical aspects of transplantation rather than an indication of homograft rejection. This assumption is based on the fact that abundant luminal debris was observed in all one hour biopsies studied regardless of whether the kidney was ultimately rejected or whether it went on to function normally.

One other feature noted in one hour tissue was the presence of large cells located in the capillary lumina of three cases (F.C., S.G., and C.J.) and in the interstitium of another (R.M.). These cells were similar in appearance to polymorphonuclear leukocytes (PMN's) but lacked the specific granules characteristic of such cells. The cells of the one hour biopsies did exhibit numerous vacuoles containing material of varying densities and it is possible that these vacuoles may represent specific granules in stages of degeneration. The presence of PMN's in this tissue is not surprising as infiltrations

of these cells have been reported in both canine (Porter, 1964 and Shorter et al., 1964) and in human (Kincard-Smith, 1964; Porter, 1964; Millard, 1970; Weymouth et al., 1970; and Busch et al., 1971) renal homotransplanted tissue. However, unlike the present study in which the biopsies were obtained one hour post transplantation, none of the canine tissue was obtained less than two days following transplantation. Busch et al. reported one case in which PMN's were observed in the peritubular capillaries one hour after renal transplantation in man. Millard et al. and Weymouth et al. reported the presence of numerous PMN's in the glomerular capillaries one hour post transplantation. Millard did not place much significance on the PMN infiltration; however, Weymouth et al. suggested that the PMN's were associated with "early renal malfunction". In the present study in which occasional PMN-like cells were observed the significance of the presence of such cells is questionable. One would normally expect the existence of some granular leukocytes in the renal vasculature. However, the observation of such cells in the interstitium (R.M.) should be regarded as an abnormal condition. In the study by Weymouth et al. PMN infiltration was observed primarily in the tissue of patients who experienced early rejection while only a

few PMN's were seen in the glomerular capillaries of patients who experienced little or no graft rejection. The present study involved 16 of the 19 cases studied by Weymouth et al. and in all except one case the PMN-like cells were observed in the tissue of patients who subsequently experienced excellent clinical course (F.C., C.J., and R.M.). In the fourth case (S.G.) the renal homograft underwent hyperacute rejection with minimal function of the graft. These results indicate that the PMN-like infiltration in the peritubular capillaries and the interstitium surrounding the capillaries in renal tissue one hour after transplantation is not correlated with the future function of the renal homograft.

The tissue of S.G. in which hyperacute rejection occurred was studied not only by this author but also by Weymouth et al. (1970) and by Williams et al. (1967 and 1968). Weymouth et al. reported that glomerular characteristics of this tissue one hour post transplantation consisted of endothelial thickening and arcing, irregular basement membranes, and fusion of the podocytes. PMN infiltration and cellular debris in Bowman's space were also observed. The present study revealed the

presence of some thickening of the basal lamina of the cortical tubules of the S.G. biopsy probably due to edema. In addition several tubules included necrotic appearing epithelial cells and the lumina of the proximal tubules were obliterated by cellular debris. Cellular debris was also observed in the lumina of the peritubular capillaries.

The presence of tubular necrosis and luminal debris in the S.G. biopsies was also reported by Williams et al. (1967) in their studies of hyperacute rejection of renal homografts in man. It was noted by Williams et al. (1968) that at one hour after transplantation numerous PMN's were present in both the peritubular capillaries and the glomerular capillaries of the S.G. biopsy. As previously mentioned, the S.G. tissue observed in the present study displayed only one PMN-like cell in the peritubular capillaries with no indication of a leukocyte accumulation. It is difficult to account for the discrepancies between the results of the two studies. The most probable explanation would be that the tissue sample used in the present study did not include an area of leukocytic infiltration. This is possible even though approximately 200 sections of the S.G. tissue were studied.

Williams et al. (1968) suggested that "hyperacute" rejection is the result of preformed humoral rather than cellular antibodies circulating in the vasculature. This suggestion was based on the discovery of serum antibodies in the blood of S.G. prior to transplantation and antibody activity in an eluate from the graft three days post transplantation at the time of the homograft nephrectomy. Similar results were reported in other patients. In these early biopsies mononuclear cell infiltration was not present. Such an infiltration would be expected in the case of a cellular antibody reaction.

Starzl et al. (1968) concluded that in five cases involving hyperacute rejection of human renal homotransplants studied by their group the rejection was due to the generalized Shwartzman reaction which is an antigen-antibody reaction reversible by heparin treatment. In each case fibrin deposition was observed in the glomerular and peritubular vasculature. PMN infiltration of the glomeruli and cortical necrosis were also observed. All of these features are characteristics of the generalized Shwartzman reaction. Serum antibodies were not found in significant amounts in the samples obtained from these patients. Myburgh et al. (1969) suggested that both the Shwartzman and Arthus reactions

could be involved in the hyperacute rejection of renal homografts. Like the Shwartzman reaction, the Arthus reaction is an acute antigen-antibody reaction. However, the Arthus reaction is not reversible with heparin and is therefore apparently not thrombotic in nature, but is rather an inflammatory process (Cochrane, 1965). Two renal homotransplants were studied one hour following transplantation by Myburgh and his co-workers. In one case, PMN infiltration was observed in the glomerular and peritubular vasculature but fibrin was not present in the tissue. The authors noted the similarity between the appearance of this tissue and that found in the Arthus reaction. The second one hour biopsy also revealed PMN infiltration of the vasculature but in this case fibrin was also present. These characteristics suggested a generalized Shwartzman reaction. Biopsies from the same cases taken at later times confirmed the presence of the rejection processes indicated by the one hour results and Myburgh et al. (1969) concluded that in some cases the Arthus reaction is responsible for hyperacute rejection of renal homografts in man and in other cases the Shwartzman reaction is involved. The question still remains as to what dictates the type of rejection process that will occur and what conditions must

be present for a particular reaction to develop. The present study involving one hour biopsies has not uncovered any facts that would aid in resolving these questions.

The majority of human renal homotransplant rejection episodes are either acute or chronic in nature (Beathard, 1969). Acute rejection occurs within days or weeks of transplantation and appears to be the result of both humoral and cellular antibody activity with vascular disruption and subsequent necrosis of the renal tissues. Chronic rejection is as a gradual process which may show itself clinically as much as two years post transplantation. This type of rejection has been reported to be a humoral (Beathard, 1969) and a cellular (Williams et al., 1968) antibody reaction. Microscopic characteristics of both acute and chronic rejection in human tissue as described by several groups (Porter et al., 1966; Shemamura et al., 1966; and Williams et al., 1967) include glomerular edema, thickening of the podocyte cytoplasm and thickening of both the glomerular and peritubular capillary membranes. Tubular necrosis and extensive mononuclear cell infiltration in the cortex were also reported. These microscopic alterations of homograft tissue were observed in renal biopsies obtained

from patients who indicated clinical signs of transplant rejection. These clinical signs include fever, tenderness and swelling in the area of the transplant, an increased blood urea nitrogen, increased serum creatinine levels, and cessation of urine flow.

Weymouth et al. (1970) observed many of the above glomerular changes in biopsies obtained one hour after transplantation from patients who subsequently experienced homograft rejection. In addition to the features mentioned previously, PMN infiltration of the glomerular capillaries was also observed. It was noted by the authors that these glomerular alterations were indicative of future renal function with correlation between the intensity of ultrastructural changes in the tissue and the severity of the rejection process.

As noted previously, the present study includes 16 of the 19 cases studied by Weymouth et al. Of these 16 cases only one demonstrated ultrastructural changes in the tubules, interstitial tissue, and peritubular vasculature which were indicative of possible homograft rejection. This patient (S.G.) experienced hyperacute rejection. The tubular, interstitial, and vascular ultrastructure of those patients who experienced chronic rejection (at least two months post transplantation) did

not differ significantly from that of patients whose transplanted kidneys went on to function normally nor from that of the control or donor tissue. An explanation for these results may be that at one hour post transplantation the combination of physiological conditions necessary for the rejection process to occur has not yet reached the tubule network of the kidney. The glomeruli, on the other hand, are the first structures of the functional unit of the kidney to come in contact with the blood of the host and therefore would be the first area in which the rejection process would occur. The increase of cellular debris in the tubule lumina of the one hour tissue was probably due to desquamation of glomerular tissue and of the proximal tubule cells in the vicinity of the glomeruli.

Cellular debris was also observed in the donor tissue although not to the same degree as in the one hour biopsies. Other morphological characteristics observed in both the donor tissue and the one hour tissue of the present study were dilated granular endoplasmic reticulum and Golgi cisternae, swollen mitochondria, disruption of the apical cell membranes and enlarged extracellular spaces especially in the tissue of J.L. These same morphological characteristics of human renal tissue have

been reported by several investigators (Pease, 1955; Brewer, 1965; Ericsson et al., 1965; Myers et al., 1966; Tisher et al., 1966; Bulger et al., 1967; and Tisher et al., 1968). It appears that these characteristics are artifacts inherent in the immersion fixation method that must necessarily be employed in the preparation of human tissue. Tisher, Rosen and Osborne (1969) worked with Rhesus monkey kidney tissue which was found to be very similar morphologically to human renal tissue. They reported that the in vivo intravascular perfusion technique of tissue preservation resulted in material that was representative of functioning tissue whereas immersion-fixed biopsies contained numerous cytological differences. Tisher and his co-workers, studying only the proximal tubule cells of the monkey kidney, found that tissue prepared by the immersion method displayed the following characteristics: 1) cellular debris in the tubule lumina; 2) disruption of the apical cell membranes; 3) enlarged extracellular spaces; 4) swollen mitochondria and microbodies; and 5) dilated endoplasmic reticulum and Golgi cisternae. These features were not present in the perfusion-fixed tissue.

The interpretation of the results of the present study were not affected by the presence of these fixation artifacts since both the donor and the one hour biopsies were prepared by the same method. Any differences between the appearance of the tissue components of the donor and one hour biopsies would be due to factors other than fixation artifact. An exception was the presence of enlarged extracellular spaces in only two cases (J.L. and C.J.) of the present study. The fact that this condition was not found in all or even the majority of cases would indicate an individual difference in the affected tissue. In the case of J.L. these spaces were observed in both the donor and the one hour tissue so it is doubtful that a reaction to the transplant was involved. Poor tissue fixation perhaps due to a hypertonic fixative, may have been responsible for the formation of the enlarged extracellular spaces. The fact that this patient experienced an erratic post transplantation clinical course should keep one from discarding the possibility of a pathological process in the tissue. In the other case (C.J.) in which enlarged extracellular spaces were observed, the patient had an excellent post transplantation clinical course giving the existence of these spaces questionable significance.

CONCLUSIONS

1. It has been reported that ultrastructural changes in the glomeruli of renal biopsies obtained one hour after transplantation may be indicative of future homograft function. This was not found to be the case with the cortical tubules, interstitial cells or peritubular vasculature of these homografts in which there was little or no correlation between pathological changes and future renal function.
2. Results of the present investigation indicate that examination of the tubular, interstitial and peritubular capillary ultrastructure during the first hour following transplantation would not aid the clinician in determining homograft function and clinical treatment.
3. It appears that hyperacute renal homograft rejection is due to an antigen-antibody reaction. There is some question as to the specific mechanism involved since the Shwartzman reaction, the Arthus reaction and preformed antitransplant humoral antibodies have all been implicated. Acute and chronic renal homograft

rejection may be the result of antigen reactions with either humoral antibodies or cellular antibodies carried by mononuclear cells.

4. Immersion fixation of renal tissue in preparation for electron microscopic viewing produces artifacts in the tissue including enlarged extracellular spaces, luminal debris, and swelling of cytoplasmic organelles. It was noted that these changes occurred in both the donor tissue and the tissue obtained one hour following transplantation. Therefore, any differences between the ultrastructure of the two biopsy types (donor and one hour) should be due to changes not associated by the fixation processes.

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APPENDIX

I. Fixation of tissue for EM

Glutaraldehyde - phosphate fixative

25% glutaraldehyde.....12.0 gm
 Phosphate buffer.....88.0 gm
 pH 7.3

Phosphate buffer

Solution a: 0.2M $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (27.6gm/liter)
 Solution b: 0.2M $\text{NaHPO}_4 \cdot \text{H}_2\text{O}$ (35.61gm/liter)
 Add 23.0 ml of solution a to 77.0 ml of
 solution b and dilute to 200 ml with
 distilled water.

Phosphate buffered osmium tetroxide

Solution a: 2.26% $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$
 Solution b: 2.52% NaOH
 Solution c: 5.4% glucose
 Solution d: Add 41.5 ml of solution a and
 8.5 ml of solution b.

OsO_4 fixative: 0.5 gm OsO_4 + 45 ml solution d
 + 5.0 ml solution c.
 pH 7.4 - 7.6

II. Embedding medium

Dow Epoxy Resin

D.E.R. 332.....7.0 ml
 D.E.R. 732.....2.0 ml
 DDSA.....5.0 ml
 DMP-30.....0.28 ml
 Mix thoroughly at room temperature.

III. Time schedule for fixation, dehydration and embedding.

- 1.* Glutaraldehyde fixative (tissue minced
into pieces no larger than 1 mm^3).. 1-2 hrs.
2. Phosphate buffer..... stored at least 1 hr.
but usually overnight
3. OsO_4 fixative..... 1 hr.
4. Distilled H_2O several washes
5. 50% ethyl alcohol (ETOH)..... 10 min.
6. 70% ETOH..... 10 min.
7. 95% ETOH..... 10 min.
8. 100% ETOH..... 10 min.
9. 100% ETOH..... 10 min.
10. 100% ETOH..... 10 min.
11. 100% ETOH: Propylene oxide (1:1)..... 10 min.
12. Propylene oxide..... 10 min.
13. Propylene oxide..... 10 min.
14. Propylene oxide: D.E.R. (1:1)..... 1 hr.
15. Propylene oxide: D.E.R. (1:2)..... 2 hrs.
16. D.E.R.....overnight
17. Embed in capsules and put in a
vacuum at 37°C 3 hrs.
18. Put in 60°C oven.....1-3 days

* Steps 1-9 should be carried out at 4°C .

IV. Stain for thin sections

Lead citrate

Solution a: 1.33 gm lead nitrate
1.76 gm sodium citrate
30 ml distilled water.

Shake for one minute and allow to stand for
30 minutes with intermittent shaking.

Lead citrate stain:

Add 8.0 ml 1N NaOH to solution a and dilute
to 50 ml with distilled water. Mix by
inversion. pH 12.0 ± 0.1

Place grids section-side down on a drop of stain on a paraffin-covered slide or a piece of dental wax in a covered petri dish. Stain sections for four minutes and rinse in a stream of distilled water. Allow to air dry.

V. Stain for thick sections (1u)

Modified toluidine blue stain

Mix:	toluidine blue.....	0.1 gm
	methylene blue.....	0.1 gm
	sodium borate.....	5.0 gm
	demineralized H ₂ O.....	100 ml

Apply a drop or two of stain to the area of a warm slide containing the sections. Leave for approximately one minute depending of intensity desired. Wash slide with running distilled water and allow to air dry. Rinse with xylene and apply cover glass with Permount.

FIGURES

Figure 1. Diagram of the renal tubular system showing the relationships of the different portions of the system and their position in the kidney. The dotted line represents the corticomedullary junction with the renal cortex located above the line (towards the periphery of the kidney) and the renal medulla located below the line.

MD, macula densa. Modified from Crouch, J. E. and J. R. McClintic (1971).

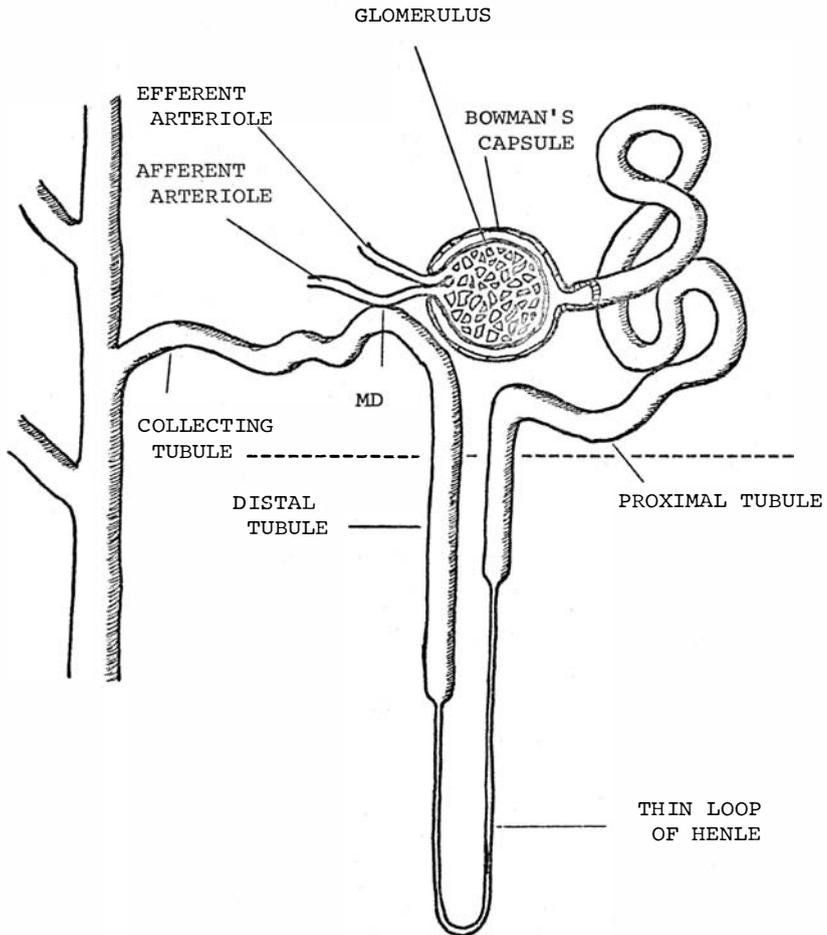


FIGURE 1.

- Figure 2. Donor biopsy. Cellular debris (d) is present in the lumina of the proximal tubules (PT). The epithelial cells of the tubules are irregular in height with cytoplasmic blebs (b) occurring in some areas. mv, microvilli; DT, distal tubules. X 470.
- Figure 3. Donor biopsy. J.L. Notice the enlarged extracellular spaces (ES) between the cells of the proximal tubules (PT). Notice the densely staining cytoplasm of these cells. L, tubule lumen. X 400.
- Figure 4. Donor biopsy. E.B. The lumina of the proximal tubules are obliterated by cellular debris (d). Notice the vacuolated appearance of many of the tubule cells. I, interstitium. X 530.

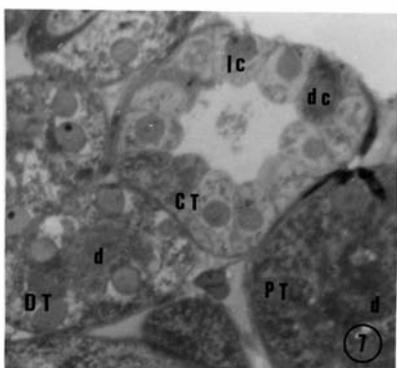
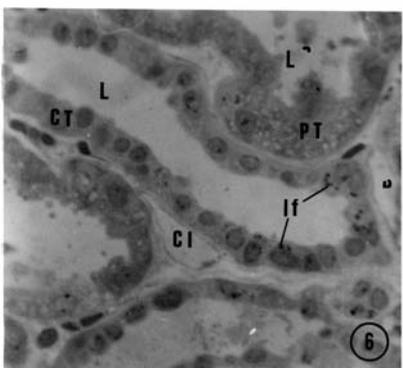
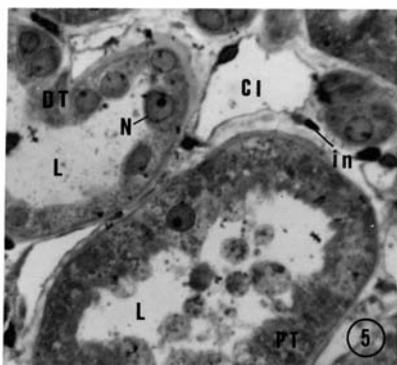
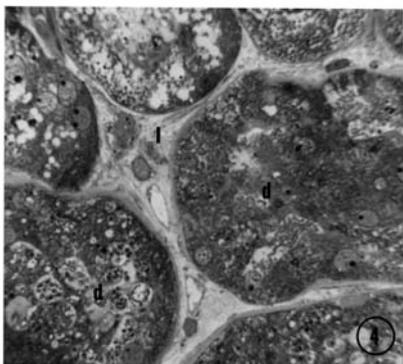
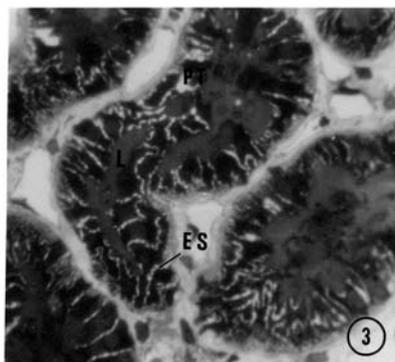
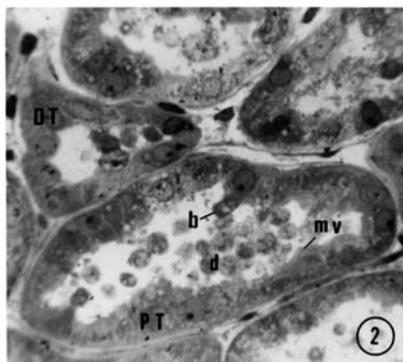


Figure 5. Donor biopsy. Little debris is present in the lumina (L) of the distal tubules (DT). Spherical nuclei (N), in many cases containing a nucleolus, are readily observed. The small, dense areas scattered throughout the cytoplasm of these cells represent mitochondria. Cl, capillary lumen; PT, proximal tubule; in, interstitial cell nucleus. X 670.

Figure 6. Donor biopsy. The lumen (L) of this collecting tubule (CT) is free of debris. The very dense particles present in the cytoplasm of some cells most likely represent lipofuscin droplets (lf). Cl, capillary lumen; PT, proximal tubule. X 600.

Figure 7. Donor biopsy. The collecting tubule (CT) contains light cells (lc) and dark cells (dc). Notice the lack of debris in the lumina of this tubule and the accumulation of debris (d) in the lumina of the proximal tubule (PT) and the distal tubule (DT). X 670.

- Figure 8. One hour biopsy. F.C. Very little debris is present in the lumina (L) of these particular proximal tubules. Blebs (b) are present, however. A small vein (vn) is also seen. mv, microvilli. X 470.
- Figure 9. One hour biopsy. P.W. The lumina of the proximal tubules are filled with cellular debris (d). Vacuoles (V) are present in the cytoplasm of some tubule cells. X 470.
- Figure 10. One hour biopsy. E.B. The lumina (L) of the distal tubules are free of debris. Notice the convex shape of the luminal surface of the tubule cells (arrow). ge, glomerular epithelium; in, interstitial cell nucleus. X 530.
- Figure 11. One hour biopsy. W.C. Necrotic appearing cells (arrows) are present in three collecting tubules. The other cells of these tubules are normal in appearance. Some debris (d) is present in the lumina of these tubules. PT, proximal tubule; Cl, capillary lumen. X 530.

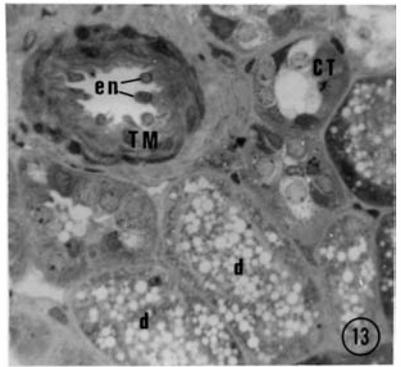
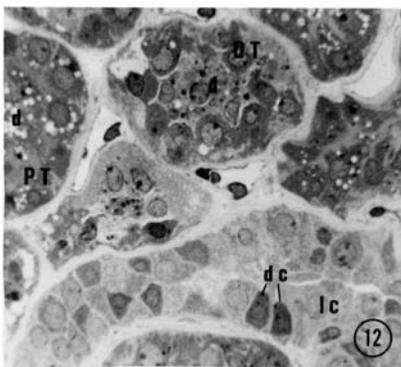
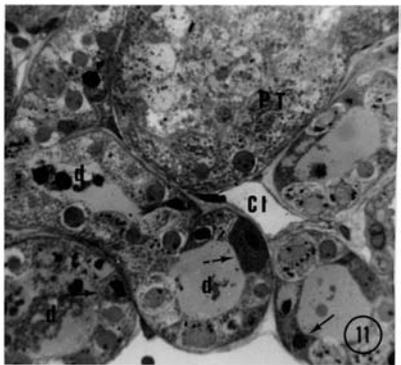
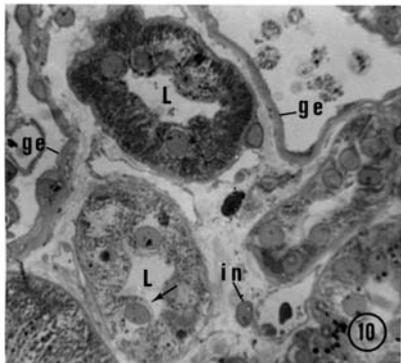
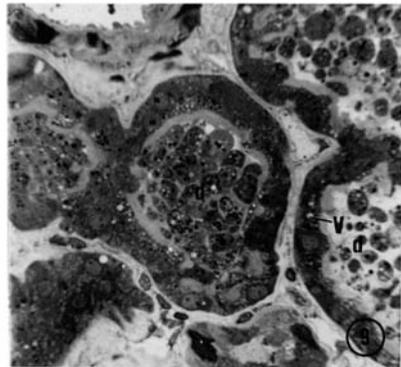
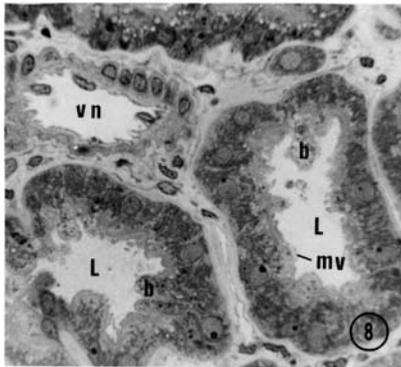


Figure 12. One hour biopsy. C.J. The cells of the collecting tubule are irregular in shape. Light cells (lc) and dark cells (dc) are present in these tubules. Debris (d) is seen in the lumina of surrounding tubules. DT, distal tubule; PT, proximal tubule. X 530.

Figure 13. One hour biopsy. E.M. The nuclei of the endothelial cells (en) project into the lumen of the tunica media (TM) can be seen surrounding the endothelial cells. Highly vacuolated debris (d) is present in the lumina of some proximal tubules. CT, collecting tubule. X 470.

Figure 14. Donor biopsy. Proximal tubule cell showing two small blebs (b) and cellular debris (d) consisting of large cytoplasmic segments as well as individual organelles. Cytosomes (C) containing dense bodies are present in the cytoplasm of the tubule cell.

L, tubule lumen; mv, microvilli; tj, tight junction; ds, desmosome; av, apical vesicle; N, nucleus; M, mitochondrion; r, ribosomes; mb, microbody; Bl, basal lamina; c, collagen; Cl, capillary lumen.

X 9,100.

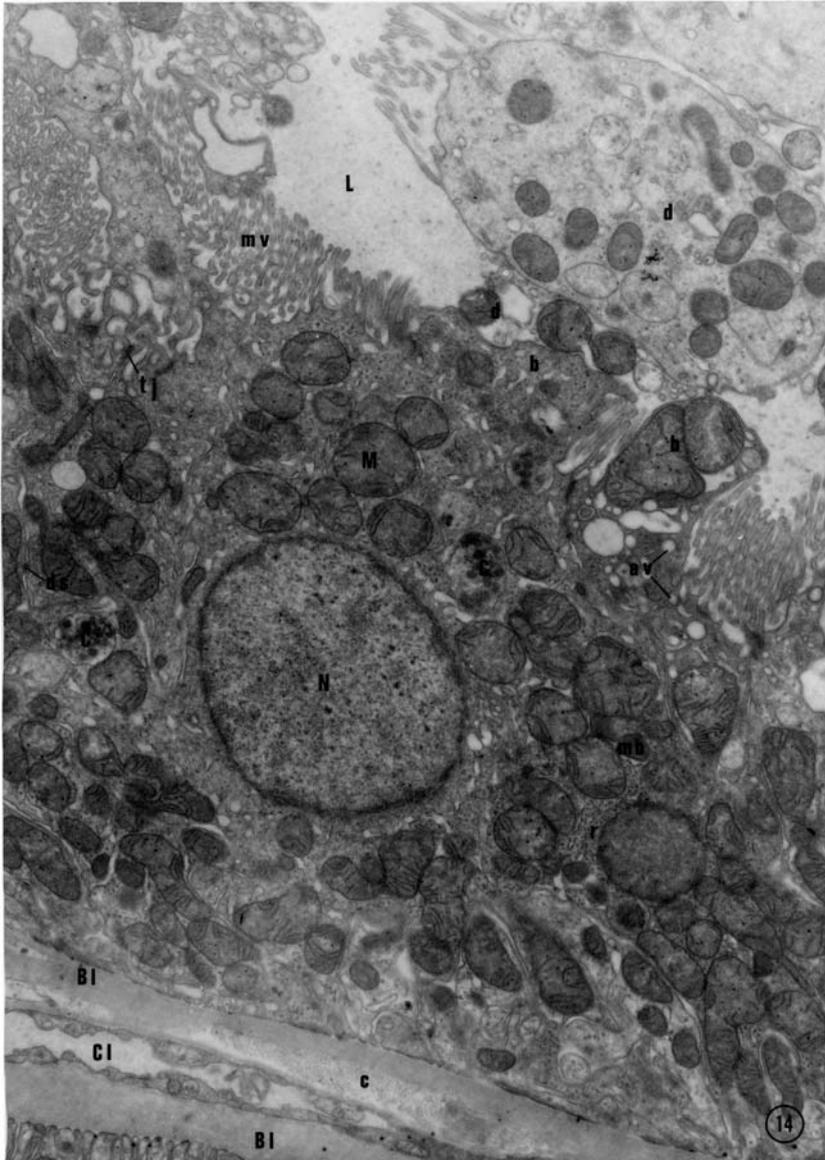


Figure 15. Donor biopsy. J.L. Notice the enlarged extracellular spaces (ES) between adjacent proximal tubule cells. Notice also the lamellated bodies (lb) often located in these spaces. Many mitochondria (M) of these cells contain matrical granules (g). mv, microvilli; ps, perinuclear space; mvb, multivesicular body; ser, agranular endoplasmic reticulum; Bl, basal lamina; Cl, capillary lumen. X 14,400.

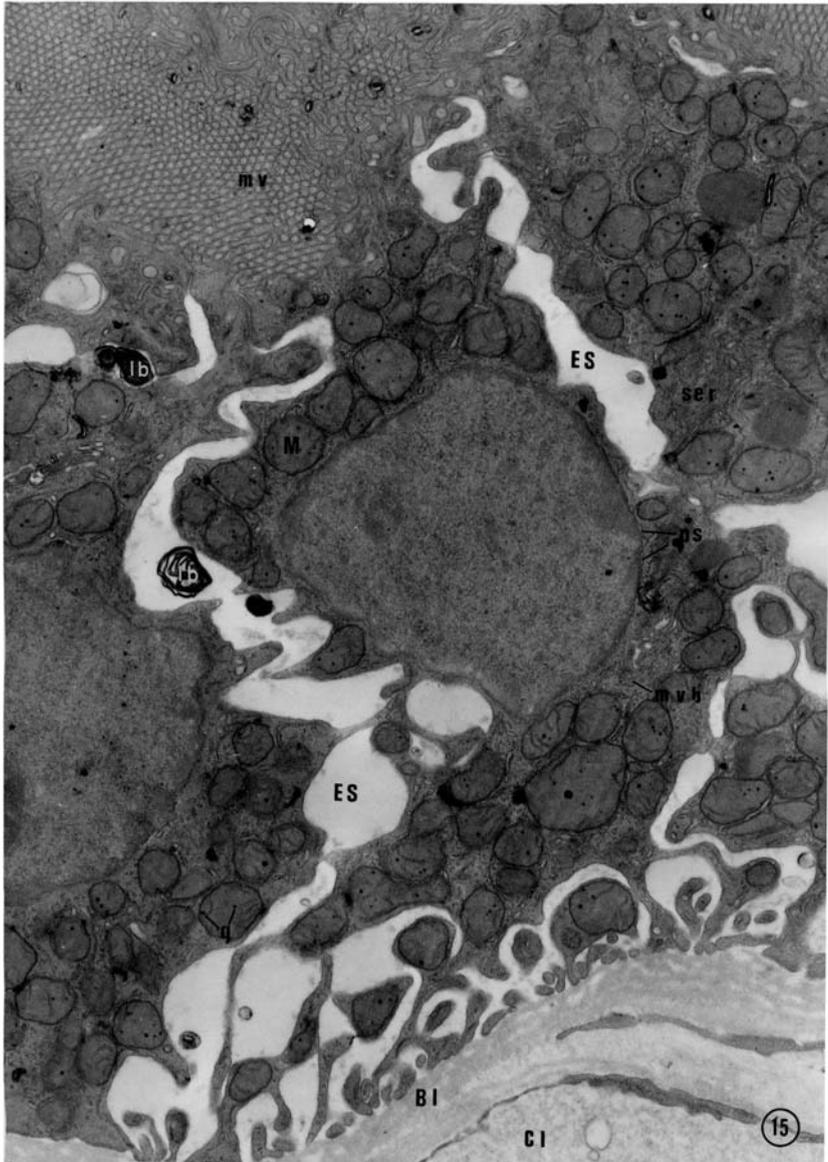


Figure 16. Donor biopsy. E.B. Many mitochondria (M) of this proximal tubule cell are irregular in shape (arrow). Granular endoplasmic reticulum (ger) is dilated and giant cytosomes (C) containing numerous vesicles (v) are located in the apical cytoplasm. p, nuclear pore; Bl, basal lamina. X 16,500.

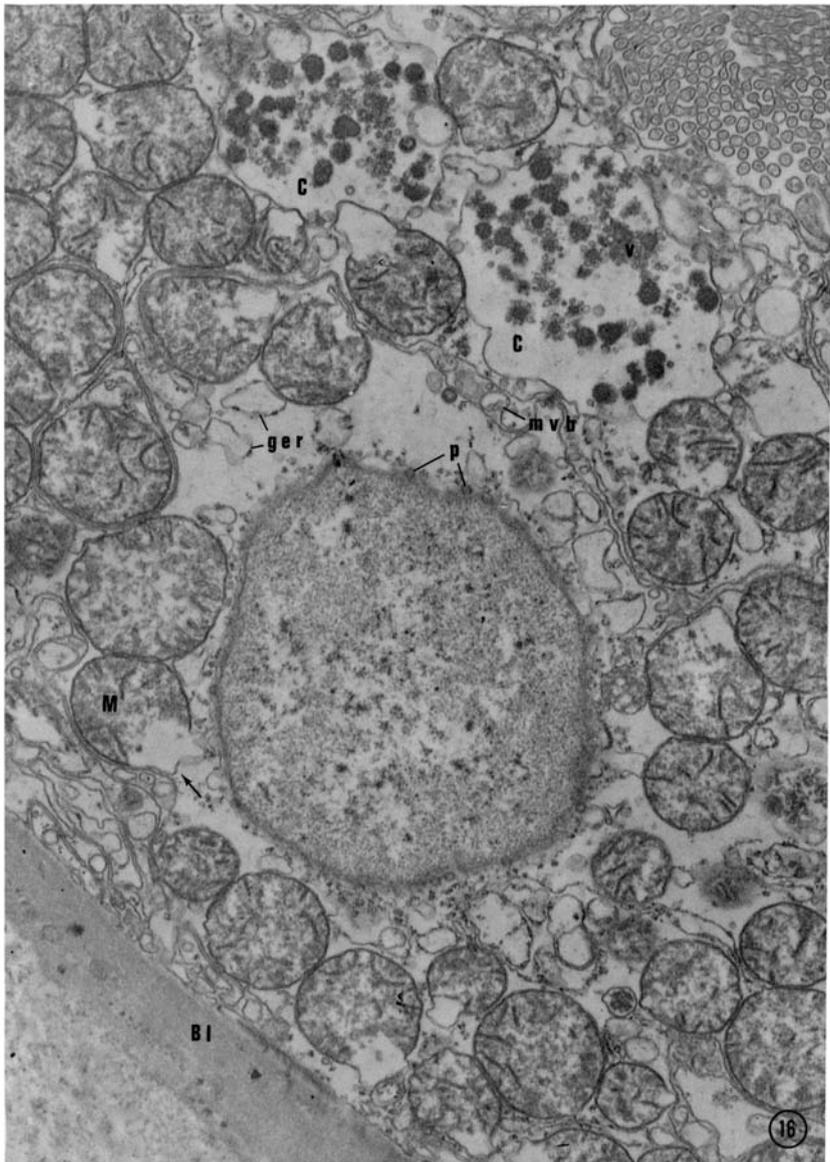


Figure 17. Donor biopsy. The apical membrane (am) of the distal tubule cells is convex in shape in the area of the nucleus (N). Notice the lack of interdigitations in the lateral cell membrane (lm) while numerous infoldings are located along the base of the cells. Large lipofuscin droplets (lf) are present in the cytoplasm of these cells. d, debris; L, lumen; M, mitochondrion; G, Golgi apparatus; ger, granular endoplasmic reticulum; v, vesicles; e, elastic fibers. X 9,800.

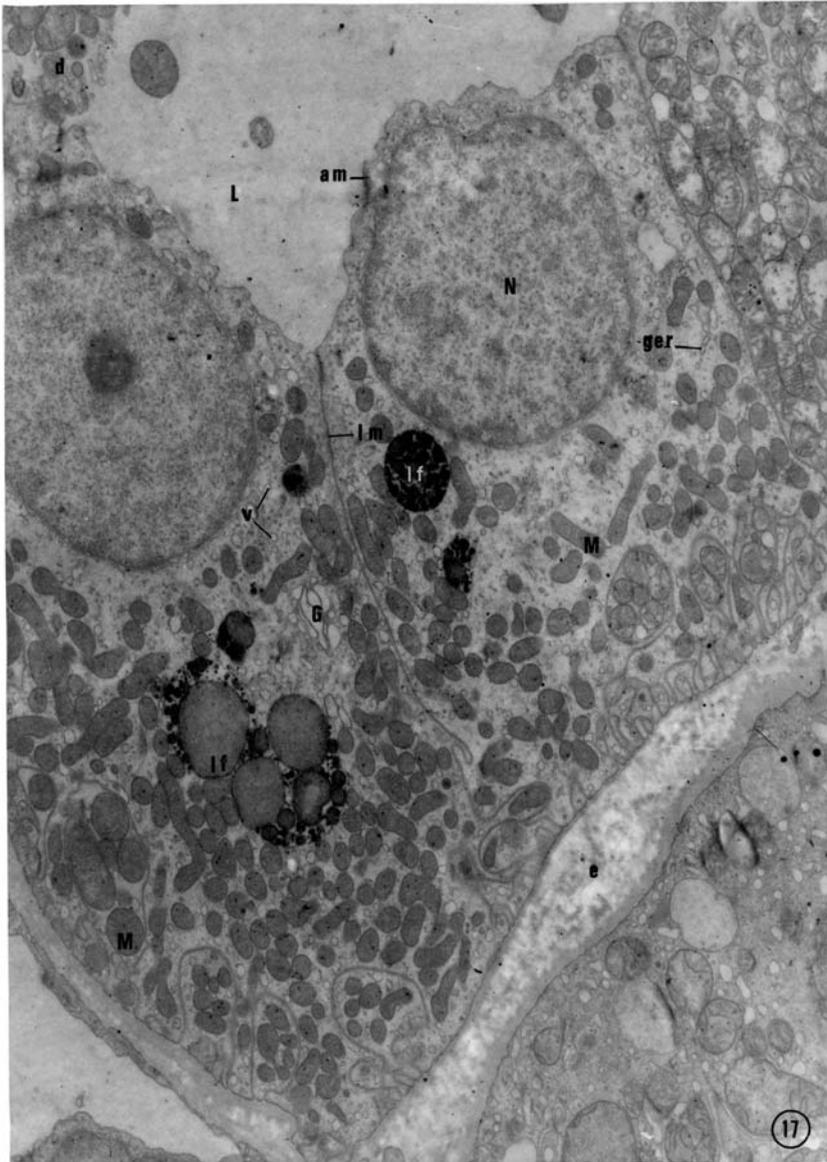


Figure 18. Donor biopsy. E.B. The apical membrane of the upper distal tubule cell is disrupted (arrows). Notice the enlarged perinuclear spaces (ps) and the dilated granular endoplasmic reticulum (ger). M, mitochondrion; lf, lipofuscin droplet; n, nucleolus; r, ribosomes; G, Golgi apparatus; Cl, capillary lumen. X 7,700.

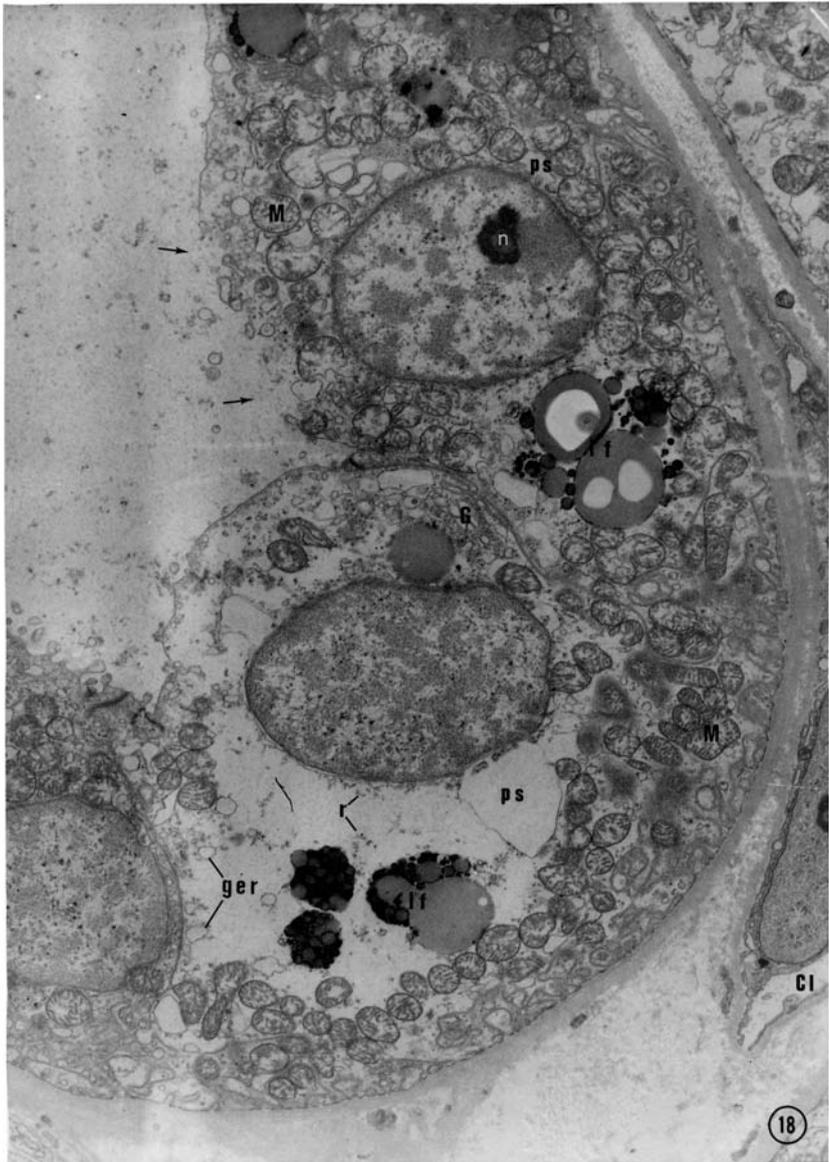


Figure 19. Donor biopsy. A dark cell (dc) is included among the light cells (lc) of this collecting tubule. Vesicles (v) and free ribosomes (r) are more plentiful in the dark cell than in the light cells. Notice the enlarged perinuclear spaces (ps) in the dark cell and in one of the light cells. mv, microvilli; mvb, multivesicular body; n, nucleolus; C, cytosome; Bl, basal lamina; e, elastic fibers. X 8,000.

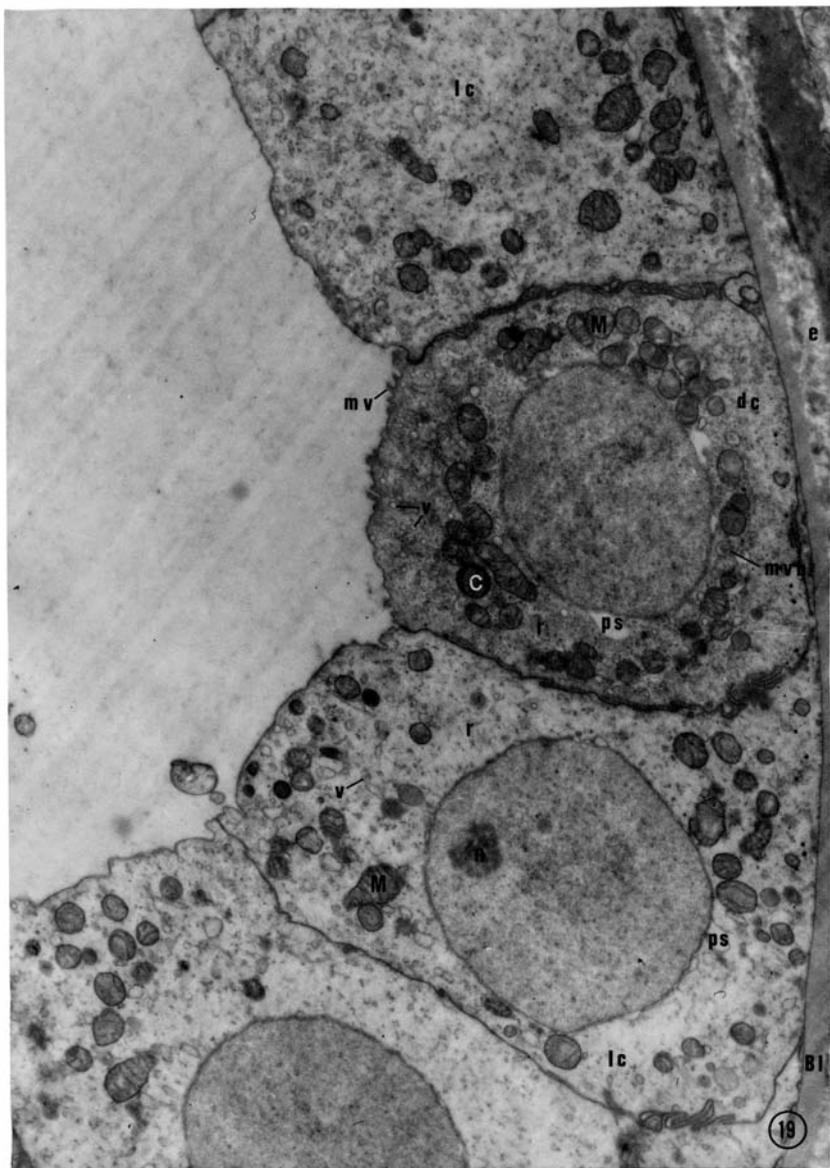


Figure 20. Donor biopsy. The endothelial cytoplasm of the capillary appears to be separated from the basal lamina (Bl) in several areas (arrows). Pores (cp) in the endothelium are clearly visible as are the thin diaphragms that bridge these pores. tj, tight junction; G, Golgi apparatus; ger, granular endoplasmic reticulum; M, mitochondrion; IC, interstitial cell; c, collagen; PT, proximal tubule.

X 12,200.

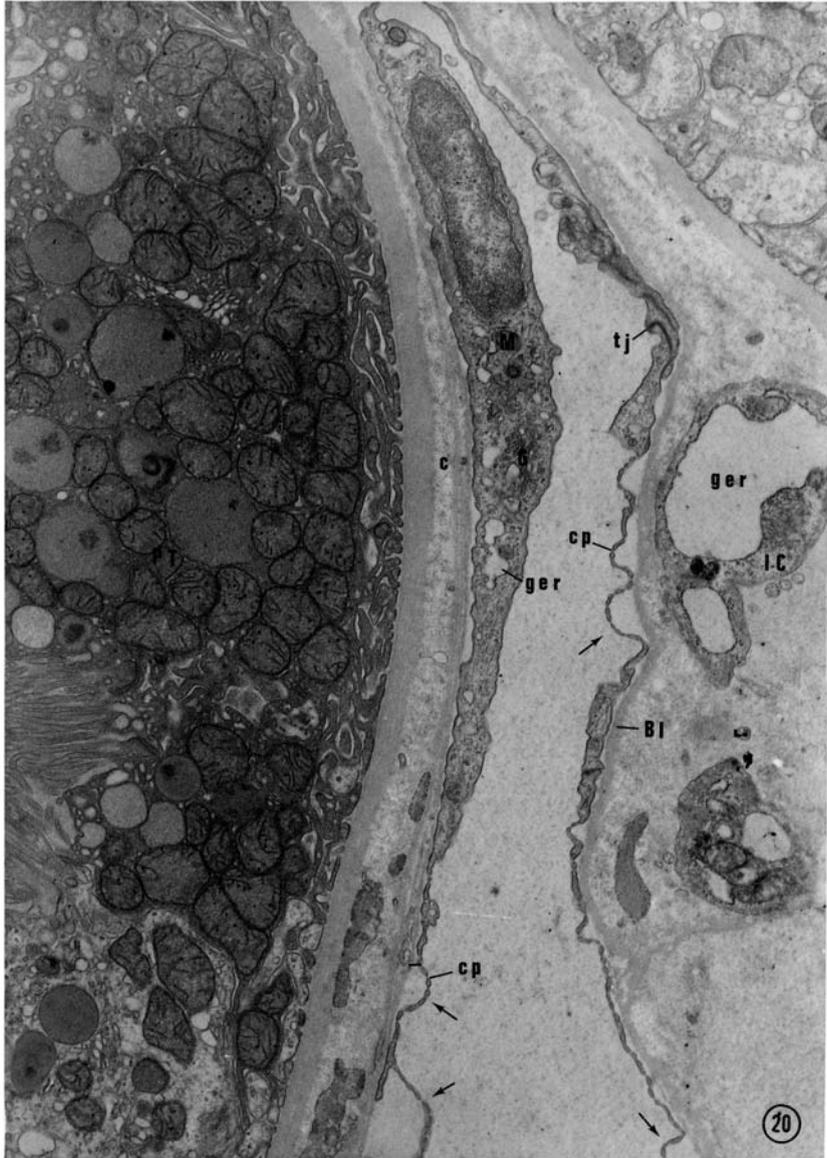


Figure 21. Donor biopsy. R.C. Erythrocyte (rbc) infiltration of the interstitium is evident. Notice the cellular debris (d) in the capillary lumina (Cl). IC, interstitial cell; c, collagen. X 2,200.

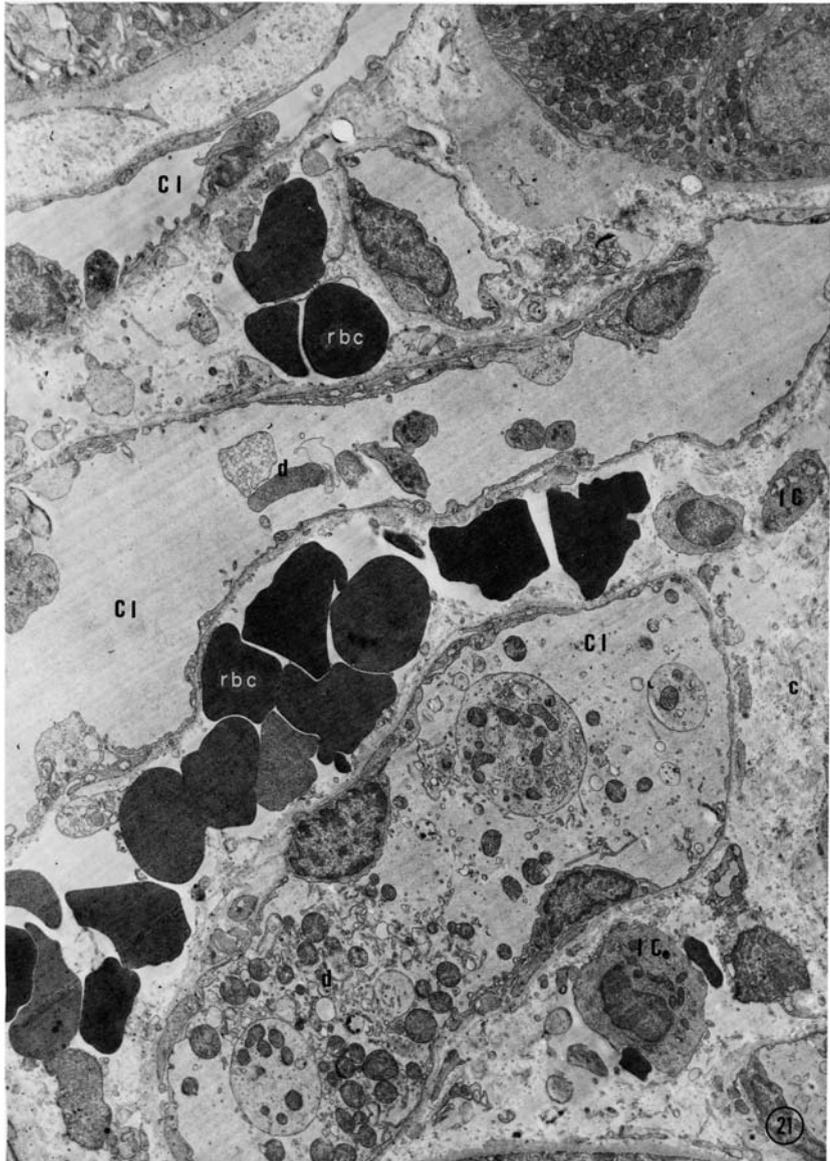


Figure 22. Donor biopsy. Smooth muscle cells (SM) of the tunica media surround the endothelial cells (en) of this small artery. The endothelial cytoplasm projects into the arterial lumen (AL) and the basal lamina (Bl) is tortuous. Small areas of vesicular appearing debris (D) are located between the basal lamina and the smooth muscle cells. v, vesicles; C, cytosome; Gly, glycogen; lf, lipofuscin droplets; IC, interstitial cell; c, collagen. X 6,500.

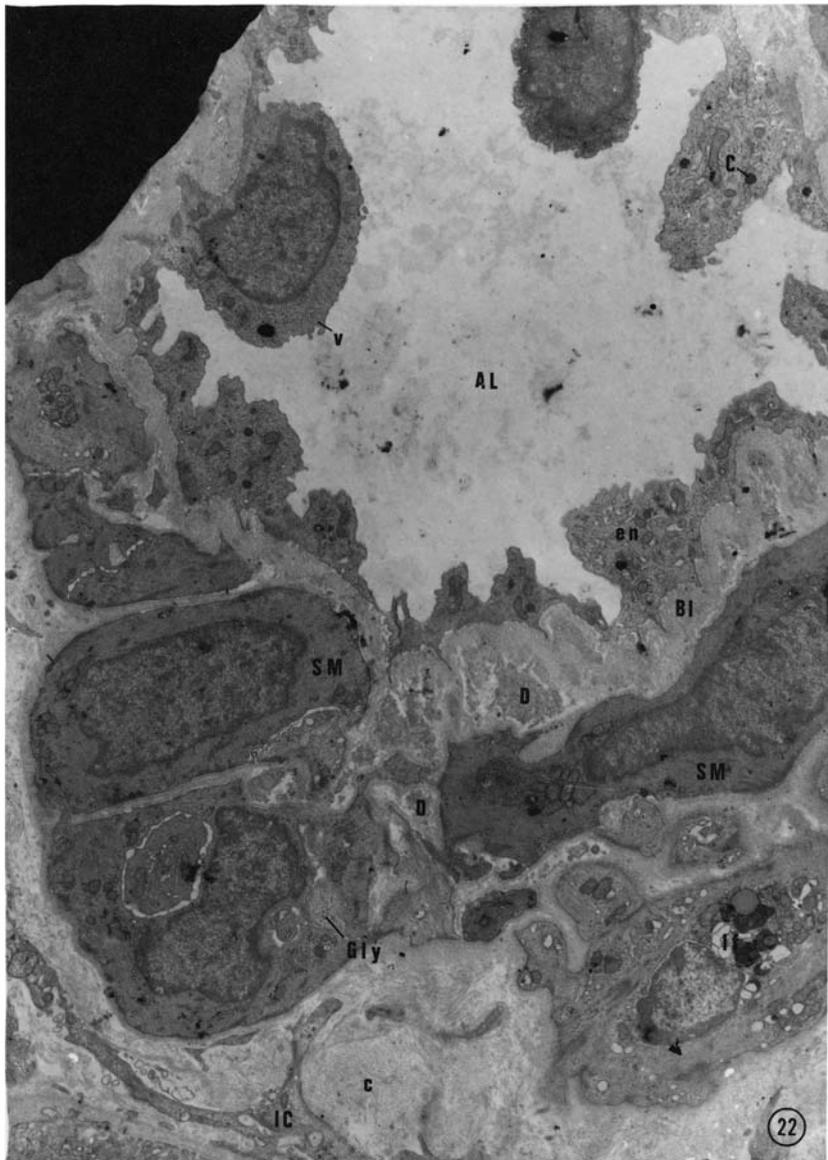


Figure 23. One hour biopsy. R.M. The internal structure of this proximal cell appears to be identical to that of proximal cells of the donor tissue. The tubule lumen is filled with cellular debris (d) which includes a nucleus (N), cytoplasmic segments, and numerous individual mitochondria (M). mv, microvilli; at, apical tubules; C, cytosome; ger, granular endoplasmic reticulum; V, vacuole; p, nuclear pore; G, Golgi apparatus; IC, interstitial cell. X 8,500.

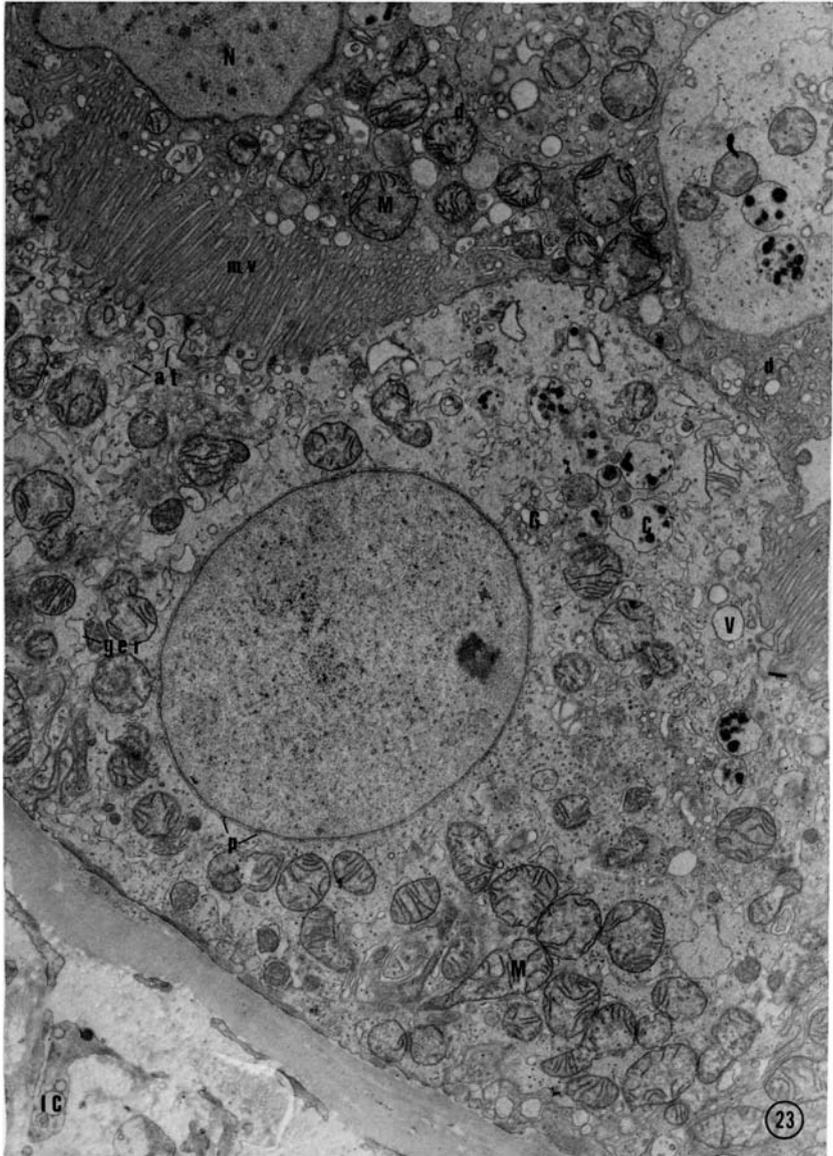


Figure 24. One hour biopsy. J.L. The proximal tubule cells in this biopsy are identical in structure to those of the donor biopsy (Figure 15) of the same case. d, cellular debris; ES, extracellular space; lb, lamellated body; n, nucleolus; C, cytosome; Cl, capillary lumen. X 8,200.

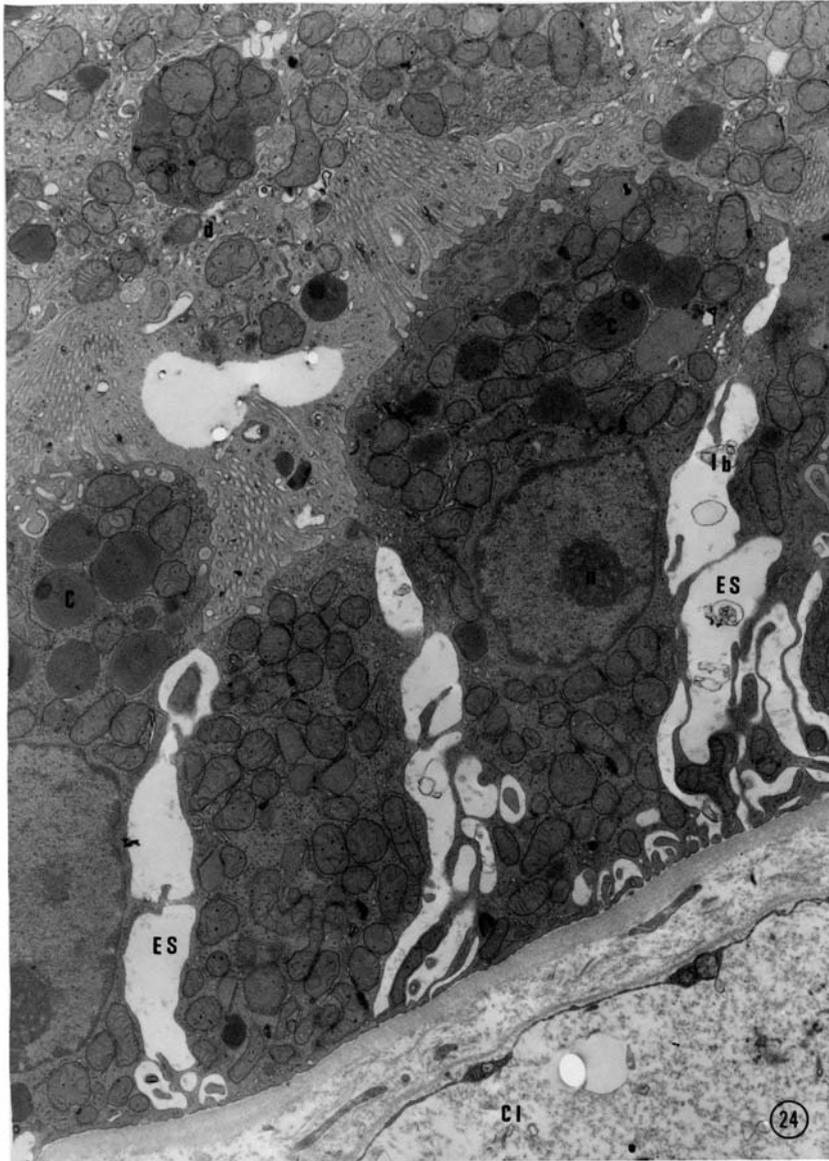


Figure 25. One hour biopsy. E.B. The cytoplasm of these proximal tubule cells (PT) appears to be compressed. Large cytosomes (C) are present both within the cytoplasm of these cells and in the tubule lumen which is obliterated by cellular debris (d).
X 11,400.

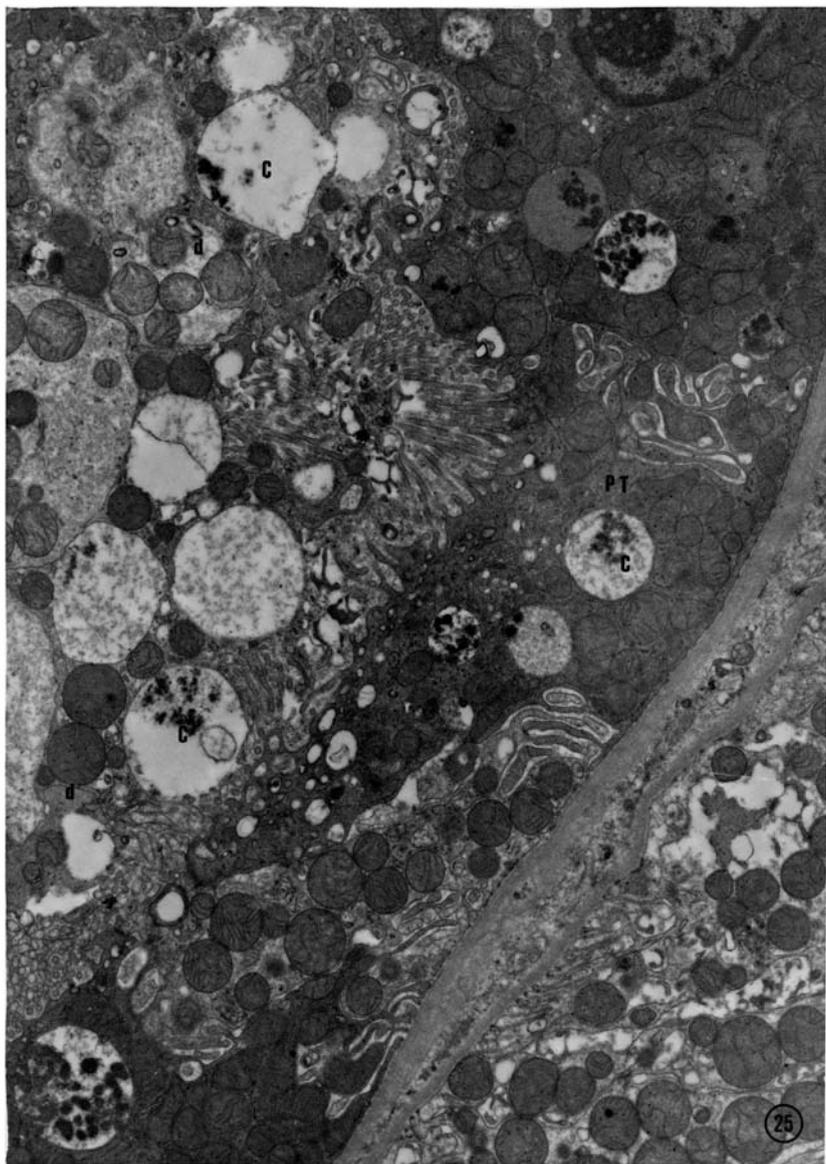


Figure 26. One hour biopsy. S.S. This distal tubule cell is typical of those observed in the one hour tissue. Notice the convex curvature of the apical membrane (am). Several cytosomes (C) are present in the apical area of the cell. M, mitochondrion; ger, granular endoplasmic reticulum; r, ribosomes; G, Golgi apparatus; L, tubule lumen; Cl, capillary lumen. X 12,300.

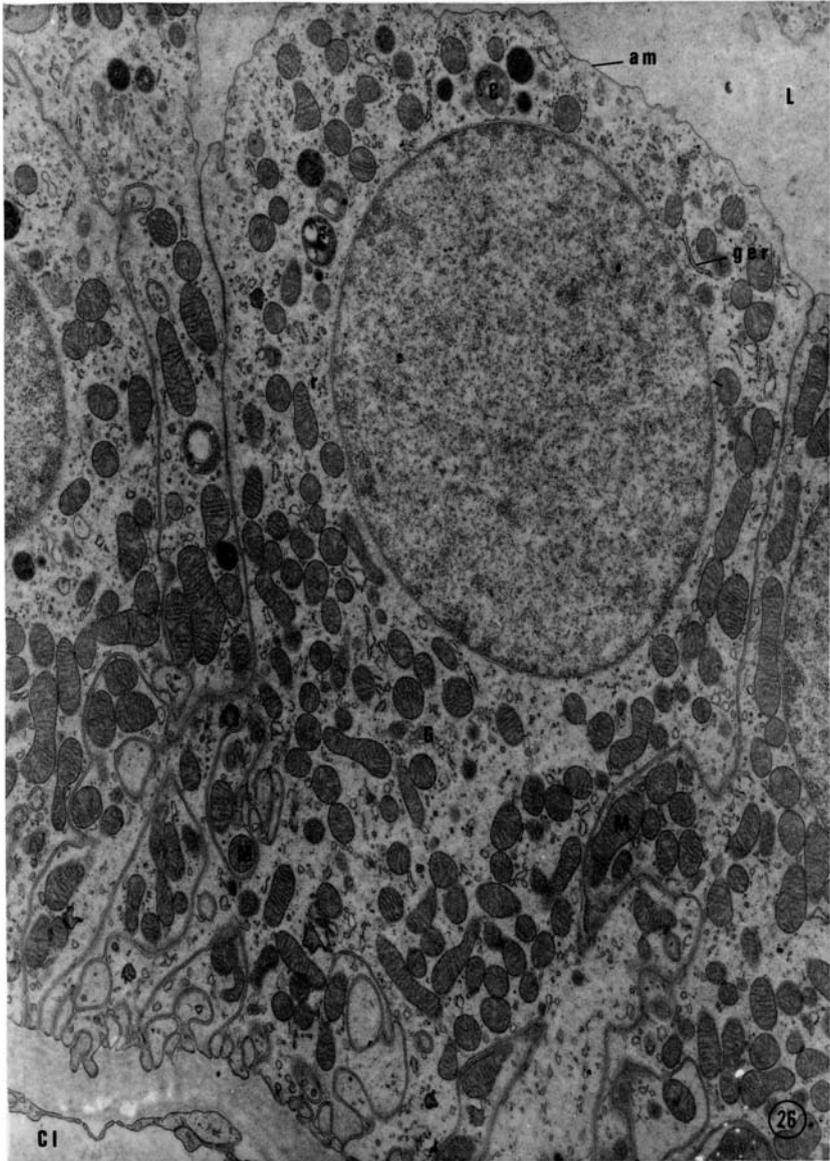


Figure 27. One hour biopsy. N.K. The cells of this tubule appear to be undergoing necrosis. The nuclei (N) are irregular in shape and numerous vacuoles (V) and vesicles (v) are present in the cytoplasm. The apical membrane of one cell is disrupted (x). Notice the basal infoldings (arrows) in some areas. X 8,800.

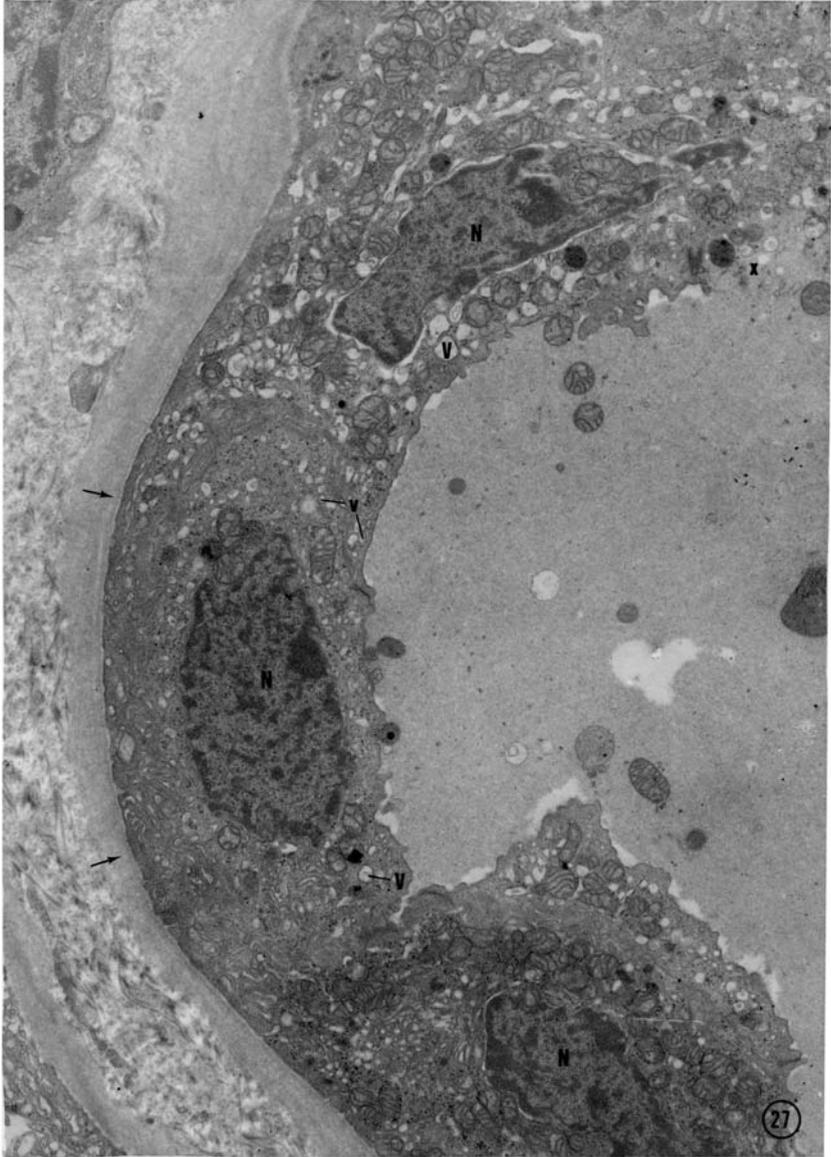


Figure 28. One hour biopsy. C.R. One cell (nc) of this collecting tubule appears to be necrotic. The cytoplasm contains many vesicles (v), irregularly shaped spaces (s), and an atypically shaped nucleus (N). Notice the disruption (x) of the apical membrane of the adjacent light cell (lc). dc, dark cell; lf, lipofuscin droplet; IC, interstitial cell. X 7,100.

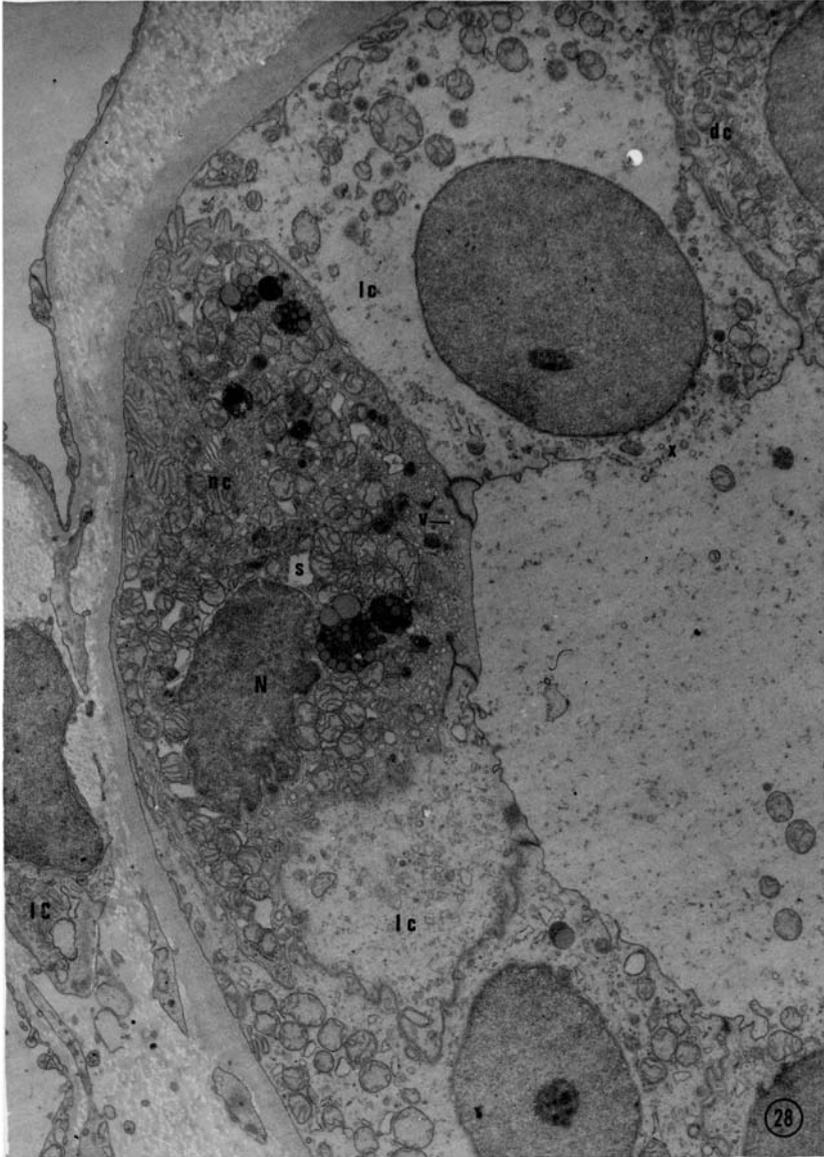


Figure 29. One hour biopsy. C.J. Located adjacent to the peritubular capillary (Cl) are two interstitial cell nuclei (ICN) each surrounded by a small amount of cytoplasm. To the left of the nuclei is a portion of a cell that has been identified as a macrophage (Ma). Large bodies (Ly) probably lysozymes can be seen in the cytoplasm of this cell. Db, dense body; M, mitochondrion; d, debris. X 14,400.

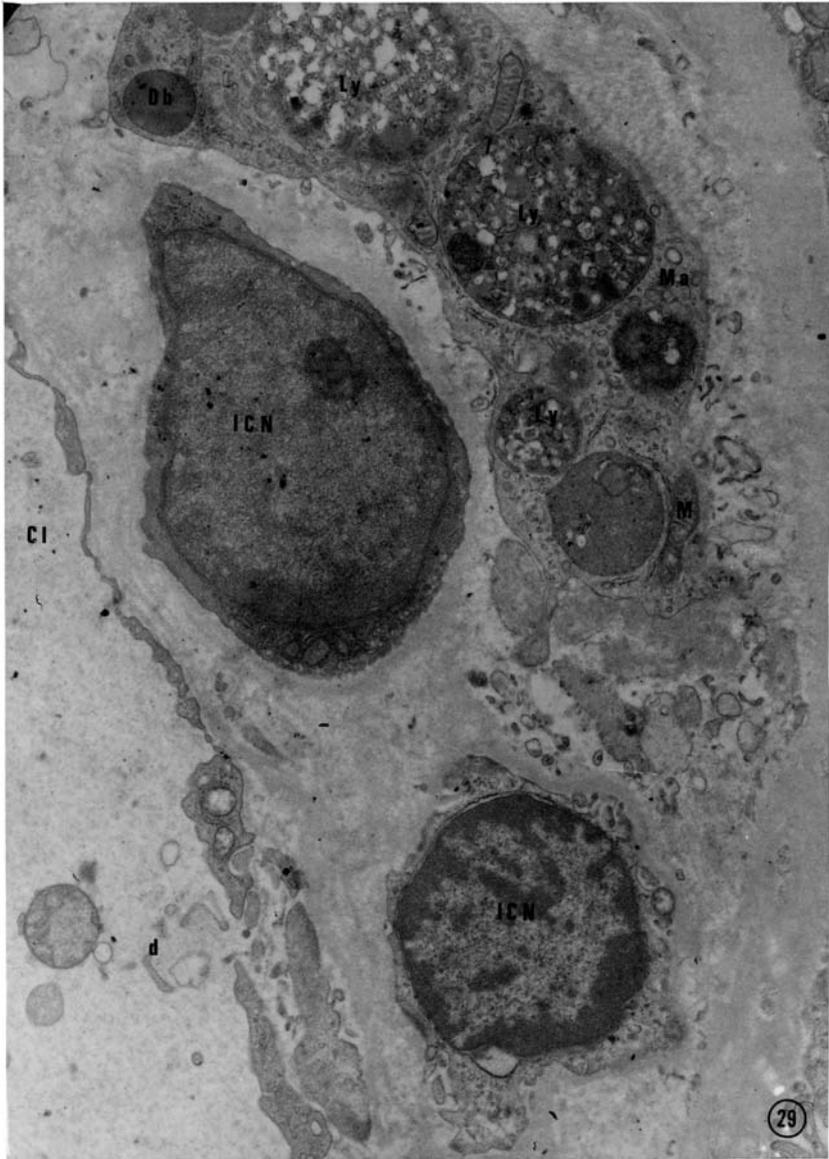


Figure 30. One hour biopsy. R.M. Two cellular segments are observed in the interstitium. Both segments contain numerous vacuoles (V) but only one displays a nucleus (N). This nucleated segment also exhibits an evagination or bleb (b). These segments probably represent leukocytes although specific granules were absent. IC, interstitial cell; Bl, basal lamina; c, collagen. X 10,200.

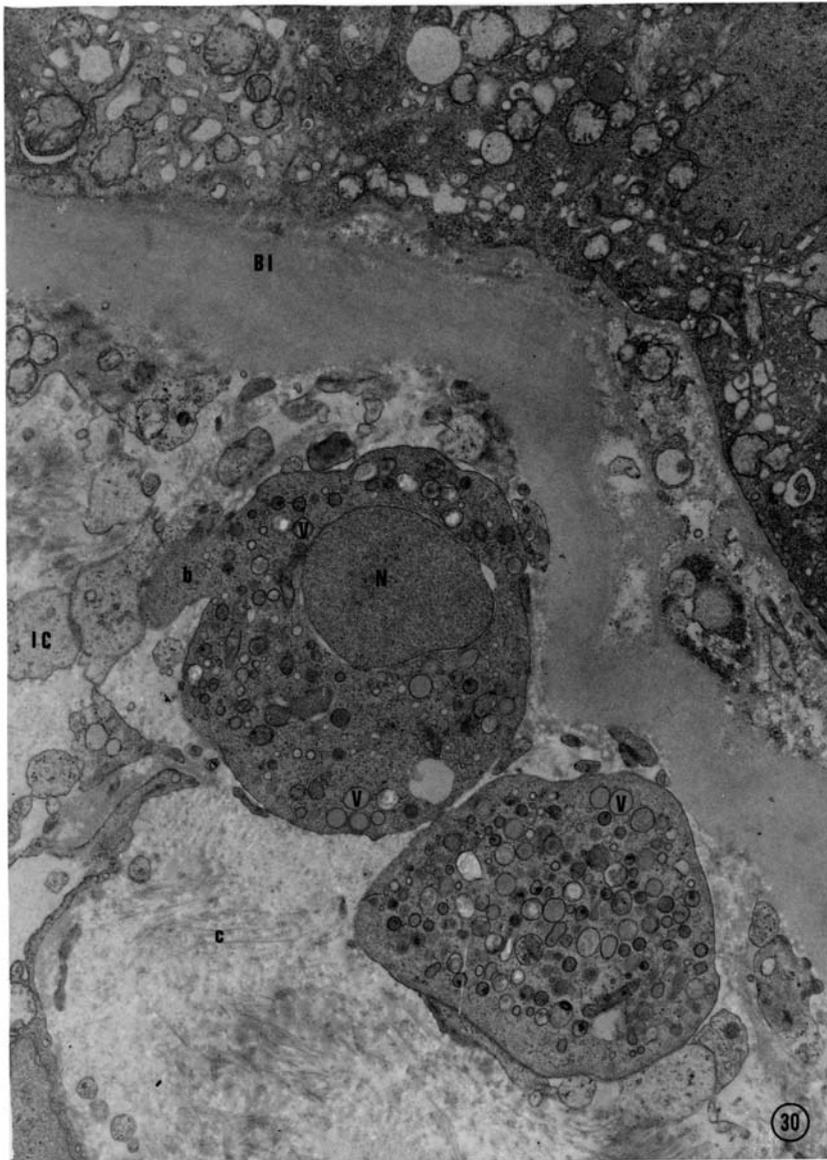


Figure 31. One hour biopsy. P.W. An erythrocyte (rbc) is present in the lumen (Cl) of this peritubular capillary. Dilated granular endoplasmic reticulum (ger) and irregularly shaped mitochondria (M) are observed in the endothelial cytoplasm. Numerous vesicle (v) were also present in the cytoplasm. d, debris; c, collagen. X 11,000.

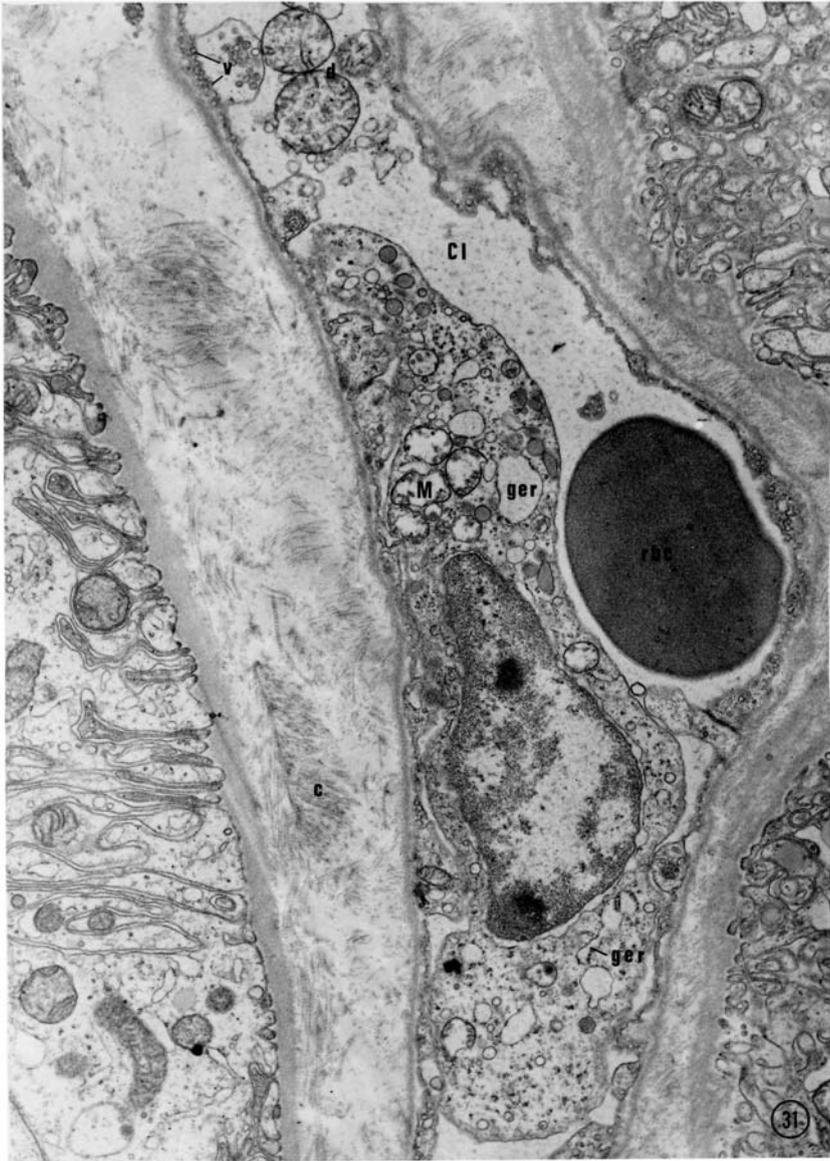


Figure 32. One hour biopsy. W.C. This cell closely resembles a polymorphonuclear leukocyte. Two nuclear segments (N) are seen. Many vacuoles (V) are also present in the cytoplasm. M, mitochondrion; ger, granular endoplasmic reticulum; Cl, capillary lumen; rbc, erythrocyte; IC, interstitial cell; Bl, basal lamina. X 15,000.

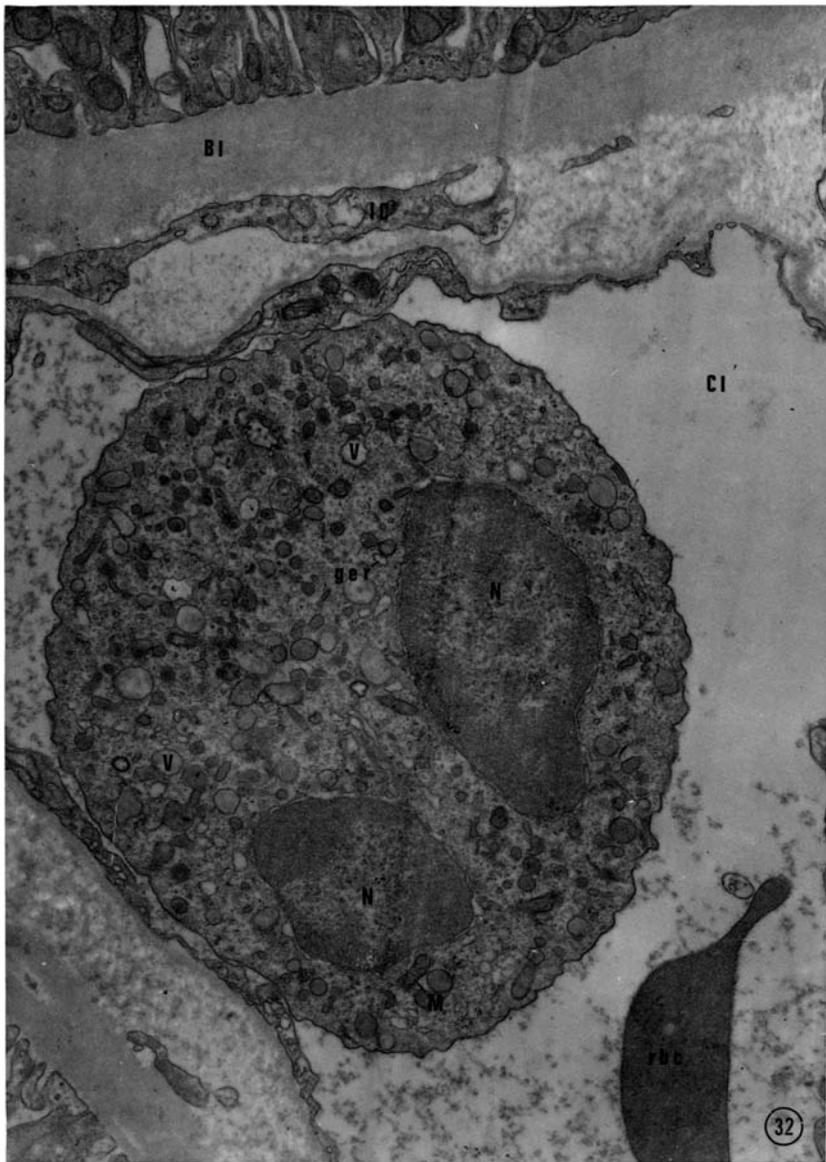


Figure 33. One hour biopsy. C.J. The cell located in the capillary lumen (Cl) is most likely a polymorphonuclear leukocyte. These nuclear segments (N) can be seen. Vacuoles (V) are located in the cytoplasm. Also observed is a lamellated body (lb). en, endothelium; cp, endothelial pore; IC, interstitial cell. X 13,300.

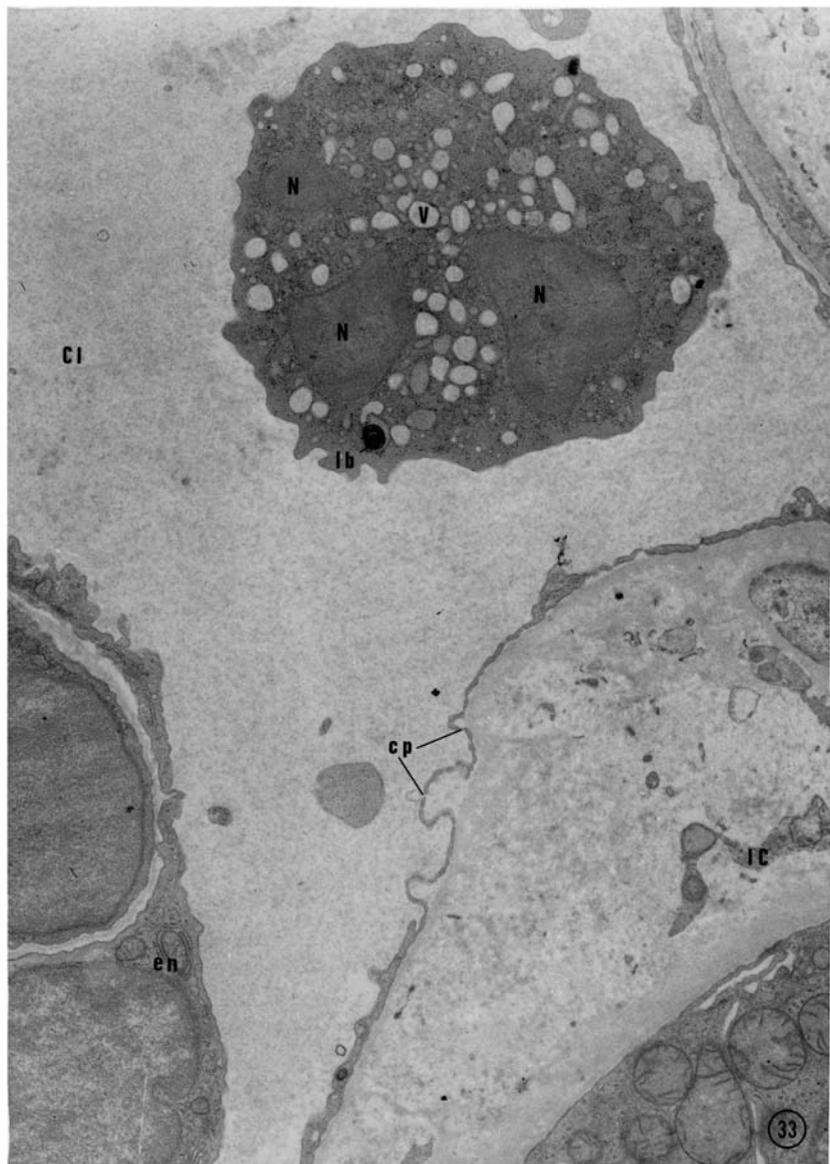


Figure 34. One hour biopsy. P.W. Possible hypertrophy (x) of the endothelial basal lamina (Bl) can be seen in this small artery. Extracellular spaces (ES) are present between many of the smooth muscle cells (SM). AL, arterial lumen; en, endothelial cell; lf, lipofuscin droplet; IC, interstitial cell. X 6,000.

