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ACUTE RENAL FAILURE:
A Morphological Study of Vascular Changes in an Ischemic Model in the Rat

Andrew L. Ries
BA Yale University, 1970

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of
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To My Grandfather

Gustav J. Axelrod

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To:

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INTRODUCTION

The entity of acute renal failure has intrigued investigators for more than a century. As the awareness of the medical community of its occurrence has increased so has the recognition of the multiplicity of events surrounding the sudden onset of oliguria and progressive renal failure. Early investigators used "primitive" means in their search for some common ground in the pathogenesis of such a diversified clinical entity, and many of their hypotheses, which have now been tested and retested with modern techniques, continue to stand. Unfortunately, however, no single mechanism has proved successful in explaining all of the findings.

The present study is an attempt to review some of the previous work which has been done in the field, to test some of the hypotheses utilizing several morphological techniques, and to synthesize some of the seemingly unrelated postulates into a model useful in understanding the pathogenesis of such a multifaceted clinical and pathological condition.

HISTORICAL ASPECTS

The entity of acute oliguric renal failure has been noted for over 100 years (Cumin, 1823 cited in Muehrcke, 1969). However, it was not until World War II that acute renal failure became a well recognized phenomenon. At this time the use of blood and plasma offered the first opportunity to resuscitate great numbers of casualties suffering severe trauma and shock. Following apparently successful resuscitation, progressive renal failure often resulting in death occurred with alarming frequency. Bywaters and coworkers (1941a, 1941b, 1943, 1944) popularized the concept of the "crush syndrome" from their studies of air raid victims during the bombing of London. They noted that persons trapped and buried with pressure on a limb were brought to the hospital with swelling of the limb, hemoconcentration, and often severe hypotension. When blood pressure was restored and the patient's condition had apparently stabilized many patients developed dark urine, oliguria, and progressive azotemia. Subsequently, patients would either die within a week or recover with a diuresis. Spectroscopic examination of urine from several of these patients revealed the presence of myoglobin. At autopsy, in addition to evidence of muscle necrosis, the kidneys showed evidence of varying degrees of desquamation, regeneration, and necrosis of tubules usually with the presence of myoglobin and hemoglobin intratubular casts. There was some evidence of interstitial edema and inflammation. Glomeruli

were apparently normal. These findings were essentially confirmed by American workers (Mallory, 1947) in studying cases of battle injury resuscitated from shock who subsequently developed oliguria, azotemia, urinary excretion of pigment and albumin and subsequent death. They emphasized the morphological findings of pigmented casts primarily in the lower portions of nephrons, dilatation of proximal and sometimes distal tubules with necrosis and/or regeneration of epithelium, and finally rupture of tubules with granuloma formation.

Prior to World War II there were only scattered references to acute renal failure. Perhaps the most complete reports in this period were made by Minami in 1923 (cited in Muehrcke, 1969) who believed that myohemoglobinuria was involved in producing renal damage, and by Kayser in 1922 (also cited in Muehrcke, 1969) summarizing the experience of the German medical corps in World War I. Councilman (1898) and Kimmelstiel (1938) emphasized the interstitial component of renal lesions found incidentally at autopsy, but occasionally seen in association with progressive oliguria and uremia, in the concept of "Acute Hematogenous Interstitial Nephritis." Councilman defined this as "an acute inflammation of the kidney characterized by cellular and fluid exudation in the interstitial tissue, accompanied by, but not dependent on, degeneration of the epithelium; the exudation is not purulent in character, and the lesions may be both diffuse and focal." He believed this condition to occur primarily in infectious diseases of children (particularly diphtheria and scarlet fever). Kimmelstiel, however, 40 years later was aware

of the multitude of conditions preceding the lesion. He also noted the presence of hemoglobin and hematin casts in several cases.

Baldwin Lucké (1946) was the first to point out the common morphological and functional characteristics in the seemingly heterogeneous group of clinical conditions with the sudden onset of renal failure as a common denominator. Tying together the many case reports to that time he noted that the renal changes noted were not specific for "crush" injuries but also were known to occur following nontraumatic muscular ischemia, burns, transfusion with incompatible blood, heat stroke, black-water fever, toxemia of pregnancy, alkalosis, sulfonamide intoxication and poisoning with certain vegetable and chemical agents. The list would be significantly longer today. He suggested the term "Lower Nephron Nephrosis" to emphasize his findings of localization of morphological changes to the distal portions of the nephron.

It is common today to classify the many causes of acute renal failure into three groups: 1) Prerenal -- acute renal circulatory failure, 2) Renal -- acute parenchymal disease, and 3) Postrenal -- acute obstructive uropathy. In spite of the myriad of causes, however, the etiology is often unknown (up to 30% in some series).

The increasing usage of the term "Acute Tubular Necrosis" in an attempt to unify the morphological syndrome was probably secondary to the emphasis in the early reports on massive

tubular necrosis as a prominent feature. Actually, this became an oversimplification when the wide spectrum of tubular changes from severe necrosis to normal tubules was noted. Even Lucké's emphasis on lower nephron lesions has become somewhat inaccurate because lesions are not always predominant in that area. In spite of this, the term "ATN" has come to be used most frequently to designate the wide variety of tubular lesions.

Two distinct types of tubular lesions in "ATN" were first noted by Dr. Jean Oliver (1944-45, 1951, 1953) in his micro-dissection studies of acute renal failure. The first he designated "Nephrotoxic," indicative of the selective desquamation and necrosis of all proximal tubules, not including the basement membrane, secondary to a direct toxic effect of a particular agent (which the nephron conveniently absorbs primarily in the proximal convolutions). The second type of lesion he called "Tubulorrhexis," and believed this to be due to renal ischemia. This lesion is a destructive, disruptive and necrotic tubular lesion occurring anywhere along the nephron and includes fragmentation of the basement membrane. Not every nephron is affected and varying lengths of different nephrons may be affected. This morphological data correlated well with the functional findings of tubular dysfunction (loss of ability to concentrate waste products, loss of ability to conserve ions, inability to extract PAH from blood, and loss of ability to reabsorb glucose) in acute renal failure. These functions are primarily located in the proximal convolution and are less well explained by distal lesions. Oliver emphasized, however, that this dichotomy is

not absolute and nephrotoxic lesions may occur with ischemia and tubulorrhexic lesions may occur with toxins. In fact, as noted by Kimmelstiel (1938) there may be no renal abnormality (even by EM) in acute renal failure.

Oliver's distinction may also have some bearing with regard to the recovery from the acute phase. Recovery from a nephrotoxic lesion may be simpler, requiring a new inner epithelial lining to cover the intact basement membrane. With the tubulorrhexic lesion, however, the basement membrane must be replaced as well.

Actually the most universal parenchymal abnormality in acute renal failure is the interstitial change -- edema and infiltration with cells. Some believe that the interstitial edema is secondary to leakage through damaged tubules, but it is more likely secondary to the primary renal insult and accompanies the inflammatory response. As noted previously, Councilman and Kimmelstiel both emphasized the interstitial edema and infiltration which may be with lymphocytes, plasma cells, histiocytes, or eosinophiles (especially in cases of drug induced acute renal failure).

ETIOLOGY

It is not surprising that in spite of the search for morphologically unifying concepts for a clinical entity with as many apparent precipitating events as acute renal failure researchers have as yet been unable to identify any common pathogenetic denominator. The principal hypotheses have attempted to attribute the etiology of acute renal failure to tubular obstruction, tubular damage, and vascular changes including diminished renal blood flow and glomerular filtration, redistribution of cortical blood flow, and vascular damage leading to the no-reflow phenomenon, any or all possibly mediated by the juxtaglomerular apparatus through the renin-angiotensin system.

The emphasis placed by early investigators on the morphological findings of intratubular hemoglobin and myoglobin casts led to the tubular obstruction theory which proposed that the oliguria was due to mechanical obstruction of urine flow by pigment casts, necrotic cellular debris, protein casts (as in cases of multiple myeloma associated acute renal failure -- Holman, 1939) and the added contribution of the external pressure of interstitial edema. As early as 1875, Ponfik (cited in DeGowin et. al., 1937) caused hemoglobinuria and renal failure in dogs by injecting heterogeneous blood. Yorke and Nauss (1911), while investigating blackwater fever, first noted the association among hemolytic disease, acute renal failure, and hemoglobin casts in tubules. They produced what

they considered to be intrarenal obstruction by injecting rabbits with hemoglobin. Baker and Dodds (1925) found that an acid urine and high (at least 1%) concentration of NaCl were required to precipitate hemoglobin in tubules when injected into rabbits. They even suggested alkalinization of the urine as treatment. This finding was later confirmed by DeGowin et. al. (1937, 1938) in dogs and by Bywaters and Stead (1944) in rabbits on an acidifying diet (no renal failure was produced on a normal diet). Baker (1937) later believed that the clinical outcome of acute renal failure was related to the amount of hemoglobin precipitated and the concentration of the urine.

There were many case reports of fatal hemolytic transfusion reactions associated with acute renal failure to support the experimental evidence and Witts (1929) noted that the commonest cause of death in these cases was "suppression of urine from hemolysis and hemoglobin infarction of the kidneys." Muehrcke (1969) notes that acute oliguric renal failure accors in approximately 45% of patients with myoglobinuria.

In his microdissection work, Oliver (1944-45) stated that the appearance of casts in dissected nephrons was much more impressive than in histological sections with whole tubules distended and completely occluded. He notes the impact (and also the limitations) of these findings:

> It is certainly true that an oliguria, due to lessened renal blood flow, might in certain cases well be the important antecedent factor in the causation of the coagulation, but the morphologist who depends on what he sees and what he can touch and feel perhaps more than on higher intellectual

> means of perception, can only believe that, in the last analysis, water cannot flow through stopped pipes. (p. 135)

Some investigators also believed that the interstitial pressure exerted by the edema and infiltration added to the obstruction of tubular urine flow. Peters (1945) found that a decrease in intrarenal pressure by only a few millimeters of mercury (effected by decapsulation) promptly restored urine output. He even suggested emergency decapsulation as treatment for acute renal failure.

In spite of this evidence, many of the same investigators had doubts about tubular obstruction as the sole cause of acute renal failure. Kimmelstiel (1938) doubted whether the process could be extensive enough to cause complete anuria. Even Bywaters, who was a proponent of the tubular obstruction theory, had doubts that mechanical blockage could be the sole responsible factor since, to produce anuria, blockage would have to include a very large number of nephrons. In addition, he noted that if this were the only factor, then any urine passed should be essentially normal in composition since it would be excreted by unblocked and, therefore, normal nephrons. Actually, the urine produced was often very dilute (Bywaters and Beall, 1941a, Bywaters and Dible, 1943). He even suggested that another factor might be the back leakage of glomerular filtrate through necrotic tubules into the blood stream. Other evidence from biopsies and autopsies indicated that insufficient numbers of nephrons were affected to account for the massive nephron failure necessary to account for the degrees of oliguria noted.

Thus, most investigators came to believe that other factors must be involved.

A second mechanism often cited to account for the pathogenesis of acute renal failure is that of nephrotoxicity. It has long been known that many substances are capable of damaging the kidney (e.g. mercury compounds, CCl_4, glycols, aniline, many drugs, etc.) and the kidney is particularly vulnerable because of its rich blood supply, its ability to concentrate substances, and its active excretory function. That acute renal failure can occur following nephrotoxic action is well recognized and, as mentioned before, Oliver used "nephrotoxic" to designate one of the two distinct classes of tubular damage he felt led to acute renal failure. Some of the mechanisms of nephrotoxic action proposed have included: 1) Direct protoplasmic poison to tubular epithelium in which the substance is filtered and passively concentrated in the tubular lumen; 2) Increase in concentration to toxic levels in the medullary interstitium by osmotic concentration of fluids (e.g. chronic phenacetin nephritis); 3) Penetration and poisoning of cells by interaction with cellular constituents (e.g. meralluride reacting with enzyme systems of sulfhydryl groups within mitochondrial wall); and 4) Hypersensitivity reactions usually of endothelium of glomerular capillaries, arterioles and arteries. Any or all of these mechanisms may be involved in cases where there is a known nephrotoxin implicated, but acute renal failure often occurs in the absence of an obvious toxin.

The area which has now attracted the most work in the

pathogenesis of acute renal failure is that of renal vascular changes. Oliver emphasized this in his designation of the so-called "tubulorrhexic" lesions he believed to be due to ischemia. It was natural to look for vascular changes in the many cases of acute renal failure which did not have some obvious toxin or massive hemolysis to afford a ready explanation. As Oliver stated (1951), "The unifying element in this heterogeneous complex, acute renal failure, is renal ischemia." In the view of many investigators (e.g. Sevitt, 1959) the tubular damage and subsequent uremic state was secondary to renal ischemia and decreased renal blood flow and glomerular filtration. However, the data on renal blood flow in acute renal failure has been poor. Several investigators (Corcoran and Page, 1943a; Selkurt, 1946; Gomori, 1964) have reported a decrease in renal blood flow to as much as 10-15% of normal in hemorrhagic shock. However, as Phillips and Hamilton (1948) have noted, the PAH method of determining renal blood flow is unreliable in acute renal failure especially since tubular function is severely impaired. Some good results have been obtained (Brun et. al., 1955; Munck, 1964) using highly diffusable gases such as Krypton^{85} or Xenon^{133}. With these renal blood flow has been observed to fall to one third normal in acute renal failure, but this should not be enough to explain the oliguria by itself. In addition, if ischemia were the sole mechanism one would expect equal distal and proximal tubular necrosis, and tubulorrhexic lesions are most pronounced in the distal portions of the nephrons.

Thus, investigators began to look for evidence of other

vascular changes through which a reduction in renal blood flow or glomerular filtration rate could mediate the production of oliguria and acute renal failure. Hesse and Filatov in 1933 (cited in Kimmelstiel, 1938) found evidence of arterial spasm in the kidney in hemolytic shock. It is known that when systemic blood pressure falls renal vasoconstriction is part of the compensatory mechanism to maintain blood flow to the heart and brain. Lauson and coworkers (1944), in investigating the renal circulation in shock, found falls in renal blood flow greater than that which could be explained solely on decreased arterial pressure. They felt that this had to represent some degree of vascular constriction. Oken and coworkers (Flanigan and Oken, 1965; Oken et. al., 1966; Thiel et. al., 1967; Wilson et. al. 1967; DiBona et. al., 1971a) used micropuncture to study mercury and glycerol induced acute renal failure and showed that the oliguria reflects decrease in glomerular filtration rate. Since intratubular pressure was decreased, and the peritubular circulation of surface nephrons available for study not decreased, they felt that this was due to aberation in the glomerular afferent-efferent arteriolar tone either by preglomerular vascular constriction, postglomerular dilatation, preglomerular shunting, or a combination of these factors. Munck (1958) measured renal pressure gradients and wedged renal vein pressures and found them to be normal in the oliguric and diuretic phase of acute renal failure. He concluded that the decreases in renal blood flow noted indicated the presence of active vasoconstriction.

A second factor contributing to the observed decrease

in renal blood flow and glomerular filtration rate was noted first by a team of investigators in World War II headed by Joseph Trueta (1947) who studied the renal vascular response to tourniquet shock in rabbits. They noted the shunting of blood from cortical to juxtamedullary glomeruli and postulated the existence of juxtamedullary shunts. Renal cortical ischemia has been confirmed by other investigators (Corcoran et. al, 1943a, 1943b; Oliver, 1944-45; Hollenberg et. al. 1968). An alternative mechanism proposed to account for this was that of plasma skimming (Pappenheimer and Kinter, 1956) in which the progressive separation of red blood cells by plasma removal in the interlobular arteries (secondary to afferent arterioles coming off at right angles) led to the deeper glomeruli being supplied with plasma rich blood and the cell rich component going to the terminal arterioles near the cortex. Therefore, the superficial glomeruli would be more susceptible to reduction in glomerular filtration rate during renal ischemia.

The third and most significant vascular change studied in renal ischemia has been the involvement of the renin-angiotensin system through which renal blood flow and glomerular filtration rate could be regulated by feedback of tubular activity. Goormaghtigh (1942, 1945, 1947) studied kidneys of patients with the crush syndrome and acute renal failure and noted the increased number and granularity of cells of the juxtaglomerular apparatus. He suggested that these features of glandular activity were associated with the production and liberation of some vasopressive substance in ischemic kidneys. He believed this substance resulted in

persistent vasoconstriction involving first the postglomerular arterioles and later the glomerular tufts, possibly followed by paralytic dilatation. As early as 1943 (Dexter et. al.) readily detectable amounts of renin were found in circulating plasma of dogs after hemorrhagic shock, in addition to decreased levels of hypertensinogen (angiotensinogen). They considered renin part of the normal compensatory mechanism to maintain normal systemic blood pressure in hypotension. Several investigators (e.g. Kokot and Kuska, 1969) have confirmed the increase in plasma renin activity in the oliguric phase of acute renal failure and the decrease in the diuretic phase. These investigators considered the estimation of plasma renin activity important in the clinical diagnosis of acute renal failure. The importance of the renin-angiotensin system in the development of acute renal failure has also been supported in work of Oken's group (McDonald et. al., 1968; Thiel et. al., 1970; DiBona et. al., 1971a, 1971b) demonstrating the protective effect of sodium loading (drinking 1% saline for one month) in rats in the development of acute renal failure induced by either mercury or glycerol as compared to rats on a low sodium diet. The sodium loaded rats (i.e. renin depleted) showed some renal ischemia and decreased glomerular filtration initially but renal function returned rapidly. They have even confirmed the increased release of renin from individual nephrons in sodium deprived animals and the decreased amount of renin extractable from single superficial glomeruli after glycerol induced acute renal failure. Brown and coworkers (J. J. Brown

et. al., 1970, 1972; Gavras et. al. 1970, 1971; Hosie et. al., 1970; W. C. Brown et. al., 1972) have demonstrated increased plasma renin concentrations in both clinical and experimental forms of ischemic acute renal failure, but not in the nephrotoxic varieties. The increase was most pronounced clinically in the early stages. However, the fact that renal vein renin concentrations do not always reflect plasma levels has also been shown. Hamilton and Collins (1942) reported that the "pressor" substance released during hemorrhagic shock was more pronounced in renal venous blood than in arterial blood. Brown's group has confirmed this in experiments in which renal venous renin concentration was measured following renal artery occlusion and found to be significantly increased while arterial renin concentrations were unchanged.

The association of increased renin release does not prove any cause and effect relationship, however, between renin and acute renal failure. It is entirely possible that renin release is a consequence of decreased renal blood flow, vasoconstriction, and renal failure or that the two are independent of each other. In addition, the vasoconstriction is presumably mediated by angiotensin II which is converted from angiotensin I in the lungs. It has been shown (Gavras et. al., 1971) that high enough levels of angiotensin II can produce acute renal failure (with histological evidence of ischemic tubular damage), possibly implying that increased renin-angiotensin activity (e.g. diuretics) should be avoided in treating some cases of acute renal failure.(provided that endogenous levels of angiotensin II as high as those known

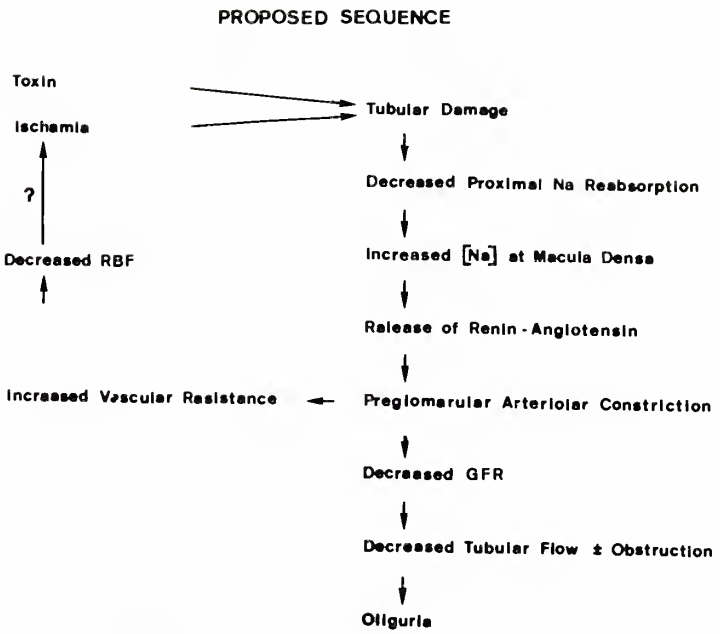
to produce acute renal failure by infusion can be achieved). The question still remains, then, of whether effective intrarenal concentration of angiotensin II can be achieved as a result of high intrarenal renin levels without transit through the pulmonary circulation. There is some evidence that this may be possible. Oparil and coworkers (1970) showed that radioactively labeled angiotensin I infused into the renal artery could be recovered to some degree as radioactive angiotensin II in the renal vein.

At this point it is obvious that no one explanation can serve to fully explain the pathogenesis of such a multifaceted clinical entity. Drs. Henry, Lane, and Kashgarian (1968) attempted to evaluate the relative roles of tubular damage, obstruction, and decreased glomerular filtration rate in a micropuncture study using two models: 1) potassium dichromate induced proximal tubule toxic cellular damage; and 2) intravenous human globin induced obstruction of the distal nephrons. The role of the renin-angiotensin system was evaluated by comparing two groups of rats made either renin poor (sodium rich) or renin rich (sodium poor) by diet. These investigators found that the renal failure produced by either model was less severe in the renin poor animals than in the renin rich animals. Oliguria was most marked in the renin rich animals in both models (in fact, the renin poor animals receiving dichromate became polyuric). Similar changes were noted in both total and individual nephron glomerular filtration rate, with the most severe changes in the renin rich dichromate group, while there was small differences in the glomerular filtration rates of the

renin rich and poor globin groups. Tubular function measured by the fractional excretion of water was also better in the renin poor animals. Thus, the protection of renin depletion was best seen in the animals with dichromate induced renal failure probably because of the important contribution of mechanical tubular obstruction (reflected by an elevation of the free flow intratubular pressure) in the animals with globin induced distal tubular obstruction. In an attempt to evaluate whether changes in glomerular capillary pressure were responsible for the changes in glomerular filtration rate, the stopped flow intratubular hydrostatic pressure was measured and found to be diminished in all animals with acute renal failure. This suggested that the decrease in glomerular filtration rate was secondary to a fall in glomerular filtration pressure which could be the result of preglomerular arteriolar constriction mediated by the renin-angiotensin system.

Thus, there seems to be considerable evidence implicating the renin-angiotensin system in the production of oliguric acute renal failure by ischemia. Henry, Lane and Kashgarian (1968) and Brown et. al. (1970) have postulated an interesting scheme in which the acute ischemic renal failure is the pathological extreme of a normal physiological response to an embarrassed renal circulation. Figure 1 illustrates the proposed scheme, the important feature of which is the self-perpetuating nature secondary to the inappropriate activation of the renin-angiotensin system.

Figure 1:



In this scheme, the initial insult is that of tubular damage leading to decreased proximal tubule reabsorption of sodium and an increased delivery of sodium to the distal portions of the nephrons. The actual control of renin release at the macula densa may not be the concentration or even load of sodium delivered to the distal tubule (DiBona and Sawin, 1971; Morgan, 1971), but the amount of sodium taken up by the macula densa (Vander, 1967). In any case, the release of renin and the action of angiotensin (either intrarenal or via the pulmonary circulation) acts on the preglomerular arterioles

to cause constriction and decreased glomerular filtration rate as well as to increase vascular resistance and decreased renal blood flow. This completes the viscious cycle by adding to the initial insult with further ischemia and by contributing to the subsequent depression of tubular flow (with or without obstruction) and oliguria. The self-perpetuating nature accounts for the prolonged effects of acute renal failure even after the initial insult has been corrected and demonstrates the inappropiate response of the renin-angiotensin system to a normal, physiological stimulus (increased sodium at macula densa).

The fourth area in which investigators have looked for vascular changes that could contribute to the pathogenesis of acute renal failure is the so called "no-reflow" phenomenon. It was on the basis of some of this work that the present investigation was based. The no-reflow phenomenon postulates that a period of ischemia to a given organ, not sufficient to cause irreversible parenchymal changes per se, may cause vascular changes severe enough to prevent the reflow of blood once blood flow is reestablished. Thus, irreversible parenchymal changes may occur secondary to vascular changes resulting from even an apparently short period of ischemia. Ames and coworkers (Majno et. al., 1967; Ames et. al., 1968) did much of the early work in this area demonstrating early changes in the cerebral vasculature of rabbits following short periods of ischemia. They showed this by carbon black perfusion after the ischemic periods and felt that the failure of reflow was due to the consistent swelling of perivascular glial cells they noted

on electron microscopic sections. Strock and Majno (1969) also demonstrated this with tourniquet ischemia in the rat hind limb. As early as 1959, Sheehan and Davis demonstrated the no-reflow phenomenon in kidneys when they noted that after compression of the renal pedicle for three hours and removal of the clamp, blood flow ceased within one half hour. Two groups of investigators, Summers and Jamison (1971) and Flores, DiBona, Beck and Leaf (1972a; also Leaf, 1970; Flores-Calle et. al., 1971; Leaf et. al., 1972; Flores et. al., 1972b) have studied the no-reflow phenomenon in renal ischemia.

Summers and Jamison clamped the renal pedicles for periods of 0, 15, and 60 minutes (in rats) and studied carbon black perfusion at the end of this period or after blood flow was returned with perfusion of red blood cells, plasma, or red cell ghosts. In some groups, the kidneys were perfused with isotonic saline prior to clamping in order to wash out all blood. They found that in the non ischemic kidneys uniform carbon perfusion was demonstrated both grossly and microscopically. In the kidneys subjected to 15 minutes of ischemia the only significant drop in carbon perfusion occurred in animals who were not heparinized prior to the clamping. Finally, in animals subjected to 60 minutes of ischemia, the no-reflow phenomenon was demonstrated (by decreased amount of glomerular filling with carbon particles) in groups in which blood flow was reestablished with whole blood, red blood cells, or red cell ghosts regardless of whether the kidneys were flushed with saline to remove blood prior to clamping. However, when carbon was infused after

60 minutes of clamping in kidneys whose vasculature had been emptied of blood by saline infusion, uniform carbon perfusion was noted indicating that the capillaries were patent enough in bloodless kidneys to allow passage of the carbon particles. The conclusions of this experiment were that no-reflow was due to trapping of red blood cells in capillaries. Summers and Jamison also studied a few animals in recovery phases up to 8 days and found that glomerular filtration rate and urine osmolality fell markedly in the first hours after ischemia and returned to normal after one week. Fractional excretion of water and urinary sodium concentration returned to normal several hours after ischemia. Carbon infusion became diffuse and uniform as early as 24 hours (although some kidneys showed spotty distribution up to one week). They concluded that normal capillary patency generally returned within 24 hours after ischemia, considerably before functional recovery was evident, so that no-reflow could not account for persistence of oliguria. Thus, in these experiments, it was found that when intravascular coagulation was controlled (administration of heparin prior to clamping), the no-reflow phenomenon involved the trapping of red blood cells when blood flow was reestablished after 60 minutes of clamping . No-reflow did not occur when kidneys were cleared of blood prior to clamping and red cells were not perfused afterwards. The capillary narrowing could be explained by either endothelial or pericapillary cellular swelling or by arteriolar spasm. By applying procaine to

the renal pedicle Summers and Jamison attempted to exclude the possibility of neural mediated vasoconstriction. However, the role of the renin-angiotensin system cannot be excluded.

Evidence of cellular swelling in the no-reflow phenomenon has been found by Flores, DiBona, Beck and Leaf (1972). They clamped the renal pedicle for periods of 60 to 180 minutes and then allowed reflow of blood for 10 minutes, 30 minutes, or 24 hours. They studied gross changes in the vasculature by making a silicone rubber cast, and also evaluated the role of cellular swelling by examining kidney sections by electron microscopy. They also studied the use of hypertonic mannitol and isotonic saline (to control for the expansion of extracellular fluid volume by mannitol) infusion just prior to the release of the clamps. They found gross evidence of diffuse, patchy ischemia in examination of the silicone casts in animals subjected to 60 minutes of ischemia and 10 or 30 minutes of reflow. The infusion of hypertonic mannitol improved the vascular pattern considerably, while the infusion of isotonic saline did not. They concluded, therefore, that the effect of mannitol was exerted osmotically rather than by expansion of extracellular volume. The beneficial effect of mannitol was seen following 120 minutes of ischemia as well, while animals subjected to 180 minutes of ischemia showed inconstant results.

Examination of kidneys by electron microscopy suggested swelling of all cellular elements and the mean endothelial thickness of renal capillaries was significantly greater

than controls. Again, the protective effect of hypertonic mannitol was noted with reduced cellular swelling. The importance of red cell trapping, as well as the observed swelling, was confirmed by clearing the kidneys of blood with saline prior to clamping. Then, with immediate perfusion of silicone, normal vascular pattern was noted. These investigators concluded that no-reflow was associated with cellular swelling and could be reversed by hypertonic mannitol through an osmotic effect of reducing cell swelling. The timing of clamping to effect irreversible renal injury was essentially confirmed as early as 1948 by Hamilton, Phillips and Hiller. They noted (in dogs) that renal ischemia of two hours was followed by gradual recovery of renal function after two to three weeks, while four hours of clamping resulted in irreversible renal injury and death within four to eight days (with three hours of clamping, some animals survived).

An explanation of the regulation of cell volume in producing the no-reflow phenomenon has been proposed by this group of investigators (Leaf et. al, 1972; Flores et. al., 1972b). They suggest that hypoxia interrupts the metabolic supply of energy which disrupts the energy requiring transport process in the cell membrane necessary to pump out intracellular sodium. This would result in an increase in intracellular sodium, chloride, and water and subsequent cell swelling.

In summary, then, the no-reflow phenomenon has been postulated as contributing to the prolongation of decreased renal

blood flow and glomerular filtration rate even after the initial ischemic insult has been removed. It must be emphasized, however, that the role of the renin-angiotensin system in producing vasoconstriction may be equally or more important in this process as cellular swelling and red cell trapping.

As indicated in the previous series of experiments, mannitol has been used extensively in the treatment of acute renal failure, although the mechanism has not been well understood. As early as 1945, Selkurt reported that mannitol improved renal function in dogs after a period of ischemia. The protective effect of mannitol has also been confirmed in some of the micropuncture work of Oken's group (Wilson et. al., 1967) who found that the combination therapy of mannitol and saline infusion almost totally prevented the development of azotemia. It is known to prevent or minimize the fall in renal plasma flow and glomerular filtration rate (Barry, 1963) and has been shown in patients with prerenal circulatory failure to increase renal blood flow (Barry et. al. 1962). Morris and coworkers (1972) studied the effects on glomerular filtration of progressive decreased renal perfusion pressure by aortic clamping in rats and found that glomerular filtration was absent in hydropenic or saline loaded rats at 40 mm. Hg pressure, while glomerular filtration continued in some nephrons in all rats infused with mannitol and in some rats infused with hypertonic saline. Urine flow persisted only in rats given mannitol.

Several mechanisms have been proposed to explain the action of mannitol including: 1) expansion of extracellular fluid space and plasma volume; 2) direct intrinsic renal vasodilatory effect; 3) dilatation of lumina of proximal and distal tubules; and 4) reduction of cellular swelling allowing for increased "reflow" of blood. Murphy and coworkers (cited in Luke and Kennedy, 1967) noted that mannitol can maintain urine flow in animals with hemorrhagic shock at blood pressures otherwise associated with anuria and felt that the increase in glomerular filtration noted in these circumstances could be explained by the expansion of extracellular fluid space. Luke and Kennedy (1967) have disputed this conclusion by noting a mannitol induced diuresis in several patients with acute renal failure and increased central venous pressure without a further increase in CVP. Thus, they felt that expansion of extracellular fluid was not important. The work of Flores, DiBona, Beck and Leaf cited above also disputes this hypothesis by noting the improved vascular flow and reduced cellular swelling in animals given a load of isotonic saline calculated to expand extracellular fluid volume by an equal or greater amount.

Goldberg and Lilienfield (1965) noted a decrease in renal vascular resistance in animals given hypertonic mannitol and felt that this was due to a direct renal vasodilatory effect.

Gottschalk and Mylle (cited in Luke and Kennedy, 1967) reported that mannitol dilates the lumina of proximal and

distal tubules by increasing the rate and volume of tubular flow. Stahl (1965) also felt that the diuresis of mannitol was by a direct action of the filtered mannitol in retaining water in the proximal tubule.

Finally, as noted previously, Flores, DiBona, Beck and Leaf have postulated the protective effect of mannitol to be due to the reduction of cellular swelling which is thought to prevent the reflow of blood after a period of ischemia. Thus the best explanations of the mechanism of the action of mannitol so far relate to 1) its ability to increase renal blood flow and glomerular filtration either by a direct vasodilatory effect or by the prevention of cellular swelling, or 2) its ability to maintain urine flow by keeping tubular lumina open.

The work at Yale, with the participation of Drs. Kashgarian, Hayslett, Siegel, Feldman and DiMeola, has evolved into a study of vascular changes in several models of acute renal failure. In one study, the distribution of outer to total cortical renal blood flow was determined using radioactive microshperes injected into rats after the administration of dichromate to induce nephrotoxic tubular damage. In addition, measurements of glomerular filtration rate and renal blood flow were made. Results indicated that the severity of renal failure was directly related to the previous dietary intake of salt (and, therefore, inversely related to renal renin content), confirming

the previous work of Henry, Lane and Kashgarian (1968) that renin content was important in determining the severity of acute renal failure. The ratio of outer cortical to total cortical blood flow was reduced to similar values in all groups, indicating that the effect of renin content was more related to changes in renal vascular resistance than to changes in distribution of blood flow (i.e. cortical shunting).

The work of Summera and Jamison, and Flores, DiBona, Beck and Leaf, referred to previously, led to studies utilizing the ischemic model of acute renal failure. In one series of experiments both renal pedicles were clamped for a period of 60 minutes and then released to allow the reflow of blood for periods of 10, 30, and 60 minutes. At the end of these periods, the ratio of outer to total cortical blood flow was determined by the injection of radioactive microspheres in rats who had been untreated or given an infusion of isotonic saline or hypertonic mannitol during the last 15 minutes of vascular obstruction. In animals who were untreated or given a saline infusion, the ratio of outer to total cortical blood flow was depressed after 10 minutes, but returned to normal after 60 minutes. The untreated animals showed a near normal ratio after 30 minutes, while the saline treated animals maintained their depressed ratio. In the mannitol infused animals, the ratio was greater than normal after 10 minutes, normal after 30 minutes, but below normal after 60 minutes. The untreated and saline treated animals remained anuric throughout, even though the ratio had returned to normal after 60 minutes, while in the mannitol treated animals

some urine flow had become established by 60 minutes. One interesting finding in this experiment was a depressed outer to total cortical ratio in animals who were not made ischemic but infused with mannitol and examined 60 minutes after the mannitol infusion (examination after 10 or 30 minutes resulted in normal ratios). This was thought to be due to a mannitol induced diuresis and subsequent plasma volume depletion by that time. This effect would not be operative in ischemic animals who had been made oliguric.

The results of this experiment indicated that, as in the dichromate treated animals, there did not appear to be a correlation between the severity of functional abnormality and the evidence for redistribution of cortical blood flow, which was definitely found to be existent.

The present study was designed to evaluate the use of several morphological techniques which have been used to study changes in renal blood flow in the ischemic model of acute renal failure outlined above, and to correlate the results morphologically with those obtained with the radioactive microshperes concerning the redistribution of blood flow. In addition, an attempt was made to confirm the results of Summers and Jamison concerning the no-reflow phenomenon, and of Flores and coworkers on the role of cellular swelling as the cause of the noreflow phenomenon and the protective effect of mannitol in reversing these changes. It was hoped that the study would shed some light on the understanding of the confusing pathogenesis of acute

renal failure. Indeed, it was begun with much of the same hope and belief so well expressed by Jean Oliver in 1951:

> And yet amidst all this elaboration of hypothesis and semantic confusion the syndrome does stand clearly outlined as the most important of the acute disturbances of the urinary system. This place it maintains not only by the frequency of its occurrence and the gravity of its ultimate effects, but more importantly by the remarkably protean aspect of its origin: it would seem that almost any clinical situation--trauma, malarial infection, obstetrical accident, transfusion reaction, postoperative complication or intoxication-- may end with its manifestations of renal failure. There must be, therefore, some pathogenetic unity behind this screen of functional, pathological, and clinical diversity. (p. 1342)

METHODS

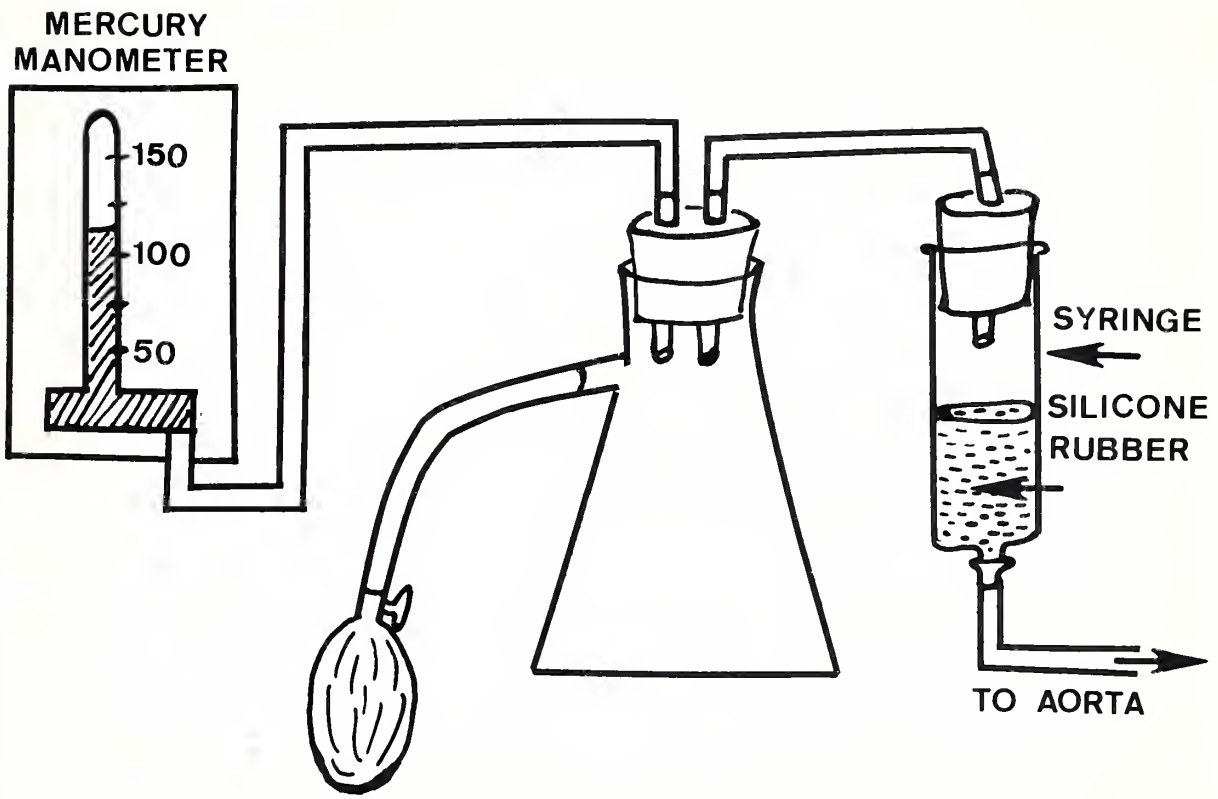
Male Sprague-Dawley rats weighing 150 to 260 grams were used in all experiments. Three morphological techniques were used to study the effects of 60 minutes of renal ischemia followed by 10, 30, or 60 minutes of reflow in animals given either isotonic saline, hypertonic mannitol, or no infusion prior to the release of the clamps.

The rats were anesthetized with intraperitoneal Inactin in a dose of 160 mg/kg. A tracheostomy was performed and a catheter was inserted into one jugular vein. Isotonic saline in a dose of 0.02 ml/min was infused via the jugular venous catheter to prevent dehydration during the course of the experiment. A laparotomy was performed and both kidneys were exposed and carefully dissected free from perirenal fat and peritoneum to eliminate all collateral circulation. The rats were then given intravenous heparin, 500 units/kg, 5 minutes prior to clamping the renal pedicles (except for the three control groups who received the heparin but were not subjected to ischemia). The clamps were left in place for 60 minutes. Fifteen minutes before the end of the ischemic period, infusions were begun (or no infusion) with either isotonic saline, 3 ml/100g, or hypertonic mannitol, 25%, 1 ml/100g. The infusions were calculated, using Harvard pumps, to end just prior to the end of the 60 minute ischemic period. At the end of 60 minutes the clamps were removed and reflow of blood through the kidneys was allowed for

periods of 10, 30, or 60 minutes. At the end of the reflow periods the animals were subjected to one of two procedures depending on whether the kidneys were to be studied by a silicone rubber suspension (Microfil, Canton Biomedical Products, Boulder, Colorado) or by carbon infusion (Pelikan Special Black Ink, Günther Wagner, Germany) for later sectioning for light and electron microscopy.

In animals subjected to the Microfil infusion, the following preparation was made during the 60 minutes of ischemia. By blunt dissection the aorta above and below the renal pedicles was exposed and loose ties were placed around the aorta just below the diaphragm, around the aorta and inferior vena cava below the renal pedicles, around the superior mesenteric artery, and around the aorta between the renal pedicles and the superior mesenteric artery. The Microfil was prepared by mixing five parts diluent with four parts Microfil compound and 3% catalyst. It was then placed in the body of a syringe attached to a sufficient length of PE 90 tubing by a 20 guage needle broken off near the base. The infusion apparatus was arranged as diagrammed in Figure 2, page 32. At the end of the reflow periods the aorta was ligated by the ties below the diaphragm and below the renal pedicles, and the superior mesenteric artery was ligated by its tie. A small incision was made in the aorta below the upper tie and the tubing inserted a short distance as rapidly as possible and secured in place with the remaining tie (all air was

Figure 2: Silicone Infusion Apparatus



removed from the system prior to infusion). The infusion proceeded at pressures between 100 and 130 mm Hg for approximately five minutes. Drainage was facilitated by a small incision in the inferior vena cava above the renal veins. Both kidneys were then removed and placed in Formalin solution for 24 hours for fixing. On successive days the kidneys were transferred into increasing ethanol concentrations (25, 50, 75, 95, and 100%) for dehydration and then into methylsalicylate for clearing, which left the tissue transparent with the silicone cast of the vasculature visible.

In the animals subjected to carbon infusion, the carbon was given intravenously immediately at the end of the reflow periods. A dose of 1 ml/100g was injected through the jugular venous catheter over a period of approximately 30 seconds. The kidneys were immediately removed and sectioned as follows. Three thin sagittal sections were made for fixation in Gluteraldehyde for electron microscopy. Each sagittal section was further sectioned leaving only a medial strip. This strip was further cut into outer, middle, and inner cortical pieces and immediately put into the cold fixative. These sections were later transferred into phosphate buffer and subsequently prepared, sectioned, and examined by electron microscopy. The remaining pieces from the first three cuts were fixed in Formalin and prepared and sectioned for examination by light microscopy.

RESULTS

Carbon Perfusion

Both right and left kidneys of all animals given intravenous carbon perfusion were sectioned and evaluated for glomerular carbon content as outlined by Summers and Jamison (1971). Between 150 and 200 glomeruli were rated on a 0 to 4 scale defined as follows:

0 No carbon

1 Carbon present in one-third or less of glomerular capillaries

2 Carbon present in between one-third and two-thirds of glomerular capillaries

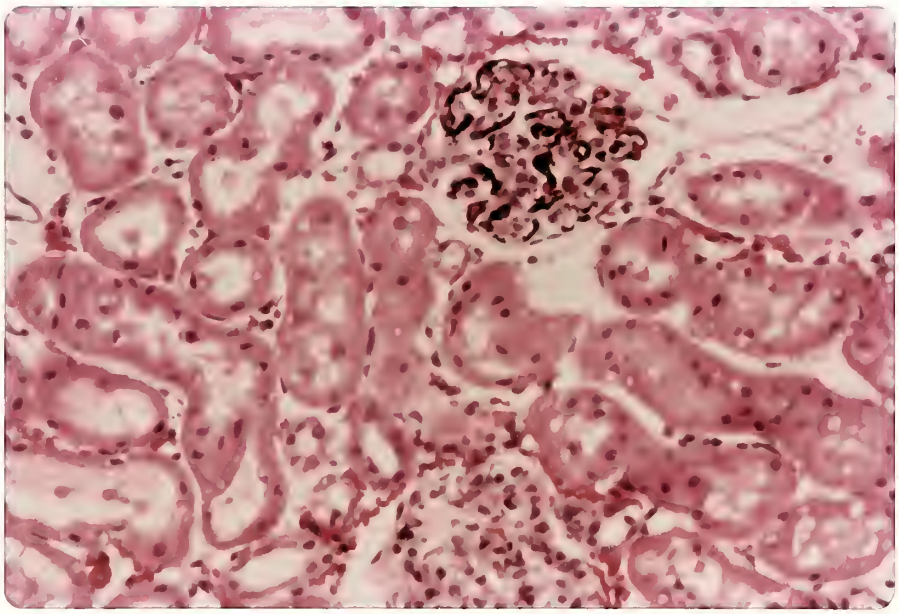
3 Carbon present in greater than two-thirds but less than all glomerular capillaries

4 Carbon present in all glomerular capillaries

Light micrographs of some of these sections are shown in Figures 3-6. All ratings were done by the author in a double blind fashion without prior knowledge of the experimental group of any section. As suggested by Summers and Jamison, and confirmed in preliminary results, there was no difference in outer versus inner cortical glomerular carbon content. There were four animals (eightkidneys) in each experimental group. The mean ratings and standard errors of glomerular carbon content of all groups is presented in Table 1 (page 38).

Figure 3: Light micrograph taken from an untreated kidney subjected to 60 minutes of ischemia and 10 minutes of reflow. The upper glomerulus is nearly filled with carbon (Rated 3) while the lower one (Rated 1) is almost empty. This demonstrates the marked variation which was seen within individual kidney sections. (H & E, X100)

Figure 4: Light micrograph taken from a saline treated kidney allowed 30 minutes of reflow after the ischemic period. Glomerular carbon content has been rated 2. (H & E, X400)



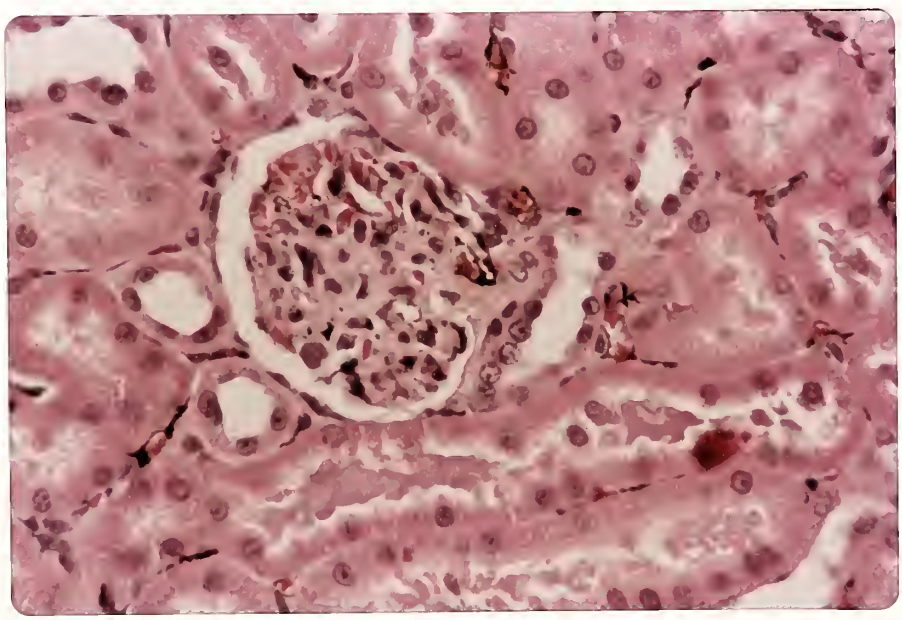
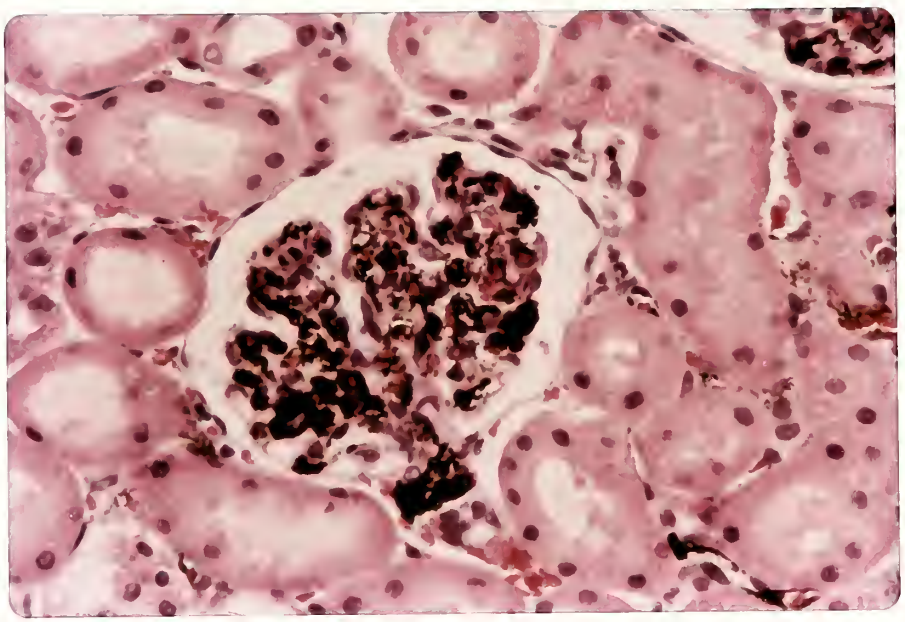


Figure 5: Light micrograph of a glomerulus taken from a saline treated, nonischemic kidney. The glomerulus is filled with carbon and has been rated 4. (H & E, X400)

Figure 6: Light micrograph of a glomerulus taken from a mannitol treated kidney subjected to 60 minutes of ischemia and 30 minutes of reflow. The glomerular carbon content has been rated 1. (H & E, X400)



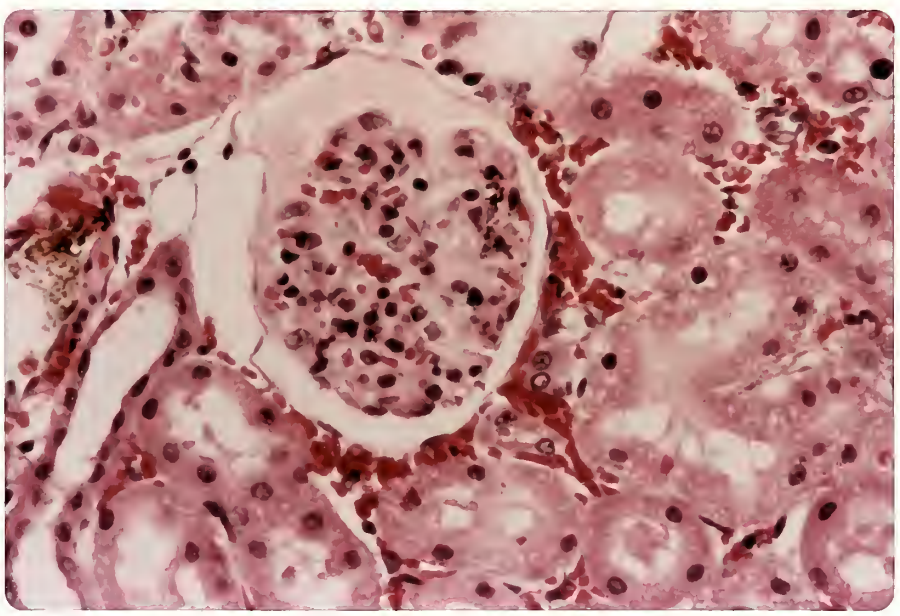


Table 1: Mean Glomerular Carbon Content

Minutes of Reflow	No Infusion Mean ± S.E.	Saline	Mannitol
Nonischemic	3.19 ± 0.13	2.69 ± 0.25	1.23 ± 0.12
10	1.24 ± 0.31	1.37 ± 0.30	1.60 ± 0.27
30	1.81 ± 0.26	1.34 ± 0.22	1.84 ± 0.23
60	1.63 ± 0.30	1.66 ± 0.27	2.69 ± 0.33

From these results it is evident that after 60 minutes of renal ischemia and reflow of blood for periods of 10, 30, or 60 minutes there is marked depression of glomerular carbon content in all groups (except for the 60 minute reflow group treated with hypertonic mannitol). The low value of glomerular carbon content in the nonischemic, mannitol treated group is interesting in that it compares with the similar finding with radioactive microspheres cited previously (although the time of carbon perfusion after mannitol infusion was not standardized in this experiment) and attributed to a mannitol induced diuresis and volume depletion.

Statistical analysis of the above data was carried out as follows. Analysis of variance performed on the No Infusion groups versus the Mannitol treated groups for the increasing reflow periods of 10, 30, and 60 minutes revealed no statistically significant differences between the two treatment groups or among the increasing periods of reflow. However, both of these comparisons were just above the $p < 0.05$ level of significance ($F_c = 3.90$ for the No Infusion versus Mannitol

Infusion and $F_r = 2.94$ for the increasing times of reflow. The corresponding F values for $p < 0.05$ level of significance are $F_c = 4.07$ and $F_r = 3.22$, respectively). The interaction variable ($F_i = 1.61$) was not significant.

In addition, a t-test analysis comparing the 60 minute reflow groups treated either with no infusion or with mannitol demonstrated a statistically significant ($t = 2.35$, $p < 0.05$) greater carbon content in the mannitol treated group. None of the other treatment groups were significantly different from the controls (i.e. no infusion).

In conclusion, therefore, the overall increase in glomerular carbon content with increasing time of reflow reached borderline statistical significance. This was due mainly to the marked increase in glomerular carbon content for the 60 minute reflow group treated with mannitol. The effect of mannitol in increasing the reflow of blood, with this model, also reached borderline significance overall, but was significant for the 60 minute reflow groups. These results confirm the "no-reflow" findings of the model used by Summers and Jamison that the reflow of blood is markedly impaired up to one hour after 60 minutes of renal ischemia. There is also some support, morphologically, for the ameliorative effect of hypertonic mannitol infusion in the return of renal blood flow through glomeruli following the ischemic period.

Silicone Perfusion

Silicone casts of the renal vasculature in all kidneys fixed and cleared as described previously were sectioned and examined for evidence of the degree of filling of all renal vessels. Four kidneys from two animals subjected to this technique were examined in each group.

The casts of all nonischemic controls (Figures 7-9) showed no evidence of ischemia and virtually all vessels and glomeruli were filled with silicone. There were no differences among the untreated, saline treated, and mannitol treated groups.

In contrast, however, the kidneys of animals subjected to 60 minutes of ischemia and either 10 minutes (Figures 10-12) or 30 minutes (Figures 13-15) of reflow demonstrated varying degrees of diffuse, patchy ischemia, especially of the outer cortical segments. There was no significant difference between the untreated and saline infused groups in this regard. However, the kidneys from animals given an infusion of hypertonic mannitol prior to the reflow periods demonstrated much less ischemia. In these kidneys there were even segments of vasculature filled as completely as the nonischemic controls. The vascular filling was slightly greater in the 30 minute reflow groups than in the 10 minute reflow groups.

The kidneys of animals allowed 60 minutes of reflow following the ischemic period (Figures 16-18) showed nearly complete recovery of blood flow in all animals. Some kidneys of the untreated group still had a few areas

Figures 7, 8, and 9: Pictures of silicone casts of the vasculature from nonischemic, control kidneys of an untreated (top), saline treated (middle), and mannitol treated (bottom) animal. They demonstrate the uniform, diffuse filling of all renal vessels and glomeruli with silicone. There is no evidence of ischemia. The renal parenchyma has been cleared with methyl-salicylate.

Figure 10: Vascular pattern, visualized by silicone rubber perfusion, of an untreated kidney subjected to 60 minutes of ischemia and 10 minutes of reflow of blood. There is evidence of a rather marked diffuse, patchy ischemia, especially of the outer cortical segments.

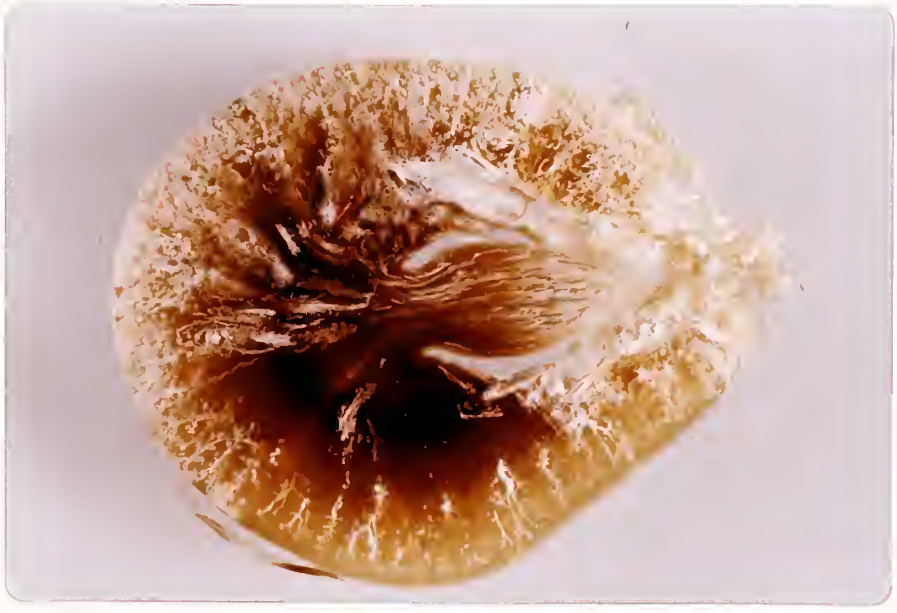
Figure 11: Silicone cast of a saline treated kidney subjected to 60 minutes of ischemia and 10 minutes of reflow. Again, as in Figure 10, there is evidence of the diffuse, patchy ischemia, indicative of the failure of blood flow to return to the cortical glomeruli.

Figure 12: Silicone cast of a kidney subjected to 60 minutes of ischemia and 10 minutes of reflow, but given an infusion of hypertonic mannitol prior to the reflow period. In contrast to the two previous pictures there is less evidence of ischemia. Some segments of the vasculature are filled through the outer cortex, indicating that mannitol has had some beneficial effect in the return of blood flow.

Figure 13: Silicone cast of the vasculature of an untreated kidney subjected to 60 minutes of ischemia and 30 minutes of reflow. There is evidence of patchy, cortical ischemia although less marked than in the 10 minute reflow groups

Figure 14: Silicone cast of a kidney allowed 30 minutes of reflow and treated with saline at the end of the ischemic period. There is further evidence of the patchy, cortical ischemia seen in these groups. There is little difference compared to the untreated kidney.

Figure 15: Silicone cast of a mannitol treated kidney in the 30 minute reflow group. There is much less ischemia than in the untreated and saline treated kidneys above, with virtually complete filling of large segments of the vasculature. This group further substantiates the ameliorative effect of mannitol in the return of blood flow in these groups.



Figures 16, 17, and 18: Silicone casts showing the vascular pattern in kidneys subjected to 60 minutes of ischemia and 60 minutes of reflow in animals either untreated (top), saline treated (middle), or mannitol treated (bottom). They demonstrate the nearly complete return of blood flow seen by 60 minutes in all treatment groups. The vascular pattern of the untreated kidney shown (top) is not as completely filled as the other two, but virtually all glomeruli are filled.

of slight ischemia although, in general, there was little difference among all three treatment groups. Thus, it seemed that by 60 minutes of reflow, blood flow appeared to be fully reestablished.

The examination of the 60 minute reflow groups extended the work of Flores, DiBona, Beck and Leaf (1972) who examined kidneys of animals allowed only 10 and 30 minutes of reflow. They also found evidence of diffuse, patchy ischemia in these groups with considerably less ischemia in the mannitol treated groups. The evidence here suggests that blood flow was re-established in all groups by 60 minutes.

Electron Microscopy

Sections for electron microscopic examination were prepared as described previously immediately following carbon perfusion. A total of 49 sections taken from outer, middle, and inner cortex were examined. In contrast to the findings cited previously of Flores, DiBona, Beck and Leaf (1972) of swelling of all cellular elements, there was marked variation among all groups, as well as within groups and within individual kidneys , with regard to the finding of cellular swelling and red blood cell sludging. There was obvious evidence of ischemic damage to tubular epithelial cells of all kidneys subjected to the 60 minute ischemic period. In addition, there did not appear to be any protective effect of mannitol in reducing cellular swelling, as swelling was noted in 8 of the 39 sections subjected to ischemia examined

and 3 of these 8 were in animals treated with mannitol (although no swelling was noted in all 5 of the mannitol treated kidneys examined given 60 minutes of reflow).

The following table summarizes the findings of vascular changes of endothelial cell swelling and red blood cell sludging noted in all kidneys examined by electron mincroscopy.

Table 2: Endothelial Cell Swelling and Red Blood Cell Sludging Found in All Sections Examined by Electron Microscopy

		Swelling		Sludging		
	# Sections Examined	Absent	Present	Absent	Slight	Marked
No Ischemia						
Untreated	4	4	0	3	1	0
Saline	3	3	0	3	0	0
Mannitol	3	3	0	2	1	0
Total	10	10	0	8	2	0
10 Minute Reflow						
Untreated	5	4	1	1	2	2
Saline	7	7	0	0	4	3
Mannitol	6	4	2	1	1	4
30 Minute Reflow						
Untreated	3	2	1	1	0	2
Saline	3	2	1	0	2	1
Mannitol	4	3	1	1	1	2
60 Minute Reflow						
Untreated	3	2	1	1	1	1
Saline	3	2	1	1	0	2
Mannitol	5	5	0	2	1	2
Total	39	31	8	8	12	19

It should be noted that there were no differences in sections taken from the outer, middle, or inner cortex of the same kidney so this division has not been made.

Nonischemic Controls (Figures 19-20)

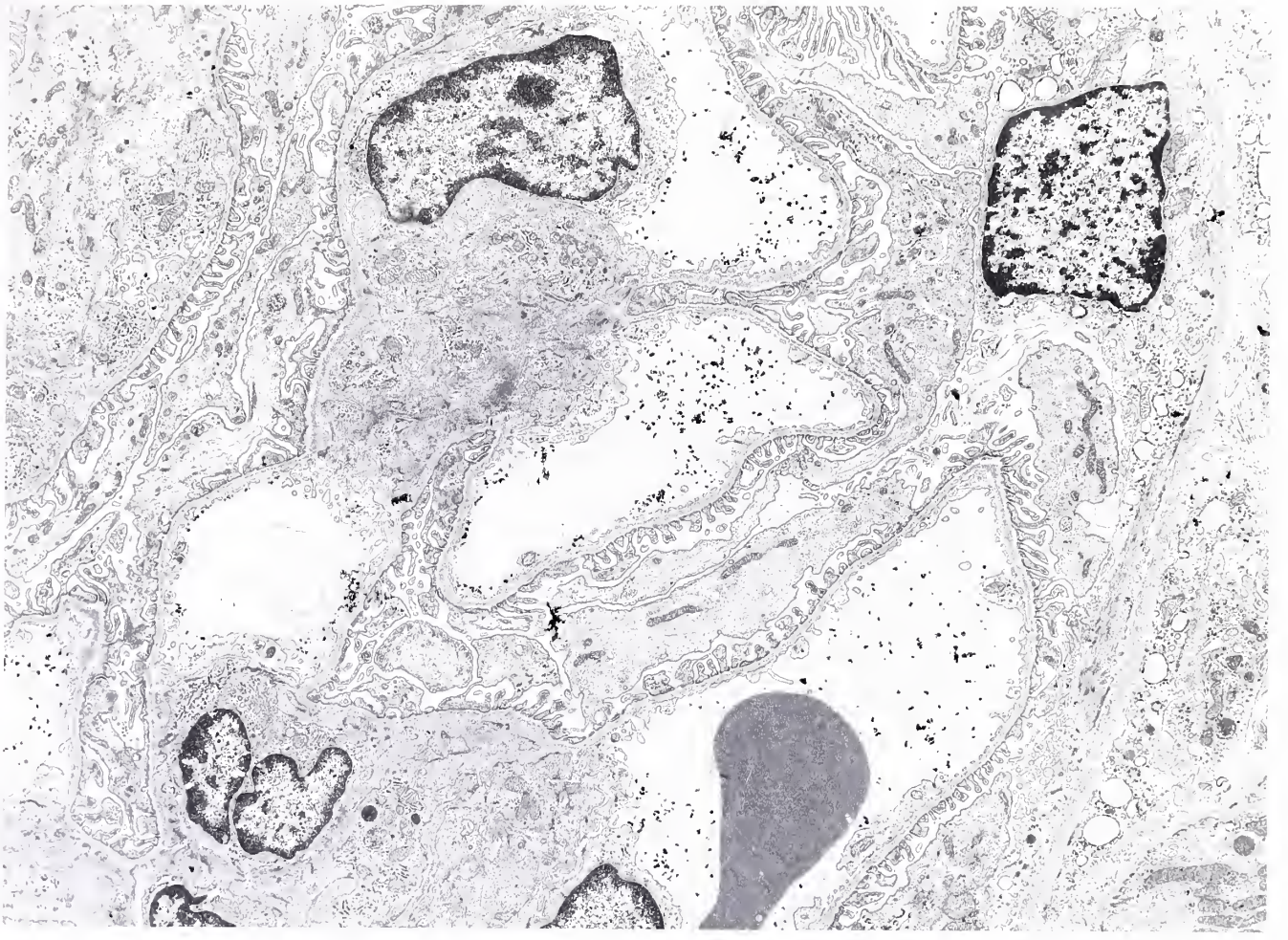
In all sections examined from animals not subjected to the ischemic period, no evidence of cellular swelling was noted. Glomerular capillaries were patent without evidence of endothelial cell swelling and carbon particles were seen within the lumina. Sections of tubules demonstrated normal epithelial cells and patent peritubular capillaries. In two sections there was some evidence of slight red cell sludging, which might have been due to the slight delay in sectioning, since there was no evidence of ischemic cellular damage.

60 Minutes Ischemia, 10 Minutes Reflow
(Figures 21-28)

Only 3 of the 18 sections from animals allowed 10 minutes of reflow showed evidence of endothelial cell swelling, and two of these were in mannitol treated animals. Red blood cell sludging, however, was noted in 16 of the 18 sections. All sections demonstrated obvious evidence of ischemic damage to the tubular epithelium with mitochondrial swelling, condensation of the mitochondrial cristae, and vacuolization and dilatation of the endoplasmic reticulum. Peritubular capillaries were generally patent without evidence of endothelial cell swelling, although red cell sludging, indicating reduced blood flow, was a frequent finding in these capillaries. There were no marked differences among the three treatment groups (untreated, saline, and mannitol) in any of the above findings. The fact that 2 of the 3 sections showing endothelial

Figure 19: Electron micrograph of a normal glomerulus from a nonischemic control kidney. The capillary lumina are wide open and contain carbon particles. There is one red blood cell visible. Endothelial cells are of normal thickness, and basement membrane and epithelial cells are also normal. (X6300)

Figure 20: Electron micrograph of a normal tubule and peritubular capillary from a nonischemic control kidney. There is no evidence of ischemic damage to the tubular cells and the peritubular capillary is patent without evidence of endothelial cell swelling. (X6300)



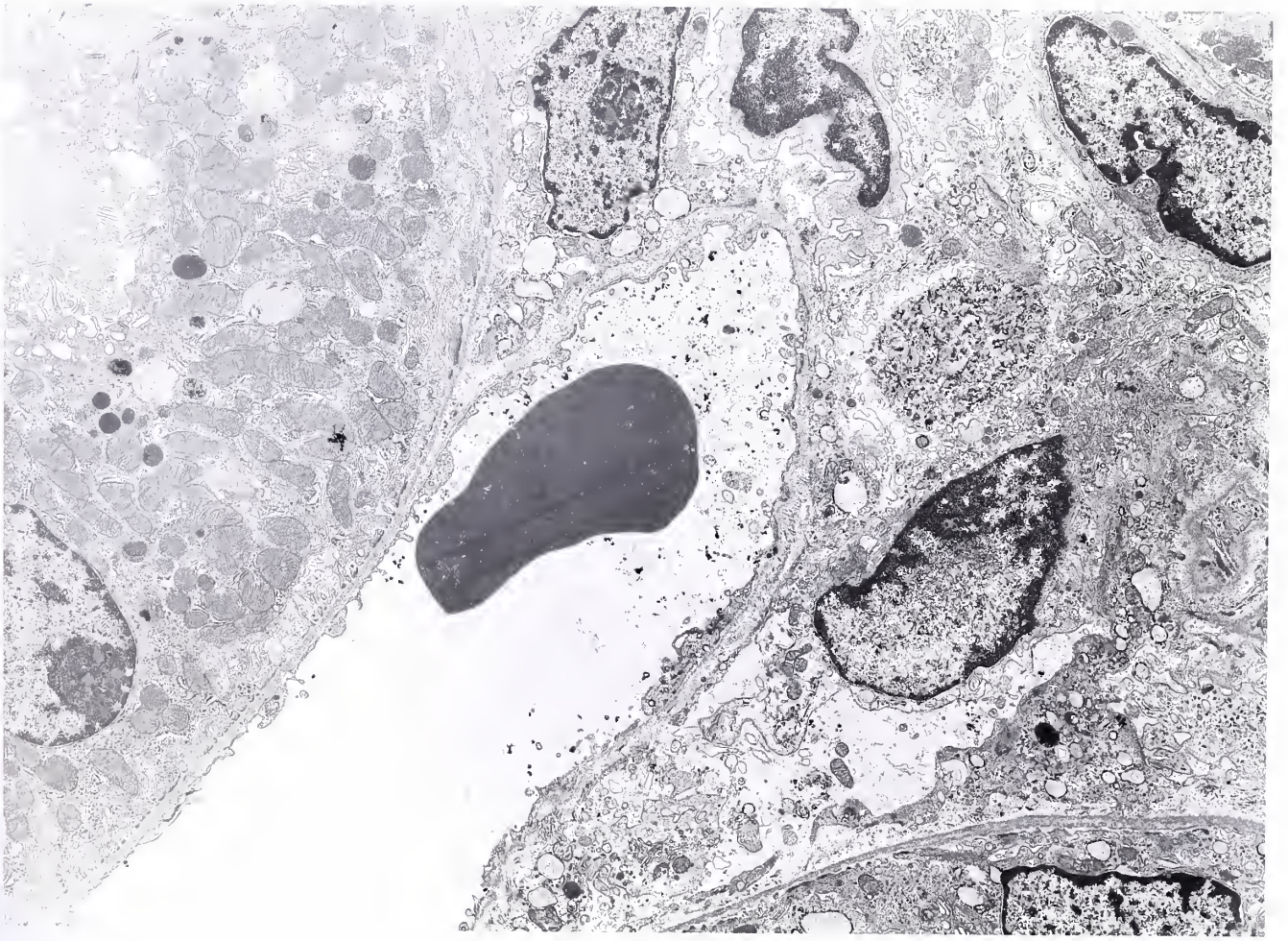
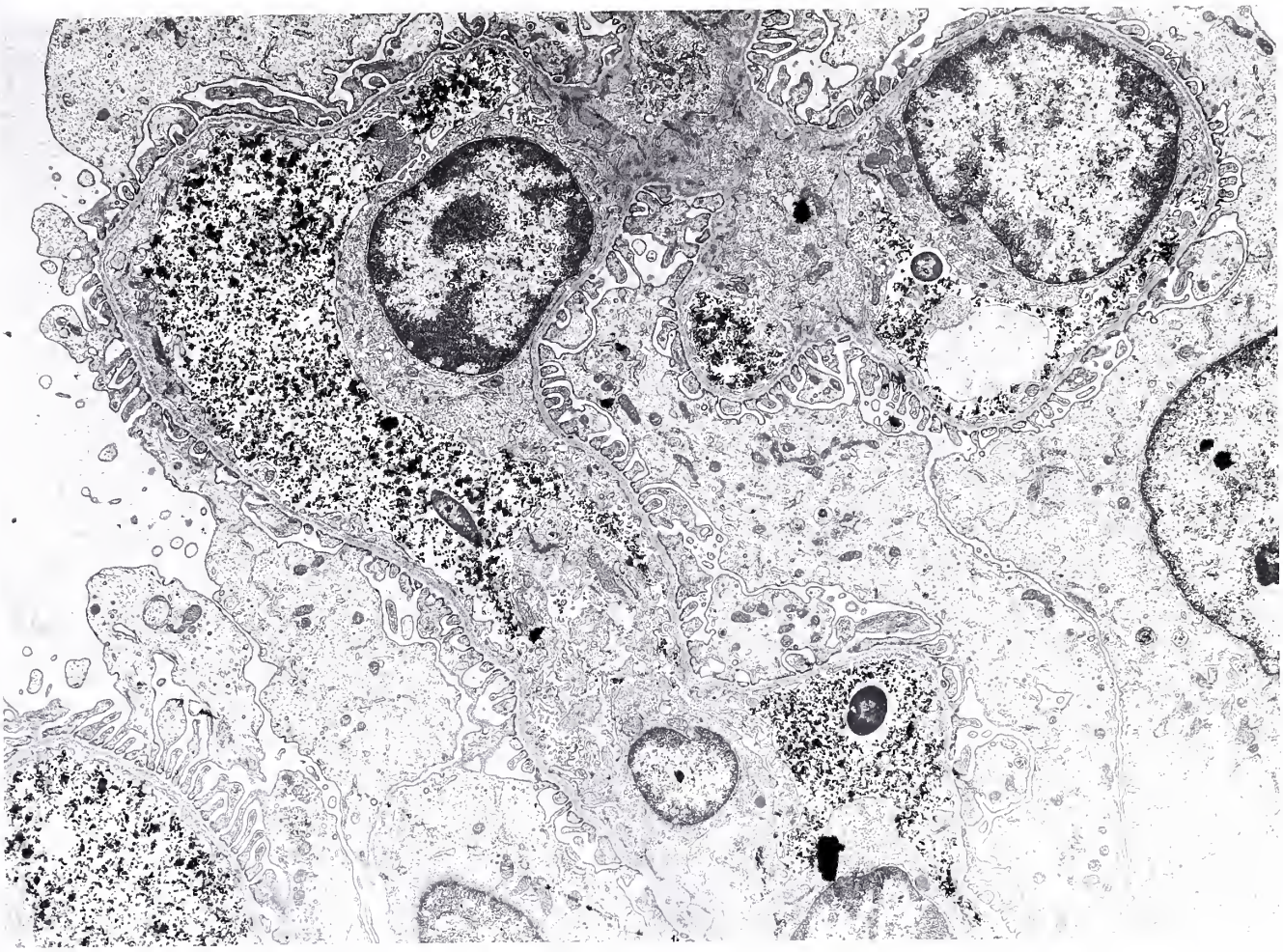


Figure 21: Electron micrograph of a cortical glomerulus from the kidney of an untreated animal subjected to 60 minutes of ischemia and 10 minutes of reflow of blood. The capillary lumina are patent, filled with carbon, and there is no evidence of endothelial or epithelial cell swelling. (X6300)

Figure 22: Electron micrograph taken from a kidney in the same experimental group as in Figure 21 showing two tubules and a peritubular capillary. There is evidence of ischemic damage to the tubular epithelium with mitochondrial swelling, condensation of the mitochondrial cristae, and vacuolization and dilatation of the endoplasmic reticulum. The peritubular capillary is patent without endothelial swelling. (X4550)



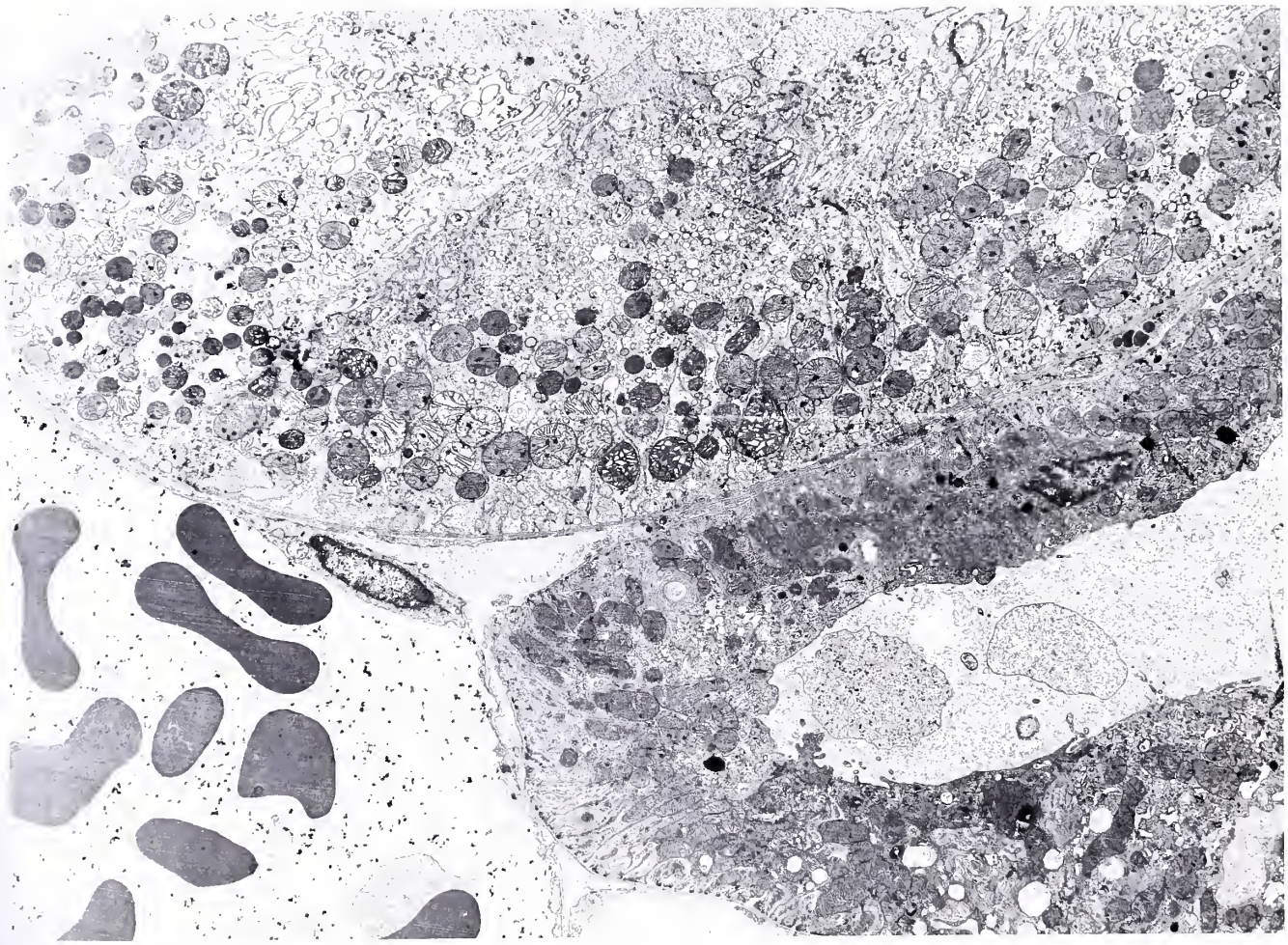
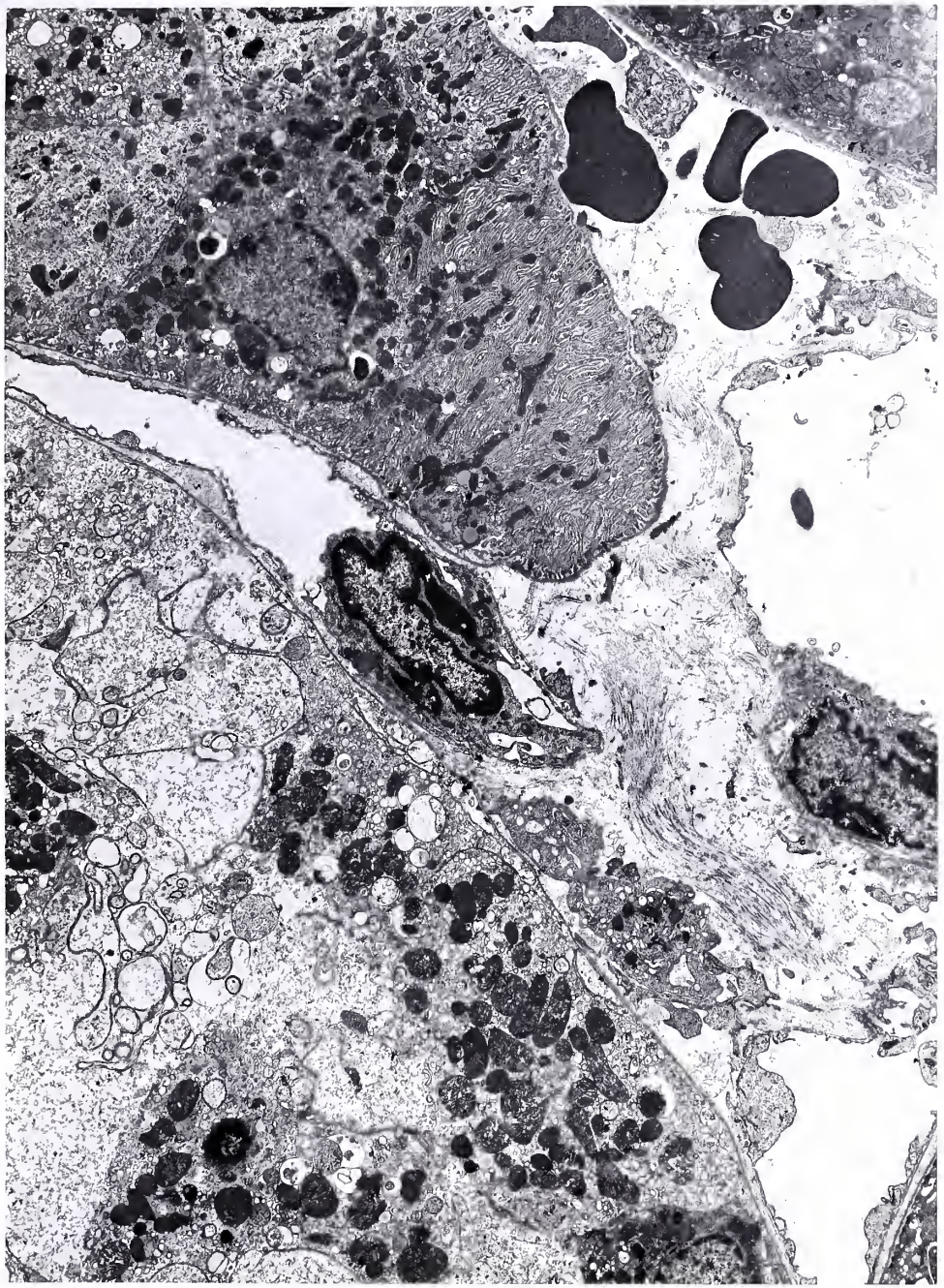


Figure 23: Electron micrograph further exemplifying the findings demonstrated in Figures 21 and 22 with ischemic damage to tubular epithelium and a patent peritubular capillary. In addition, there is slight sludging of red blood cells within the lumen of the capillary indicating some reduction of blood flow. (X6300)

Figure 24: Electron micrograph from a kidney subjected to 60 minutes of ischemia and given an infusion of isotonic saline prior to the reflow period of 10 minutes. There is evidence of ischemic damage to the tubular epithelium and patent peritubular capillaries without cellular swelling or red cell sludging. (X4550)



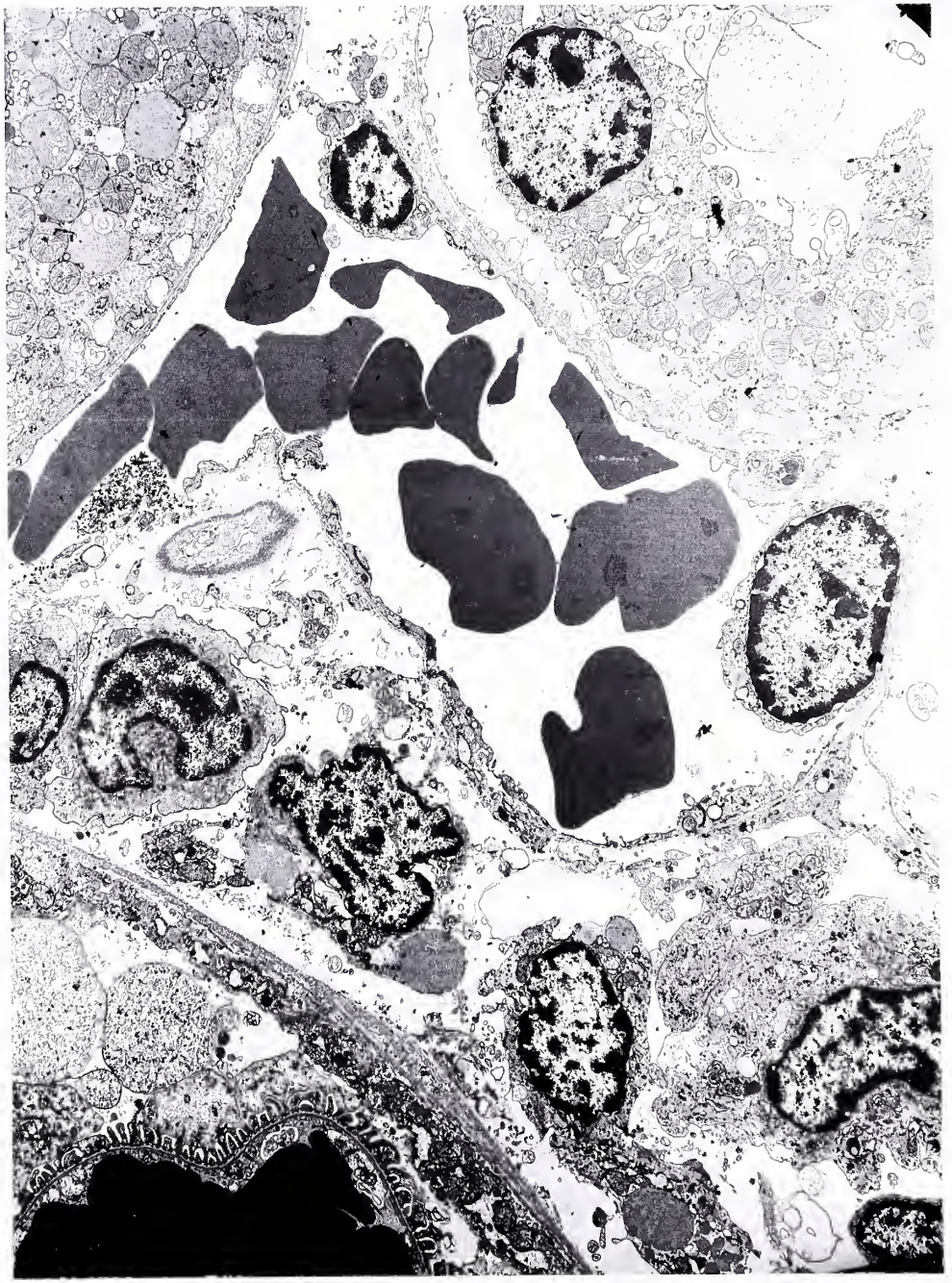
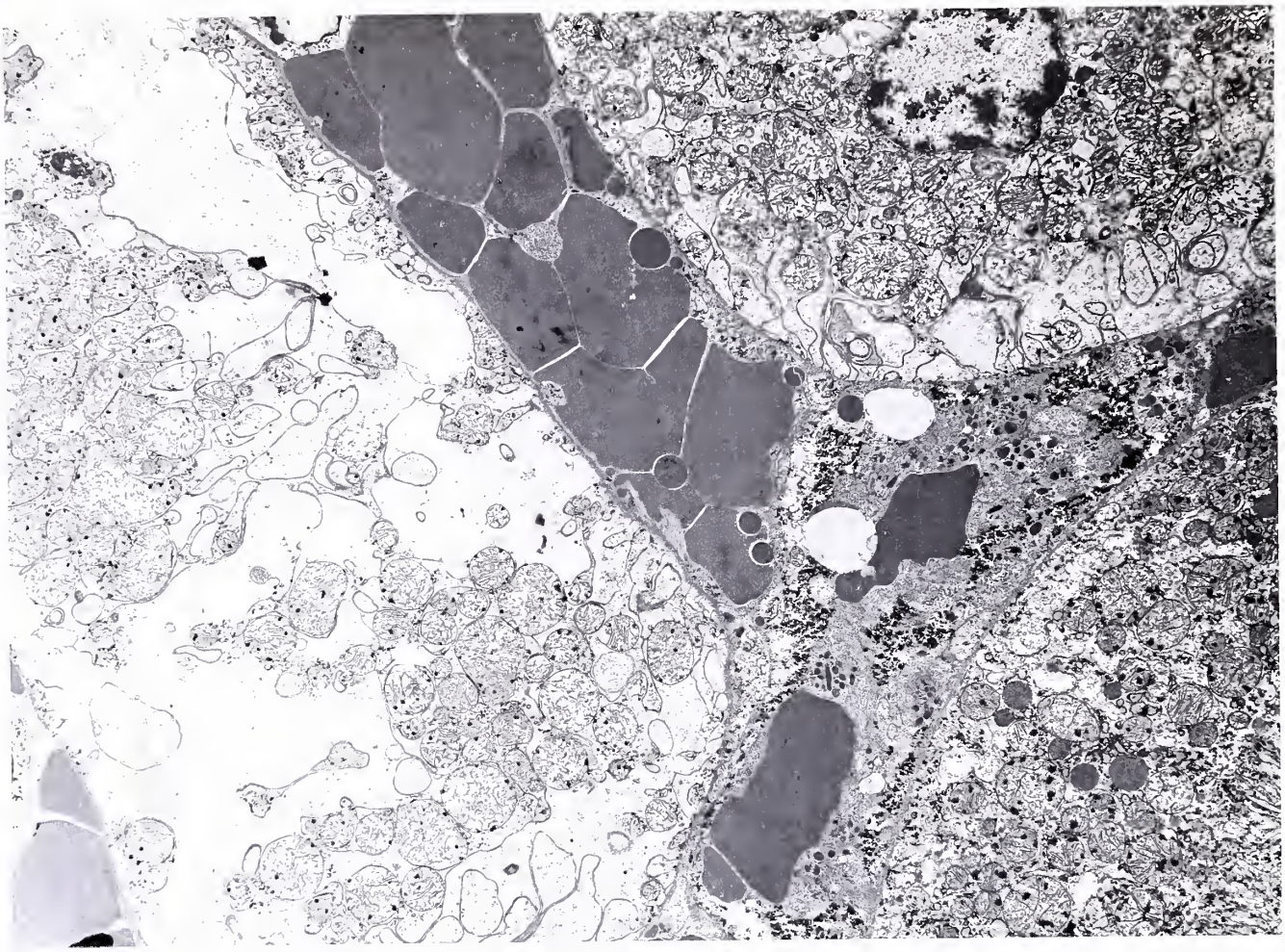


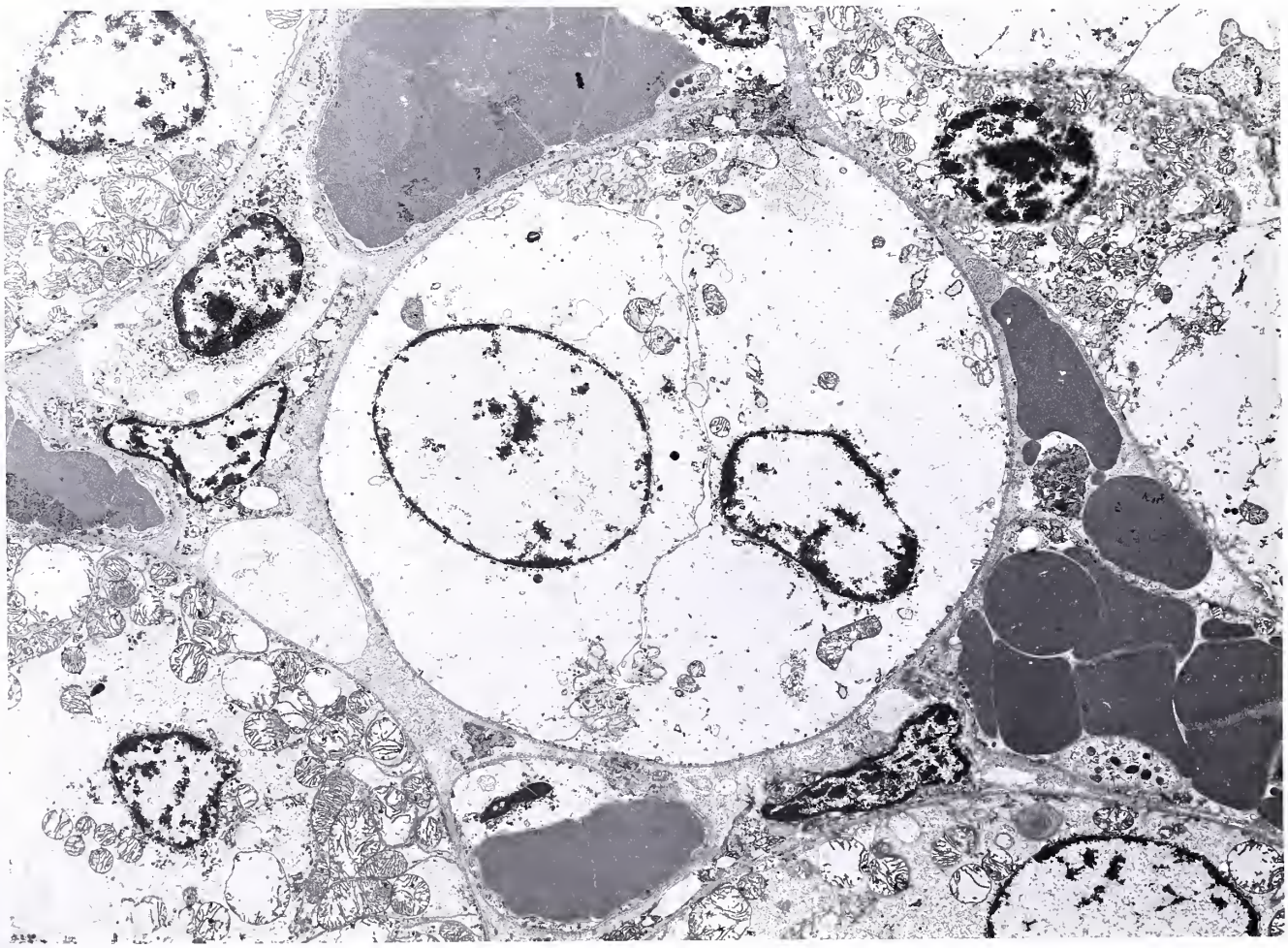
Figure 25: High power electron micrograph from a saline treated kidney given 10 minutes of reflow after the ischemic period demonstrating a patent glomerular capillary and no evidence of cellular swelling or disruption of the basement membrane. (X16,100)

Figure 26: Electron micrograph from the kidney of an animal given a hypertonic mannitol infusion near the end of the 60 minute ischemic period and allowed 10 minutes of reflow. As in the previous pictures there is evidence of epithelial cell damage and a patent peritubular capillary with slight red cell sludging. No endothelial cell swelling is noted. (X4550)

Figure 27: Electron micrograph from a mannitol treated kidney prior to 10 minutes of reflow. In addition to the tubular epithelial cell damage, however, there is marked evidence of red blood cell sludging and of endothelial cell swelling in the peritubular capillary. The red cells, with carbon particles, appear "trapped" in the midst of the swollen cells. (X4550)

Figure 28: Electron micrograph from another mannitol treated, 10 minute reflow animal further demonstrating the red cell sludging and endothelial cell swelling in the peritubular capillaries. The tubule visible in the center of the picture is from a loop of Henle and appears swollen shut. (X4550)





cell swelling were in mannitol treated animals certainly suggests that at 10 minutes of reflow any improvement in renal blood flow associated with mannitol treatment could not be explained solely on the reduction of cellular swelling. In fact the findings of red cell sludging without endothelial cell swelling suggests that some other explanation, e.g. vasoconstriction, is necessary to account for the trapping of red blood cells.

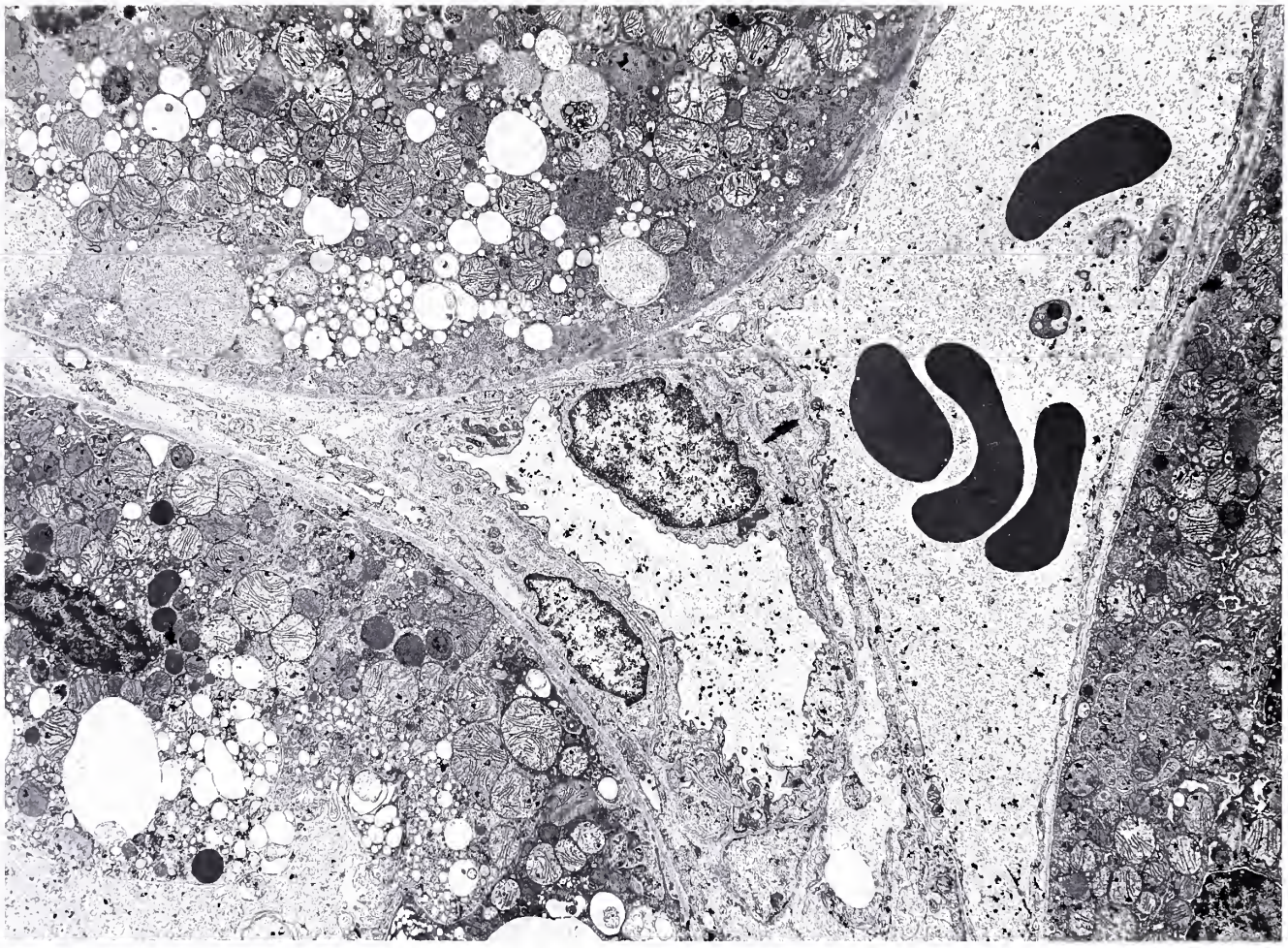
60 Minutes Ischemia, 30 Minutes Reflow
(Figures 29-35)

In the 10 sections examined from animals allowed 30 minutes of reflow, 3 (one in each treatment group) showed evidence of endothelial cell swelling. Evidence of red cell sludging was found in 8 of the 10, and all sections demonstrated the same findings of ischemic damage to tubular epithelium noted in the previous sections. This confirms the marked variation within each group. In addition, Figures 29-31, taken from the same kidney, show the variation which was seen within one kidney. Again, any protective effect of mannitol could not be attributed to the reduction in cellular swelling as the absence of swelling was found in all treatment groups.

One very interesting lesion which was noted in a few of the 30 minute reflow sections, and not in any of the 10 minute group, is shown in Figure 35. In this picture there is extravasation of red blood cells into the arteriolar wall. This type of lesion is similar to the intramural hemorrhage

Figure 29: Electron micrograph from the kidney of an untreated animal subjected to 60 minutes of ischemia and 30 minutes of reflow. The tubular epithelial cells show the same evidence of ischemic damage and the peritubular capillary is widely patent. (X4550)

Figure 30: Electron micrograph of a glomerulus from an untreated kidney allowed 30 minutes of reflow showing red cell sludging and endothelial cell swelling within the glomerular capillary. (X4550)



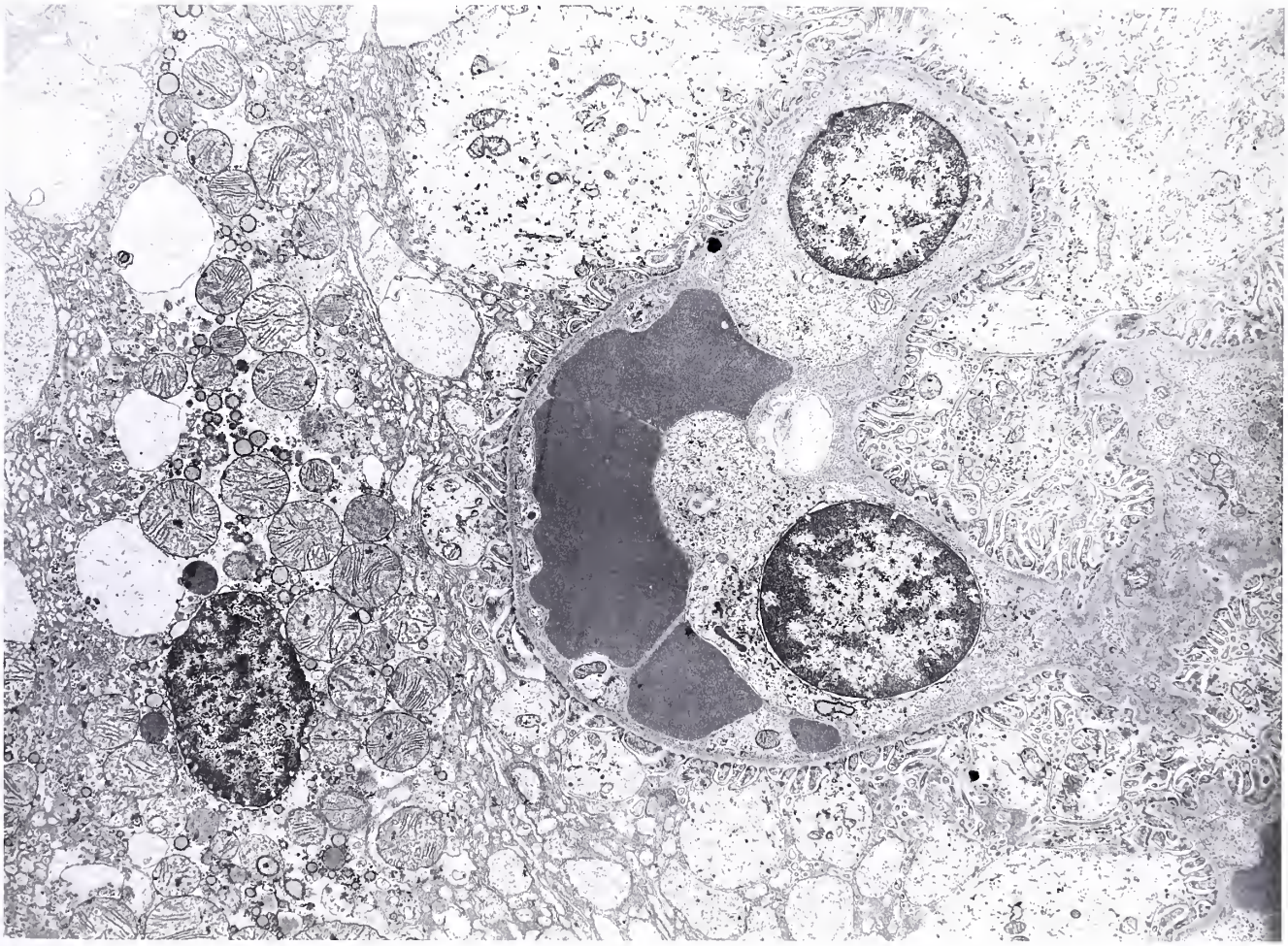
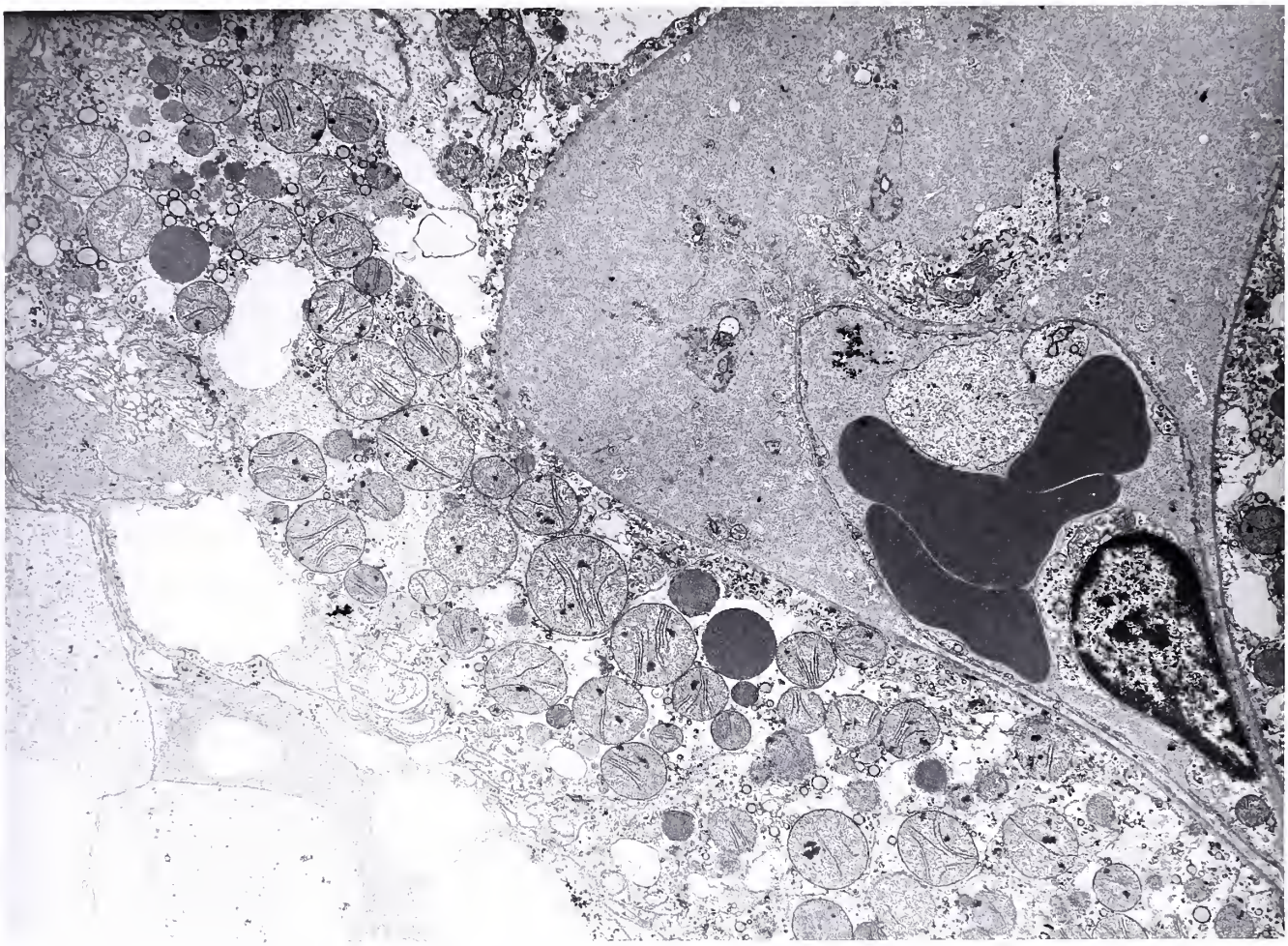
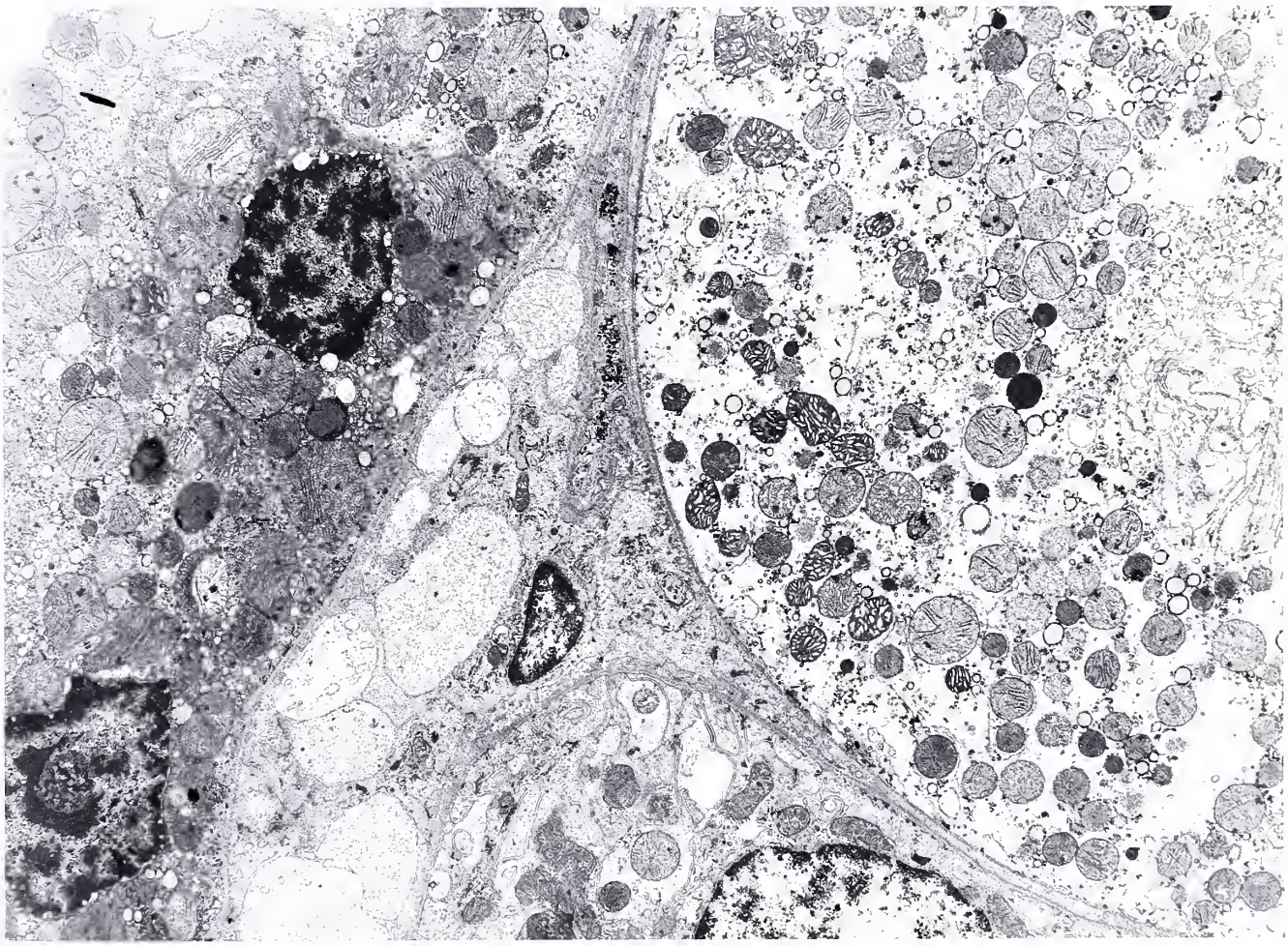


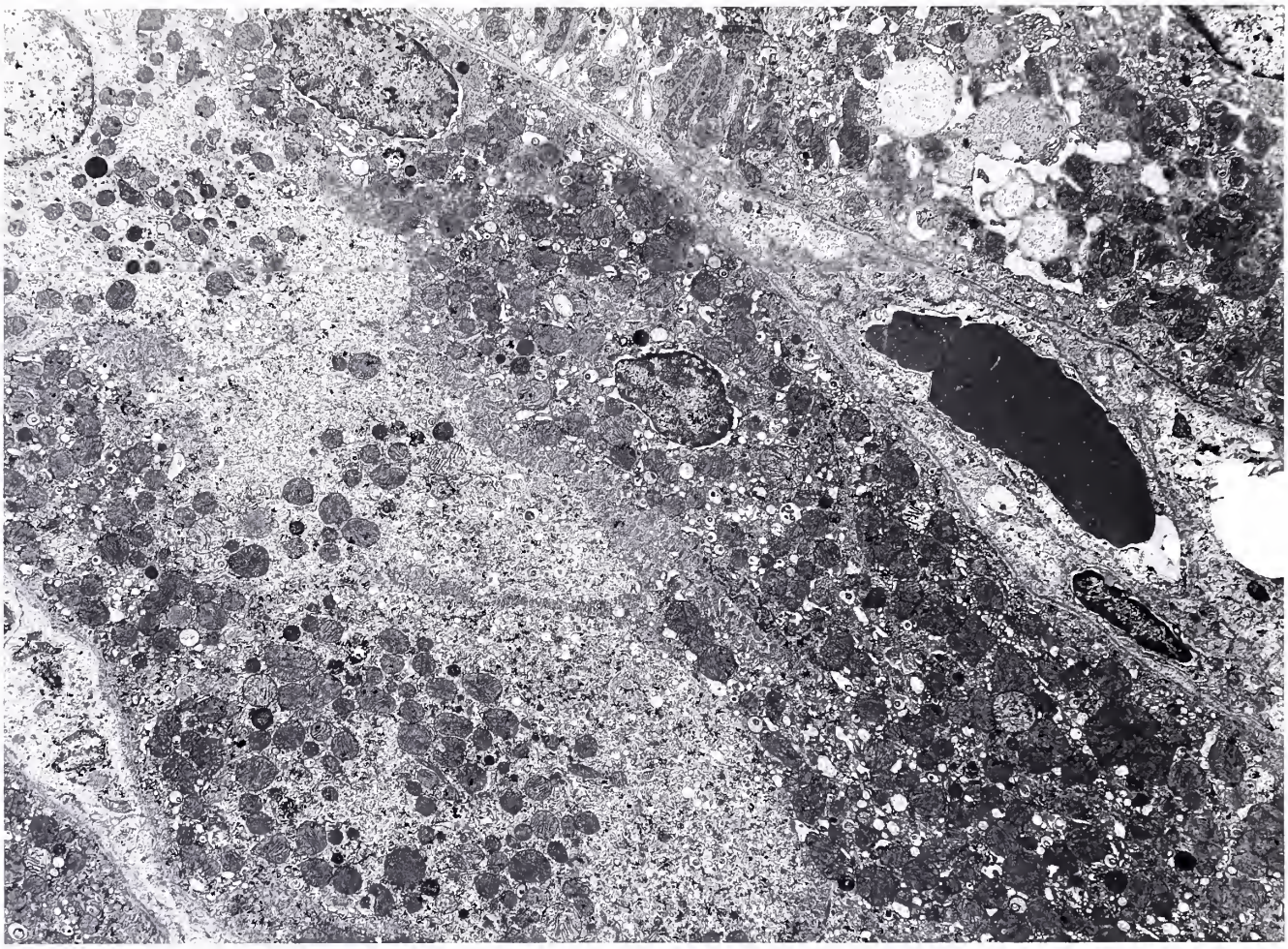
Figure 31: Electron micrograph taken from the same kidney as Figures 29 and 30 and demonstrating red cell sludging and endothelial cell swelling in the peritubular capillary. Comparing this picture with Figure 29 illustrates the variation in morphological evidence of vascular obstruction noted within the same kidney. (X4550)

Figure 32: Electron micrograph from the kidney of an animal given an isotonic saline infusion prior to the 30 minute reflow period. There is evidence of severe ischemic tubular epithelial cell damage and some indication of endothelial cell swelling in the peritubular capillary. (X6300)





Figures 33 and 34: Electron micrographs taken from the same kidney of an animal given hypertonic mannitol and 30 minutes of reflow following the ischemic period. There is evidence of tubular cell ischemic damage (more severe in Figure 34), red cell sludging, and endothelial cell swelling in both pictures. (X4550)



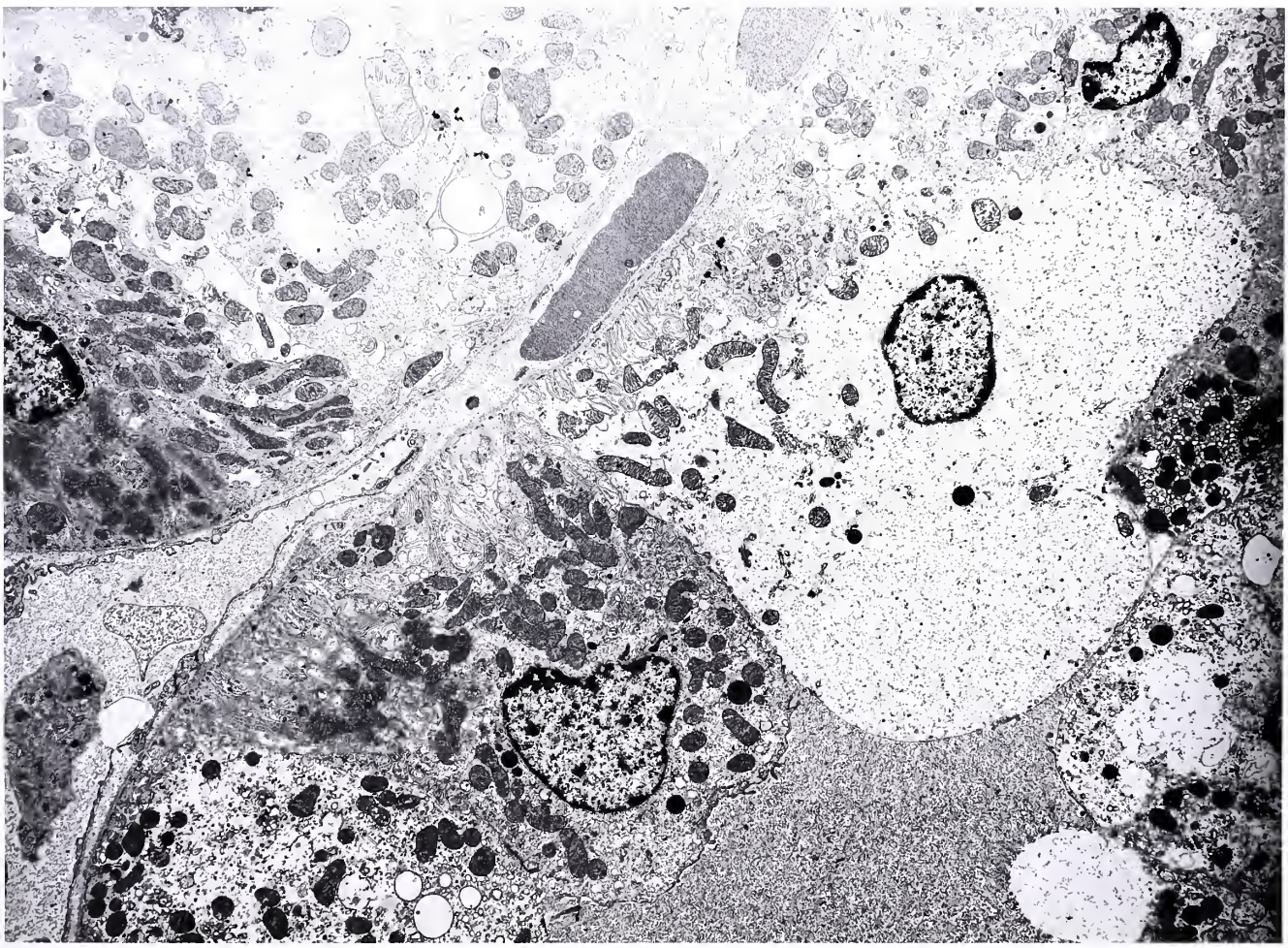
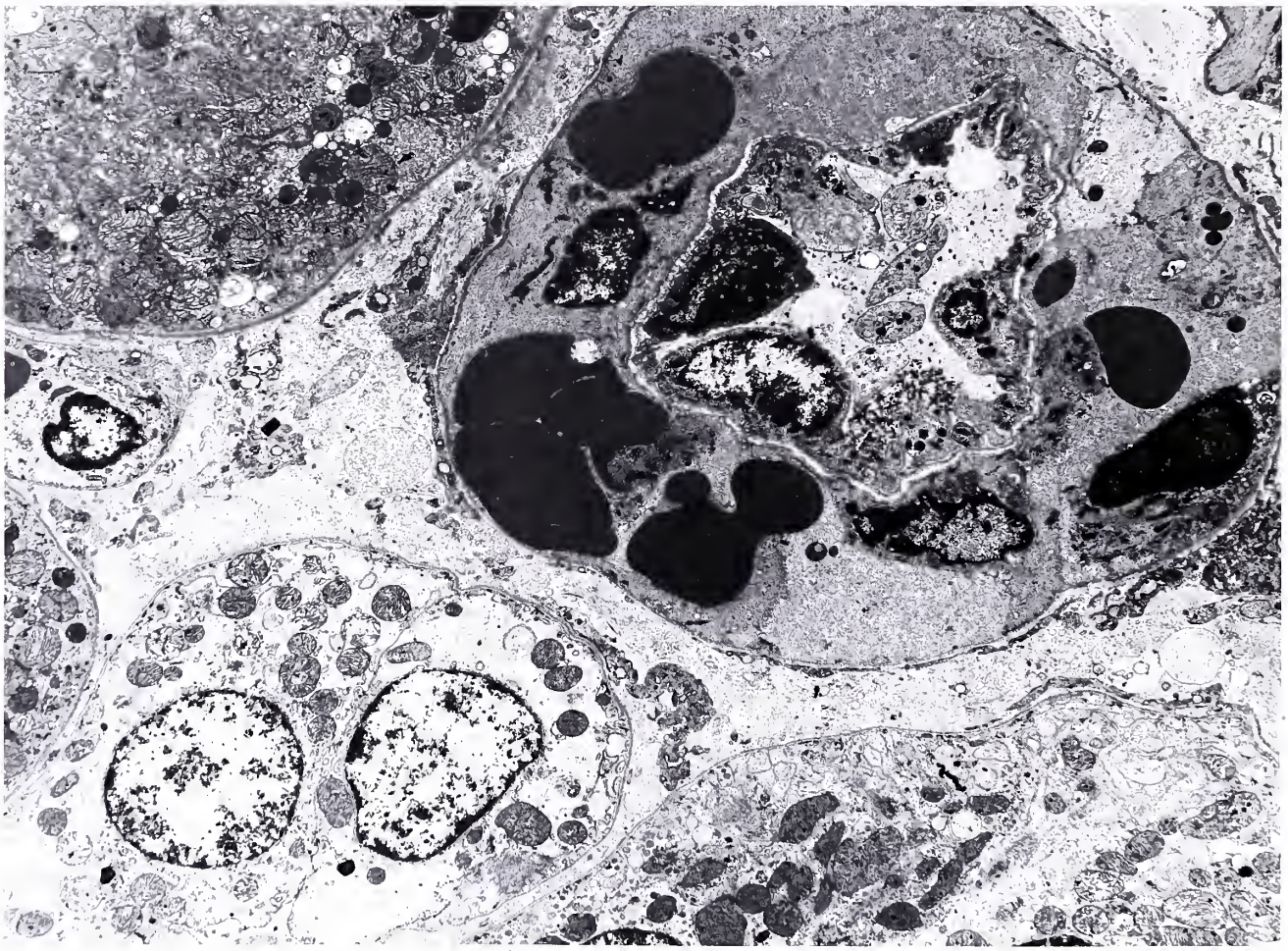


Figure 35: Electron micrograph of an interesting lesion noted in the kidney of an animal given an isotonic saline infusion and 30 minutes of reflow. There is extravasation of red blood cells into the arteriolar wall. The presence of carbon particles within the lumen indicates that the vessel was patent at the time of carbon infusion. As mentioned in the text this lesion is similar to the intramural hemorrhage seen in patients with malignant hypertension and suggests intense vasospasm of the vessel. (X4550)



seen in patients with malignant hypertension and suggests that intense vasospasm may be present in these kidneys. The presence of carbon particles within the arteriolar lumen suggests that there has been some return of blood flow through this vessel despite the intramural damage.

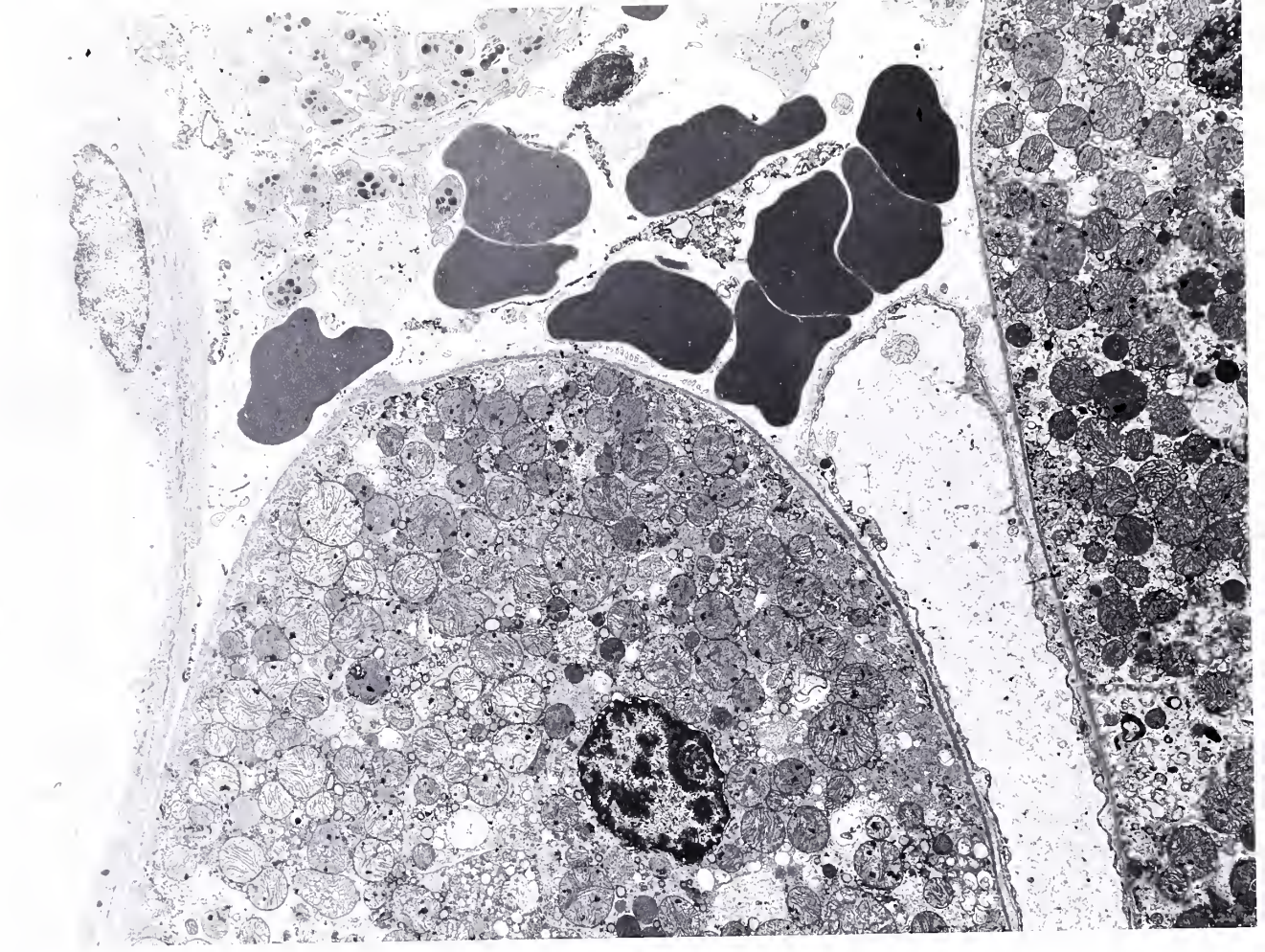
60 Minutes Ischemia, 60 Minutes Reflow
(Figures 36-43)

In the 11 sections examined from animals in the 60 minute reflow group, endothelial cell swelling was noted in only 2, and there was no swelling found in any of the 5 mannitol treated sections examined. Increased renal blood flow was possibly evident in the slight decreased proportion of sections showing red cell sludging (7 out of 11). Tubular epithelial damage was again noted in all sections. Figures 38-39 further demonstrates the variation in findings within the same kidney. The mannitol treated group showed no evidence of cell swelling, which might indicate that by 60 minutes of reflow mannitol did produce some reduction in cell swelling, but the absence of cell swelling in the untreated and saline treated groups does not allow the conclusion that any increase in renal blood flow in mannitol treated animals was due to the reduction in cell swelling. In addition, red cell sludging was still found in 3 of the 5 mannitol treated sections so the absence of cell swelling was not always correlated with evidence of blood flow return.

The finding of intramural hemorrhage is again demonstrated in Figures 42 and 43, indicating intense vasospasm. In

Figure 36: Electron micrograph taken from the kidney of an untreated animal subjected to 60 minutes of ischemia and 60 minutes of reflow. In addition to the tubular epithelial damage there is evidence of interstitial hemorrhage with the extrusion of red blood cells and the formation of a platelet thrombus. The peritubular capillary visible below the red blood cells is patent. (X4550)

Figure 37: Electron micrograph from the same kidney as in Figure 36 further demonstrating the interstitial hemorrhage found in this kidney. Again the tubule is severely damaged. (X6300)



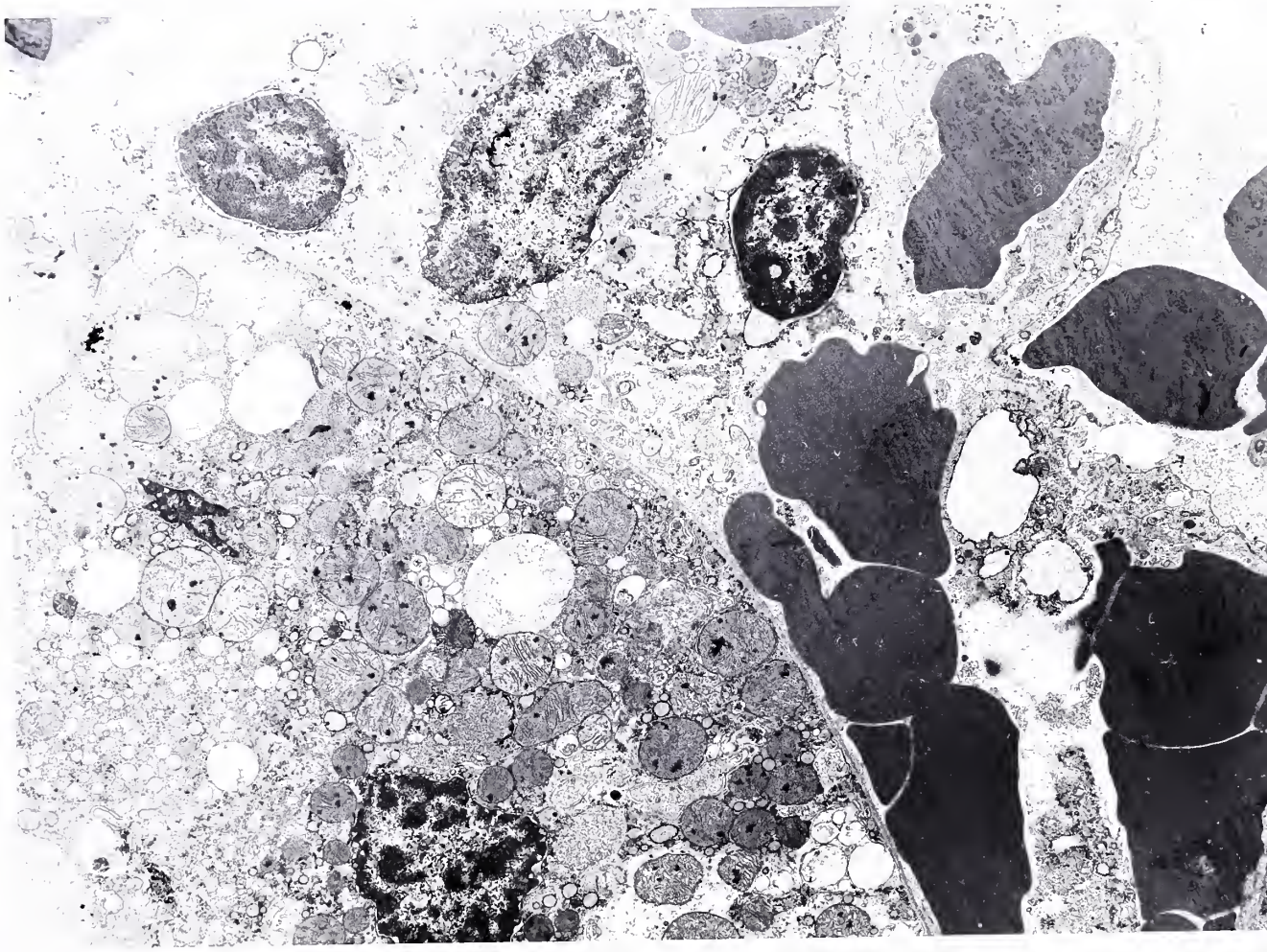
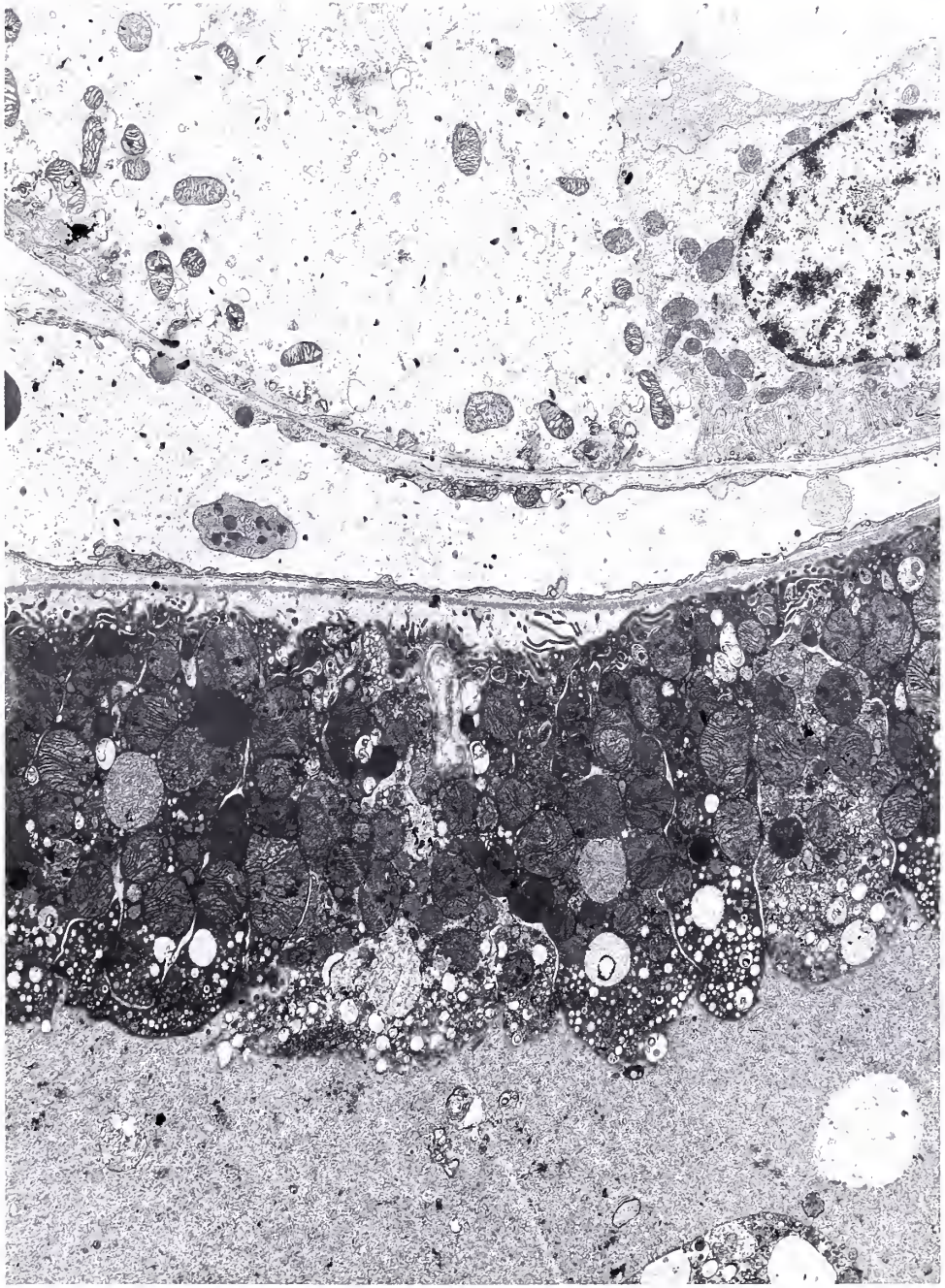
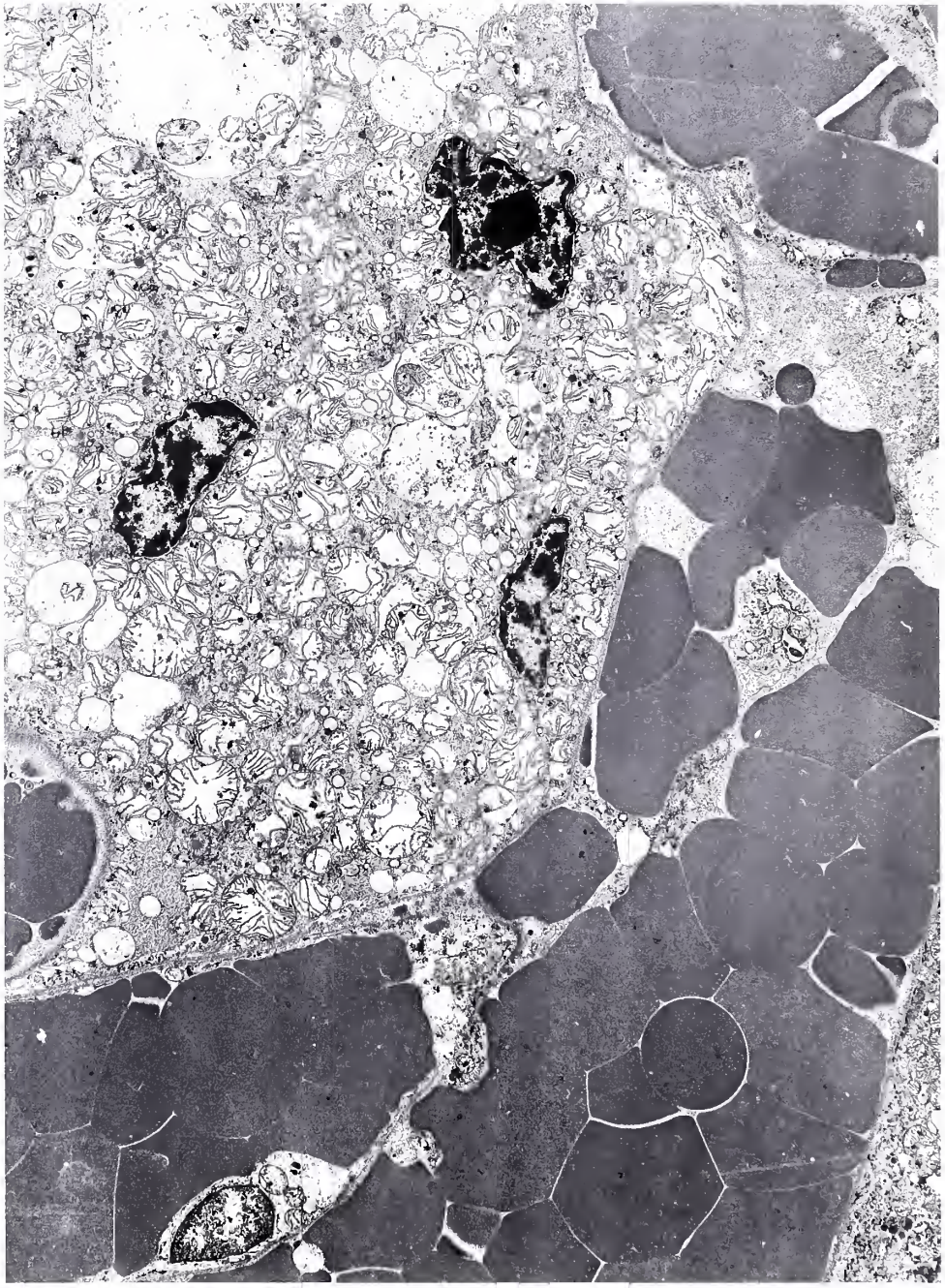


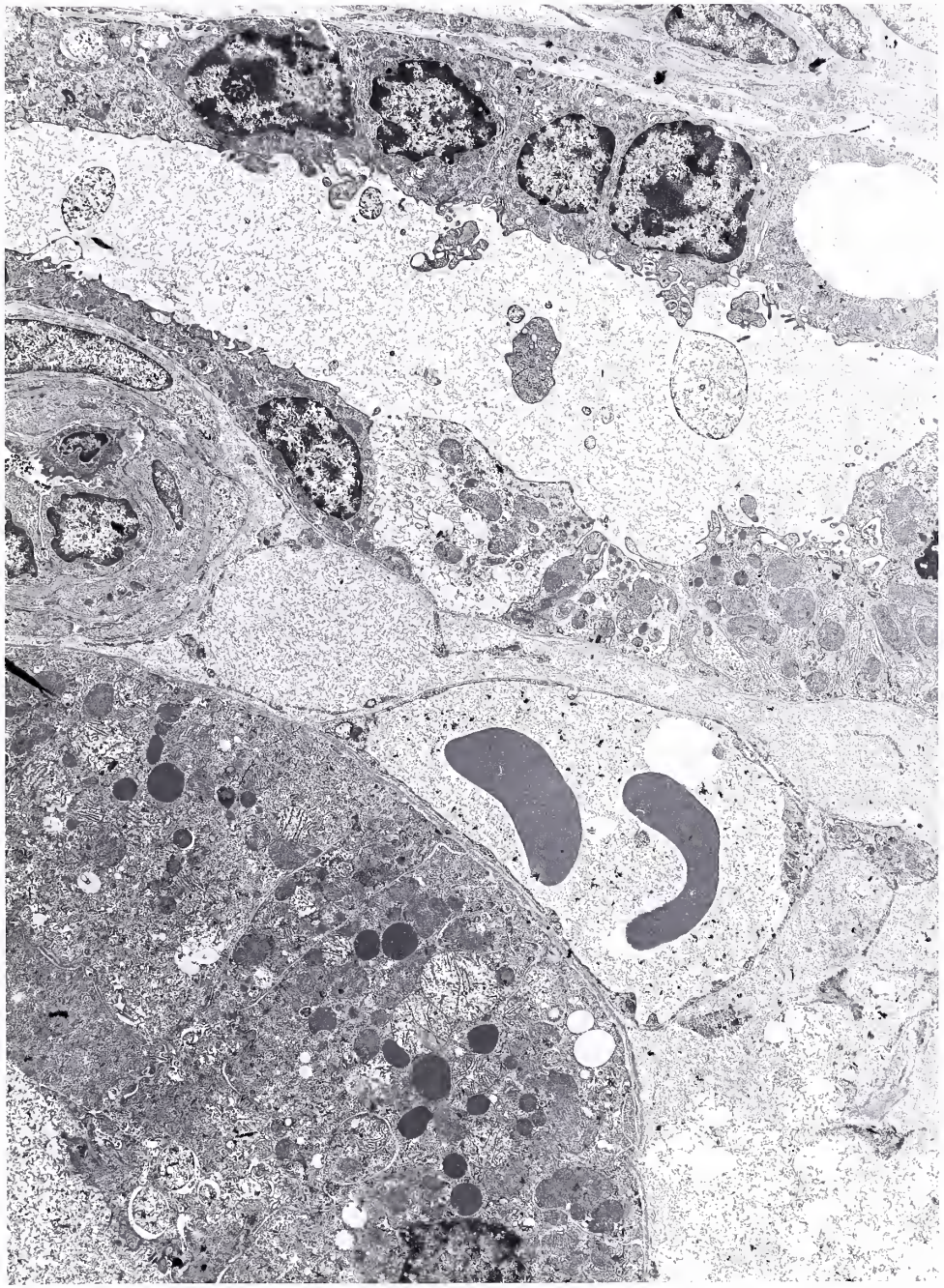
Figure 38: Electron micrograph from a saline treated kidney allowed 60 minutes of reflow and showing severe changes of tubular damage, red cell sludging and endothelial swelling. This contrasts markedly with Figure 39 taken from the same kidney. (X4550)

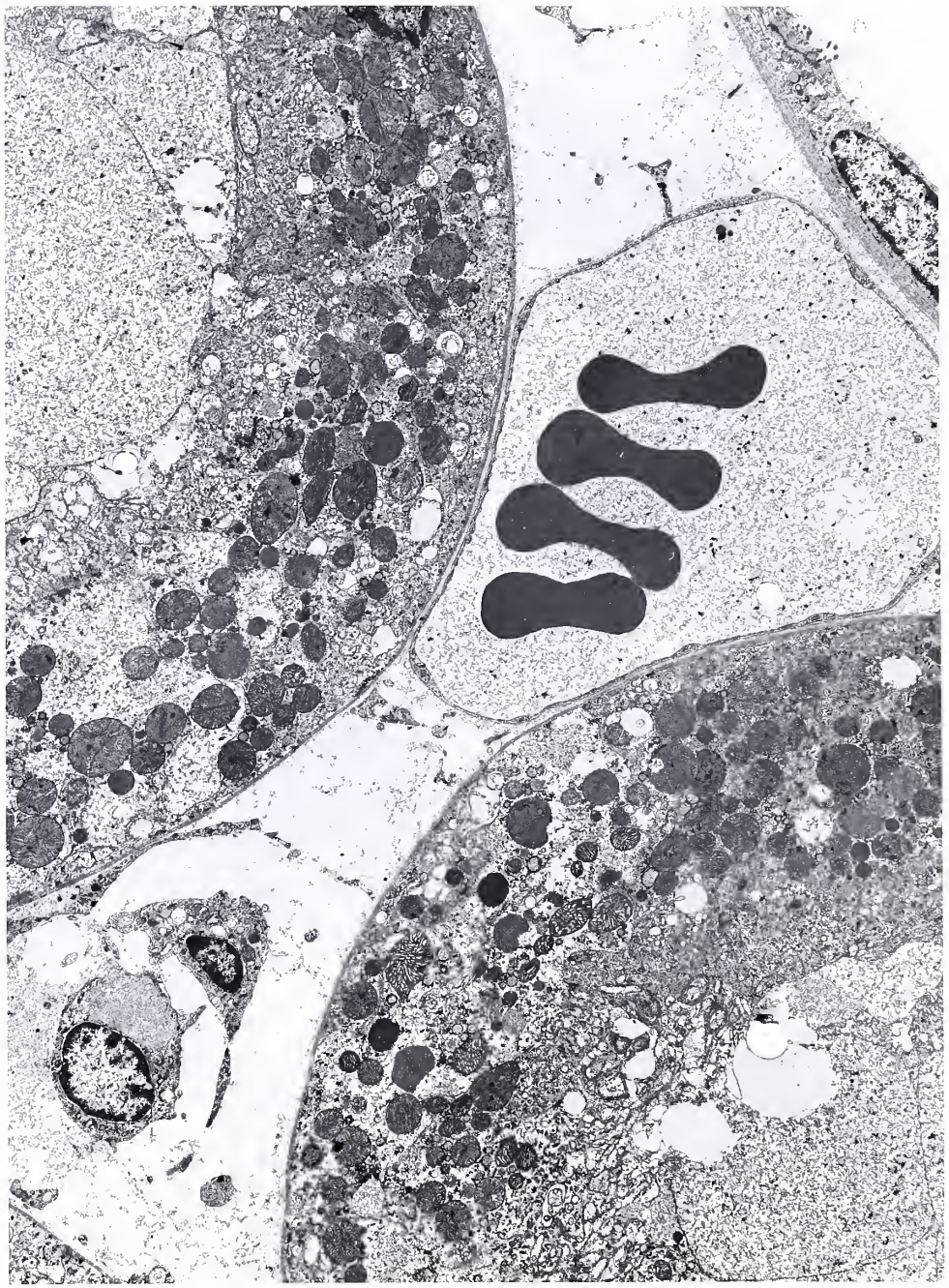
Figure 39: Electron micrograph taken from the same kidney as in Figure 38. The peritubular capillary is patent without evidence of endothelial cell swelling. (X6300)



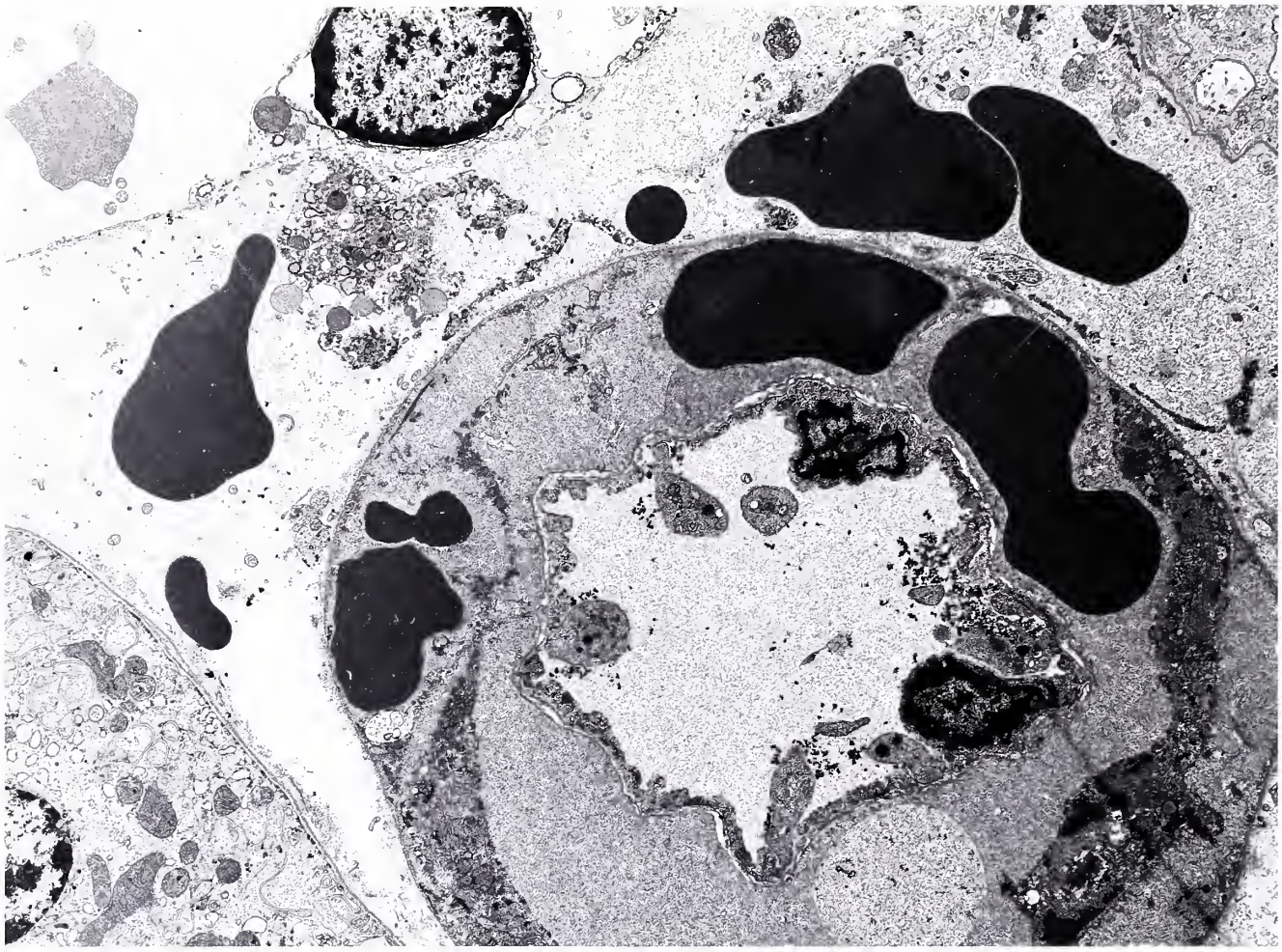


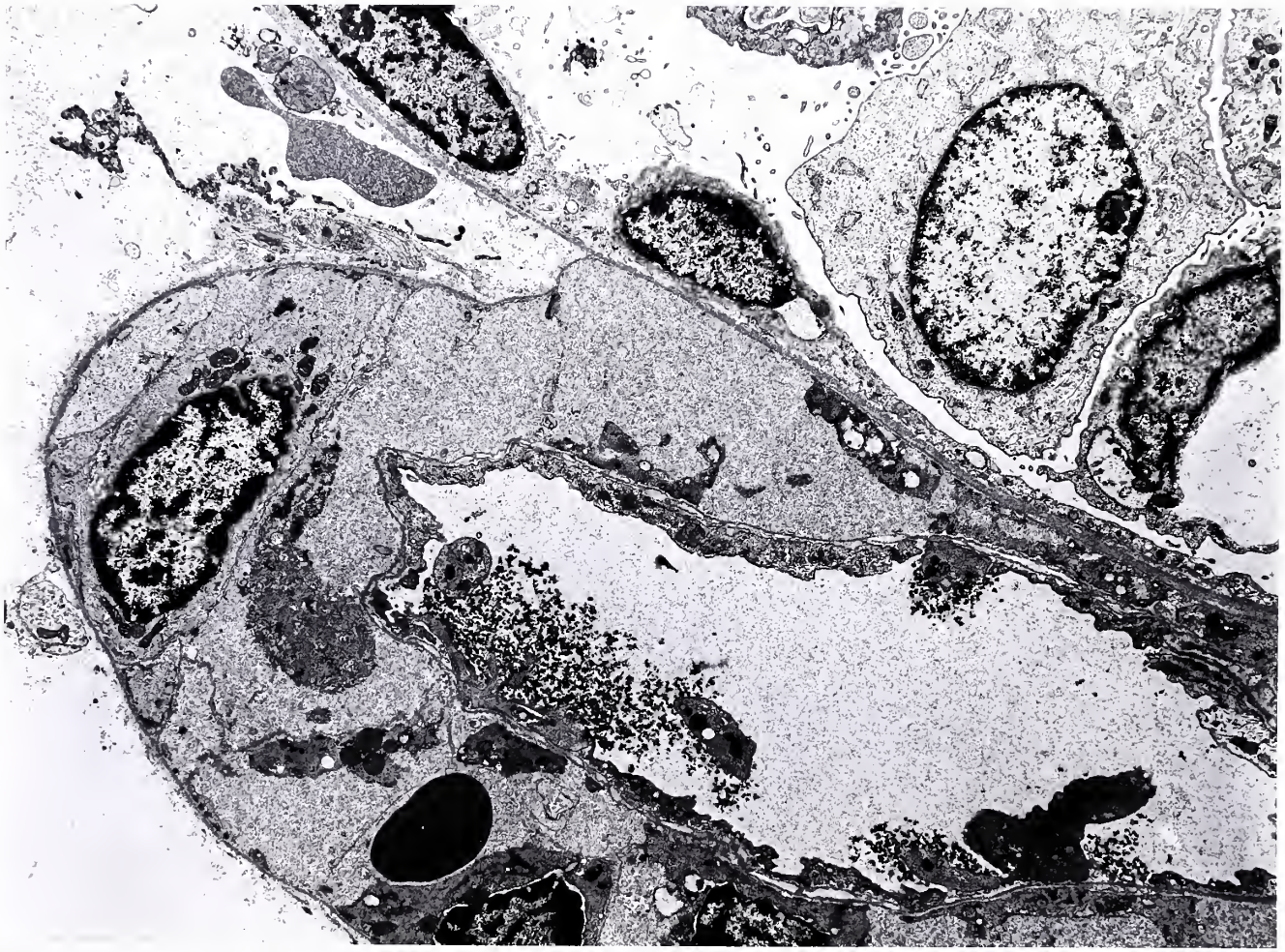
Figures 40 and 41: Electron micrographs taken from the same kidney of a mannitol treated animal given 60 minutes of reflow. The tubular epithelial cells are, again, damaged and the peritubular capillaries are patent without evidence of endothelial swelling. A small arteriole in Figure 41 appears closed, possibly by vasoconstriction. (X4550)





Figures 42 and 43: Electron micrographs taken from an untreated kidney allowed 60 minutes of reflow. Both demonstrate the interesting lesion of intramural hemorrhage in arterioles, suggestive of intense vasospasm. In addition, Figure 42 shows evidence of interstitial hemorrhage around the arteriole, although the basement membrane of the arteriole appears intact. (X6300)





addition, Figures 36 and 37 show evidence of hemorrhage into the interstitium with extrusion of red blood cells and platelets and the formation of a platelet thrombus (Figure 36). Interstitial hemorrhage was also seen in Figure 42 but was not seen in sections from any other kidneys and was considered an isolated finding.

DISCUSSION

The results of this experiment, using several morphological techniques to study an ischemic model of acute renal failure, confirm the existence of diminished renal blood flow and depressed cortical flow after a 60 minute ischemic period. The carbon perfusion demonstrated a marked diminution in glomerular carbon content after 60 minutes of ischemia and 10, 30 or 60 minutes of reflow (except for the mannitol treated, 60 minute reflow group). The silicone casts revealed evidence of diffuse patchy ischemia after 10 and 30 minutes of reflow, but indicated the nearly complete reestablishment of blood flow by 60 minutes.

The redistribution of blood flow away from cortical glomeruli was confirmed by the silicone casts which showed that after 60 minutes of ischemia and 10 and 30 minutes of reflow there was marked cortical ischemia. This correlates well with the radioactive microsphere data presented previously which demonstrated a depressed ratio of outer to total cortical blood flow after 10 and 30 minutes of reflow. Both models indicated the return of blood flow to the cortical glomeruli after the 60 minute reflow period. The carbon perfusion technique was unable to detect a difference in glomerular carbon content between outer and inner cortical glomeruli.

The suggestion of Flores, DiBona, Beck and Leaf (1972) that the "no-reflow" phenomenon could be explained on the

basis of cellular swelling leading to red blood cell trapping could not be confirmed by the present data. The finding of cellular swelling was an inconstant feature of the sections examined by electron microscopy. The finding of red cell sludging in 31 of 39 sections from animals subjected to the ischemic period indicated that blood flow was reduced, but swelling was observed in only 8 of the 39 sections. Ischemic damage was evident in the tubular epithelium of all ischemic kidneys. Interstitial hemorrhage was noted in only one section examined and, therefore, was not considered important in contributing to diminished renal blood flow.

The protective effect of a hypertonic mannitol infusion in the restoration of renal blood flow after the 60 minute ischemic period was confirmed by this data, but could not be attributed to the reduction in cellular swelling as suggested previously. The glomerular carbon content of the mannitol treated groups was higher than in groups given either no infusion or an isotonic saline infusion calculated to control for the expansion of extracellular volume. The overall increase in the mannitol treated groups reached borderline statistical significance. However, the 60 minute reflow group treated with mannitol had a statistically significant greater glomerular carbon content than the untreated group. These findings correlated well with the silicone casts which showed a definite improvement in the ischemic pattern after 10 and 30 minutes of reflow with mannitol treatment. The sections examined by electron microscopy did not demonstrate any reduction in cellular swelling for the mannitol

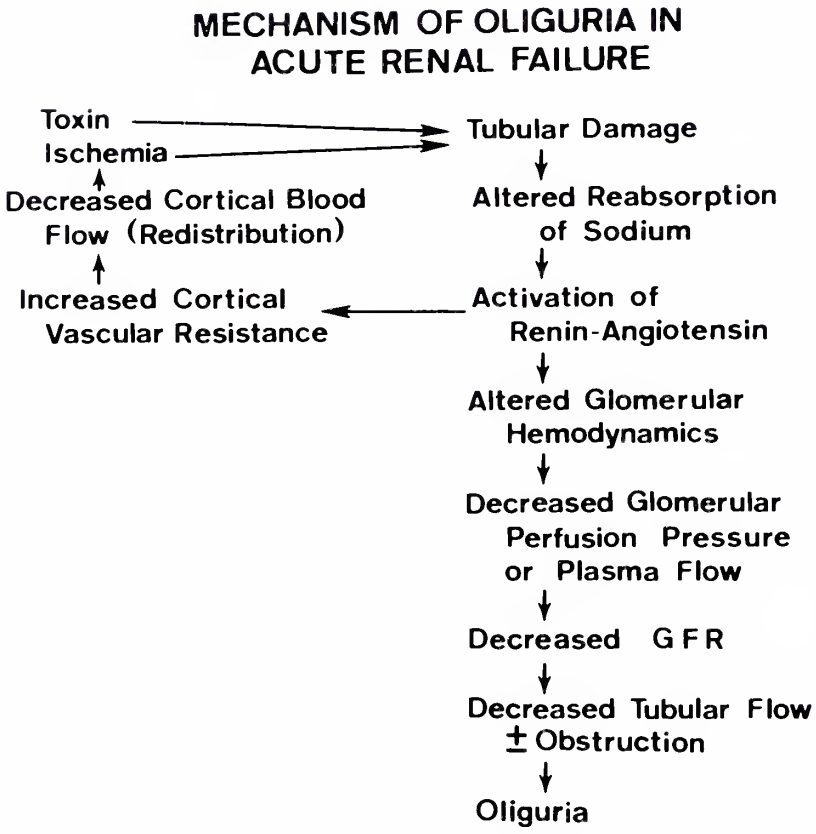
treated kidneys as 3 of the 8 sections showing evidence of endothelial cell swelling were in mannitol treated animals. Thus, from this data, one can conclude that any protective effect of mannitol was not due to expansion of extracellular volume or to reduction of cellular swelling. This means that mannitol's effect could be due to some renal vasodilatory mechanism leading to the improved renal blood flow or to dilatation of tubular lumina in cases where there may be some degree of tubular obstruction.

It is suggested, therefore, that the reduction of renal blood flow and glomerular filtration which occurs after renal ischemia is due to some degree of vasoconstriction, probably of the preglomerular arteriole. The finding of intramural hemorrhage in several of the sections was an interesting discovery suggesting the presence of intense vasospasm in these kidneys. Further support for this idea comes from a recent paper by Venketachelon (1973) who used morphological techniques to examine vascular changes in a model of glycerol induced acute renal failure and found evidence of significant interlobular and afferent arteriolar vasoconstriction without endothelial swelling by electron microscopy. The vasoconstriction may well be mediated by the renin-angiotensin system as suggested by work of several investigators cited previously.

The following scheme is proposed to account for the sequence of events leading to the functional disturbances in acute renal failure. It is modified from that previously

suggested by Drs. Henry, Lane and Kashgarian (1968) on the basis of data recently published (Daugharty et. al., 1974) which indicates that the fall in glomerular filtration rate which occurs with ischemia can be attributed solely to a fall in glomerular plasma flow.

Figure 44:



This scheme emphasizes the multiplicity of events which contribute to the production of acute oliguric renal failure. The initial insult may be a nephrotoxic agent or renal ischemia which leads to tubular damage and altered reabsorption

of sodium. Activation of the renin-angiotensin system results in arteriolar constriction and subsequent depressed cortical blood flow which completes the cycle and leads to self-perpetuation of the scheme. In addition, the vasoconstriction alters glomerular hemodynamics either by decreasing glomerular perfusion pressure or glomerular plasma flow. This results in diminished glomerular filtration which, with the possible added presence of tubular obstruction, leads to oliguria.

The proposed scheme incorporates most of the theories which have been proposed in the past century to account for the production of acute renal failure including tubular obstruction, nephrotoxicity, and vascular changes of diminished renal blood flow and glomerular filtration, redistribution of cortical blood flow, and the important contribution of the renin-angiotensin system. The no-reflow phenomenon observed in this and previous experiments can be explained on the basis of the vascular changes suggested in this scheme and not on the basis of endothelial cell swelling as previously suggested.

SUMMARY AND CONCLUSIONS

Acute renal failure was induced in rats by clamping the renal pedicles for 60 minutes. Toward the end of this period the animals were given an infusion of either isotonic saline or hypertonic mannitol (or no infusion). Blood flow was then reestablished for periods of 10, 30, or 60 minutes and the kidneys were examined using three morphological techniques: light and electron microscopic sectioning following the infusion of carbon particles, and silicone casts of the vascular system obtained from the infusion of liquid Microfil.

The results confirmed the diminished renal blood flow and cortical to medullary redistribution of blood flow which occurs following this period of ischemia. Mannitol was shown to have an ameliorative effect in the return of renal blood flow when compared to the saline and untreated groups. These findings, however, could not be explained on the basis of cellular swelling leading to red blood cell trapping, as suggested by previous investigators. Nor could the protective effect of mannitol be shown to be due to the reduction in cellular swelling.

It is suggested that renal vasoconstriction is the more important mechanism leading to reduced renal blood flow, and a scheme is proposed implicating the activation, by tubular damage, of the renin-angiotensin system and the subsequent changes in vascular resistance and glomerular hemodynamics as the important contributors to the continued renal ischemia and the production of oliguria in acute renal failure.

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