EXPERIMENTAL STUDIES IN ACUTE RENAL FAILURE
II. Fine Structure Changes in Tubules Associated with Renal Failure Induced by Globin*

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An association between hemolytic disease, acute renal insufficiency, and hemoglobin casts in kidney tubules was first recognized by Yorke and Nauss in 1911 (1). Baker and Dodds (2) reported the first experimental work on obstruction of renal tubules by hemoglobin casts and observed that rabbits with alkaline urine did not form casts whereas those with acid urine did so. They postulated that the renal lesion resulting from transfusion reactions was based on the formation of intratubular casts with resulting obstruction. DeGowin, Osterhagen, and Andersch (3) also concluded that the renal lesion of transfusion reactions in man was based on tubular obstruction by hemoglobin casts. Dunn, Gillespie, and Niven (4) described the histopathology of kidneys from persons sustaining crushing injuries and noted that these lesions were characterized by tubular casts confined to the lower portions of the nephron. They also observed that a toxic reaction appeared to occur in the cells near the casts of involved tubules. Bywaters *et al.* (5) studied casualties who had sustained crushing injuries and found that myoglobin was present in the urine and that pigment casts were in the kidney tubules. This observation led Bywaters and Stead (6) to study the effect of injected myohemoglobin in normal and acidotic rabbits. They obtained no lesions in normal rabbits but found characteristic cast formation and oliguria in acidotic animals.

The similarity of lesions resulting from several different conditions, *e.g.* massive tissue destroying injuries, blackwater fever, transfusion reaction, and injection of hemoglobin or related compounds, led Lucké (7) to propose the term "lower nephron nephrosis" as being descriptive of the renal lesion resulting from these several causes.

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Other authors (4, 8) have applied the name "crush syndrome" to the same lesion, with the obvious implication that the etiology is restricted to crushing injuries. Still other workers have called the same syndrome by several different names such as: shock kidney, acute tubular necrosis, or hemoglobinuric nephrosis (9).

Some workers have observed that dehydration and acidosis without any other manipulation resulted in transient tubular damage, and they therefore proposed that antecedent damage was necessary for the production of lower nephron nephrosis (10–13). Acidosis as a factor predisposing to cast formation in the dog has been denied by Flink (14), who showed that equal damage results from hemoglobin injection whether the urine is acid or alkaline. Kidney ischemia has been considered of major importance in acute kidney failure by some (15–17) although it has been adequately demonstrated by others that acute renal failure can develop without any diminution in renal blood flow (18, 19). Meroney and Rubini (20) presented a theory of the development of lower nephron nephrosis based on their clinical studies as well as the observations of previous investigators. They consider that the lumens of collecting tubules, distal tubules, and ascending loops of Henle are plugged by precipitated protein and cell debris and that these plugs are held firmly in place by edema. The result of the tubular obstruction is oliguria or anuria. When the edema diminishes and the plugs of debris are released, diuresis results. This theory was extended by Parry, Schaeffer, and Mueller (21) who demonstrated that in experimental animals the tubular casts were much more extensive in animals which had a low rate of urine flow than in animals which had a high rate of urine flow induced by an osmotic diuretic, and that the mortality and morbidity were reduced in animals with high urine flow.

Human globin injected into rats to produce the typical renal failure lesion was first carried out by Mason *et al.* (22). The present work was undertaken to study further the role of globin in casting light on the etiology of "lower nephron nephrosis." Globin is a component of all the compounds previously implicated in the production of this syndrome; *e.g.*, hemoglobin, myoglobin, methemoglobin. Specific reference has been made by others to an apparent toxic reaction by tubule cells associated with development of the lesion (4, 19). Our present report confirms that there is an early, toxic effect on the cells of the lower nephron when globin is present in the tubules in any appreciable concentration. We present herewith the fine structure changes occurring in the early, acute phase of development of lower nephron nephrosis induced by the intravenous administration of globin in the rat.

Materials and Methods

Female Sprague-Dawley rats weighing 110 to 160 gm were used in these experiments. Purified human globin (23) was injected intravenously in the amount of 0.75 gm per kg of body weight into the saphenous vein of rats treated in one of the following five ways.

1. *Dehydrated.*—This is our standard model and groups of 6 to 10 of these animals were used to accompany each of the other four groups and serve as references. These rats had commercial rat chow and water available at all times until 24 hours prior to injection at which time both food and water were removed and not made available again until 4 hours after injection of globin.

2. *Water Ad Libitum.*—Free access to commercial rat chow and water at all times. Fourteen of these animals were used.

3. *Water-Loaded.*—Water was given by stomach tube to the amount of 5 per cent of body weight on the morning of the experiment and again 3 hours later. Forty minutes after the second water load, globin was injected. Twenty-one of these animals were used.

4. *Water-Loaded Plus Antidiuretic Hormone.*—These were treated as the water-loaded group above and in addition 0.2 unit of pitressin was given subcutaneously 1 hour before the second water load. Twenty-one of these animals were used.

5. *Dehydrated Plus Mannitol.*—These animals were dehydrated as above and were given 0.8 gm of mannitol per kg of body weight 10 minutes before injecting the globin. Fifteen of these animals were used.

Following the globin injection all animals were placed in metabolic cages and fluid intake and output were measured daily until death or the end of the acute phase of the disease at 7

TABLE I

Clinical Characteristics of Acute Renal Failure

	Experimental disease	Human disease
Mortality	20 to 50 per cent	40 to 60 per cent
Reversibility	Reversible	Reversible
Oliguria	Short duration (less than 24 hrs.)	Usually 2 to 5 days
Low urinary osmolality	300 to 500 mOsM/Kg H_2O	300 to 500 mOsM/Kg H_2O
Azotemia	BUN = 100 to 300 mg per cent	BUN = 100 to 300 mg per cent
Hyperkalemia	Serum K^+ = 6 to 10 mEq/liter	Serum K^+ elevated
Hyponatremia	Not present	If present is secondary to overhydration
Acidosis	Present	Present

TABLE II

Urine Flow at the Time of Globin Injection Related to Disease Severity after Different Pretreatments

	Urine flow	Disease severity	Mortality
			per cent
Dehydration	Low	+++	20
Water load plus ADH	Low	+++	48
Water *ad libitum*	Normal	+++	29
Water load	High	++	0
Mannitol	High	+	0

days. Urine and plasma osmolality were measured in a Fiske osmometer and sodium and potassium were measured in a Coleman flame photometer.

The clinical characteristics of the experimental disease are compared to the human in Table I. A comparison of urine flow and disease severity after globin injection in the five groups

of animals is given in Table II. Histological observations by light and electron microscopy were made on animals from three groups: dehydrated, water *ad libitum*, and dehydrated plus manitol. Light microscopy revealed that the protective effect of mannitol was associated with fewer damaged tubules in that group than in the dehydrated water or water *ad libitum* groups (24). Electron microscopy demonstrated that when the tubules were damaged to any degree there was no difference in the type of damage in any of the groups. For this reason the observations reported here are based on lesions of the dehydrated animals. Tissues were taken at the same time intervals and with the same treatment as described in the first paper of this series (23).

OBSERVATIONS

One minute after injecting the globin solution, spherical aggregates of the same type as those seen in the glomerulus associated with globin transport are seen in the proximal tubules (Fig. 7) in addition to a granular electron-opaque material (Figs. 7 and 7 *a*) and a diffuse electron-opaque material (Figs. 9 and 9 *a*) which may represent previously dissolved globin which did not aggregate before reaching the position in which it was fixed (Figs. 7, 9, and 10). Within 5 minutes after injection a granular density is seen in the distal tubule lumens, and it is assumed that this is also globin (Fig. 14). The demonstration that the lumen contents are globin is made by autoradiography (Figs. 1 and 2).

The diffuse globin in the proximal tubule fills the lumen uniformly and extends between the microvilli. At the base of the microvilli, pinocytotic vesicles are seen in various stages of extension into the cytoplasm (Figs. 7, 9, and 10). At 10 minutes after injection, larger aggregates are seen deeper in the cytoplasm but the majority are still on the lumen side of the cells (Fig. 8). All of this intracellular globin is surrounded by membranes; some membranes are closely applied to the aggregates while other aggregates are free within what appears to be an extensive membrane-limited system of cytoplasmic channels (Figs. 9, 11, and 12). The cytoplasmic membranes surrounding the globin do not have any associated granules. There is occasional close relationship between the channel system and the Golgi apparatus, but no continuity between them has been seen. The globin density is not seen between the proximal tubule cells but is seen to extend on the lumen side to the region of the tight junction (Figs. 9 and 10) in a manner similar to that described for hemoglobin (25, 26). More globin appears on the basal side of the proximal tubule cells with the passage of time and it is eventually seen in the connective tissue space on the basal side of the tubules (Figs. 12 and 13).

The cells of the thick limb of Henle's loop and distal convoluted tubule (for convenience, we shall call these two components the lower nephron) apparently take up the globin by pinocytosis (Figs. 15 and 16). The large number of membrane-bounded vesicles typical of the proximal tubule is not found within the cells of the lower nephron, however. Various stages of degeneration of the lower nephron cells associated with the presence of more than a minimal amount of

intracellular globin are seen in Figs. 19 to 22. In many places a single cell is found to have taken up globin and subsequently degenerated in position with little apparent damage to the adjacent cells (Fig. 19). With the passage of time, more and more of the lower nephron cells become involved in the toxic reaction so that as early as 1½ hours many entire tubules are devoid of living cells and the confines of the basement membrane are seen to be filled with cell debris and globin (Figs. 21 and 22). It is still not certain if globin is transported by the lower nephron cells in any manner similar to that of the proximal tubule cells. Some globin is occasionally observed in the connective tissue space adjacent to portions of the lower nephrons, but no correlation between time and location with respect to the lumen can be made in this segment as it can in the proximal tubule.

The mechanism of toxic effect on the lower nephron cells has not been ascertained as yet, although some pertinent observations may be made. The large aggregates of globin appearing in the tubule lumens are not found to be taken up by the cells. Pinocytotic uptake of small quantities of globin appears to be the means by which entry into the cell is obtained. In the early stages of uptake, globin is not found free in the cytoplasm. In cells which are beginning to degenerate, there are many small intracytoplasmic globin aggregates without surrounding membranes (Figs. 19 and 20). It is not obvious if the membranes break down first and release globin into the cytoplasm or if the membranes are lost as a correlate of the degenerative process. Another impressive but nonspecific, degenerative reaction by the lower nephron cells is the formation of many myelin figures (Figs. 20 and 22).

Mitochondria are involved in globin uptake to a considerable extent in both the proximal and distal portions of the nephron. Autoradiographs following injection of tritium-labeled globin frequently show evidence of radioactivity in mitochondria (Figs. 4 and 5). Mitochondria in various stages of degeneration are seen to contain dense material resembling globin (Figs. 5, 17, and 20), and some of the involved mitochondria in more advanced stages of degeneration approach the appearance of cytolysomes (27, 28) (Figs. 6 and 18).

At the time when lumens of the lower nephrons are beginning to be filled with protein precipitate and cell debris, the proximal tubules begin to show dilatation and in some instances even disruption by tearing of the cell membranes (Fig. 24). Tearing apart of the proximal tubule cells occurs at some point other than the terminal bar and can be distinguished from basal compartment swelling resulting from fixation with hypertonic fixative (29).

Six hours after globin injection, when the proximal tubules are dilating, there is very little globin remaining within either the lumen or cells of the proximal tubules (Fig. 23). This suggests that, in distinction to the lower nephron, any destruction of these proximal tubule cells results from pressure necrosis or actual disruption by tearing rather than from the toxic effect of globin.

DISCUSSION

Within 1 minute after intravenous injection of purified human globin into rats it has passed the glomerular barrier (23) and appeared within the lumens of proximal tubules. The base of the microvilli appears to be the site of uptake of globin just as it is for hemoglobin (25). The globin appears in at least three forms in the proximal tubule lumens: (*a*) dense, spherical droplets; (*b*) fine granular; and (*c*) homogeneous dense material filling all the available space. The granular and homogeneous forms of globin are found within small pinocytotic vesicles or channels leading from between the bases of the microvilli, but no indication of uptake of the large aggregates has been observed. The small vesicles which originate at the base of the brush border appear to pinch off and coalesce with others of the same type, thus forming larger vacuoles within the tubule cells. After a lapse of time the globin is seen on the basal side of the tubule cells. This fact suggests that there is an oriented path of migration from the lumen to the basal side, since the reverse sequence is not seen.

A system of channels leads at least part way through the cell and globin is found within the profiles of this system. There are no granules associated with the membranes of these channels so it is not part of the ergastoplasm. This channel system might best be termed "microlabyrinth" in the sense with which that term was used by Dempsey (30). A Golgi body is sometimes seen in close relationship to the elements of this microlabyrinth but the significance of the relationship was not ascertained in our present study. With the close packing of organelles within the proximal tubule cell, many other structures such as mitochondria, infolded cell membranes, and ergastoplasm are also in close relationship with the channels but are not necessarily functionally or structurally connected to them.

After traversing the proximal tubule cells, globin is next seen within the tubular basement membranes and in the connective tissue spaces around the tubules. Large accumulations of globin are not seen in the peritubular spaces and therefore the globin must be carried away, probably by the peritubular capillaries. Globin is not extruded between the tubule cells and does not enter the space between tubule cells from the lumen so that when it is absorbed the only pathway available for it is through the cell.

Much of the globin passing the glomerular barrier also traverses the proximal tubule lumen without being resorbed, and within 5 minutes after injection globin is found in the lumen of the distal tubule. It appears that globin has a toxic effect on cells of the lower nephron while other cells of the nephron are relatively unaffected by it. Fairly large quantities of globin can be taken up by the proximal tubule cells without identifiable damage, but whenever a lower nephron cell contains more than a small amount of globin it manifests obvious degenerative changes (compare Figs. 8, 9, and 12 with Figs. 19 to 22). The degenerative changes in the distal tubule do not appear to be specific,

e.g. the myelin figures are also found in such conditions as uranium poisoning (31), but the combination of degenerating cell components with intermixed globin droplets is pathognomic.

Mitochondria are involved with globin absorption in both proximal and distal tubule cells. This fact is demonstrated by autoradiography and by the presence of mitochondria which contain unusual accumulations of material having density and texture like that of globin. All stages of transition ranging from mitochondria with scant globin inclusion to structures which are morphologically indistinguishable from cytolysomes (27, 28, 32) are found during globin absorption in kidney tubules and in cells of other tissues as well (unpublished data). It appears that cytolysomes may arise directly from mitochondria by the incorporation of foreign protein in addition to the accidental inclusion of mitochondria within a region of cytoplasmic degeneration. Previous evidence for the participation of mitochondria in protein resorption by kidney tubule cells has been obtained by histochemical methods (33–35) and by electron microscopy (36, 37) although some investigators have noted a failure to find such evidence (25, 38).

The role of mitochondria in globin metabolism may be accidental in the tubule cells. The major portion of intracellular globin in the proximal tubules is found within vacuoles and cytoplasmic channels, and it is discharged from the cells on the basal side without apparent change. It is possible that a small quantity of globin escapes from the enveloping membranes and is taken up by the mitochondria for subsequent digestion while that portion which does not leave the vacuole is unaffected by the cell. Another possible, but less likely, explanation is that some of the globin is taken up by pinocytosis while a smaller amount is absorbed directly through the membrane and that it is only the globin entering directly by the latter route which is subsequently taken up by mitochondria. Mitochondria of the lower nephron become involved with globin in the same way and with roughly the same frequency as those of the proximal tubule so it would appear that globin exerts its toxic effect on some component of the lower nephron cells other than mitochondria.

After sufficient destruction of lower nephron cells has occurred, at about 2 hours' postinjection, the entire lumen of the most affected tubules becomes plugged with cell debris and globin. Dilated proximal tubules are first seen at the time of plugging of the distal tubules. These observations are consistent with those of others who produced lesions with hemoglobin (3, 10–14, 39, 40). Wichstein and Lange (41) induced proteinuria in the rat by two different methods and found a direct correlation between the number of distal tubule casts and the amount of renal damage.

Some of the proximal tubules in our experiments are seen to be disrupted by the pulling apart of originally adjacent cells and tearing which occurs in the part of the cells nearest the lumen. Separation of cells by disruption of des-

mosomes is never seen; the tear is always at some other point. We conclude that in these cells a critical pressure has been exceeded and that the cells are unable to flatten beyond a certain limit because of the cytoplasmic structure at the base of the brush border which appears analogous to the terminal web of the intestine at the base of the microvilli (42).

Our observations support the concept of intratubular obstruction and concomitant toxic cellular damage as the primary etiology of acute lower nephron nephrosis. Meroney and Rubini (20) presented a detailed theory of acute kidney failure in tubular necrosis which involved the concept of plugging of tubules with debris and accompanying tissue edema. If all of the tubules are plugged, according to this theory, anuria results; if some of the tubules are plugged but some are still patent, oliguria results. The theory further states that when the edema decreases, and the plugs of debris come out, diuresis results. Conn, Wilds, and Helwig (18) have demonstrated that anuria can occur without any decrease in perfusion flow of blood in the kidney. This fact is explainable on the basis of nephron plugging without having to invoke a concept of shunting of blood to bypass the glomerulus. Goldberg (19) has added evidence to the concept of nephron plugging in an elegant experiment using hemolyzed erythrocytes and determinations of blood flow with krypton plus measurements of various clearance factors. He observed an acute decrease in urine flow, creatinine clearance, and hippuran clearance, but no decrease in renal blood flow. Tubular casts were formed as the lesion developed. His conclusions were, "The changes appear to be secondary to intratubular obstruction and concomitant toxic tubular damage." Our observations on the course of events in the animals given globin are in complete agreement with those of Goldberg. It is of interest that we arrived at the concept of toxic reaction of the lower nephron cells on the basis of their fine structure cytopathology independently of the physiological and biochemical basis upon which Goldberg based his conclusions.

Our concept of the development of acute renal failure associated with conditions which release products containing globin, *i.e.* hemoglobin, myoglobin, methemoglobin, and globin itself, is as follows: Globin and any associated compound is transported through the glomerular wall (23) and is carried through the lumen of the nephron by the moving stream of glomerular fluid. Some of the material is resorbed by the proximal tubule but much is not, and the proximal tubule cells are not adversely affected by the resorption process. In the lower portions of the nephron, material is also taken up by the tubule cells. If the animal has been deprived of water, relatively more globin-associated material is taken up than in the normal animal, and some moderate amount of globin is toxic to the cells of the lower segments of the nephron. The resulting debris from dying and disintegrating tubule cells plus the globin in the lumen combine to form plugs in the collecting tubules, distal tubules, and ascending

loops of Henle. These plugs then effectively stop urine flow in the affected nephrons and create back pressure which causes the proximal tubules to undergo dilatation, pressure necrosis, or disruption. The animal will become oliguric when sufficient nephrons are plugged or anuric if still more are plugged. Further support is added to this concept by the observation that fewer nephrons are plugged or damaged in animals which have a high urine flow rate and thus have reduced transit time and possibly a lower concentration of toxic material in the urine (43). Either of these conditions results in a decreased uptake by the tubule cells and thus the amount of toxic material in any lower nephron cell would be more likely to remain below the threshold required to produce damage.

The protective effect of the high urine flow rate is independent of its cause, whether by water loading or solute diuresis. Conversely, more damage is caused in those animals with an original low rate of urine flow, and this is also independent of the cause. Equal damage is produced in water-loaded animals which have received antidiuretic hormone and animals which are dehydrated because of restricted intake (44). The differences that do occur are in the number of involved nephrons rather than in a different degree of damage to any one nephron.

SUMMARY

When purified human globin is injected intravenously into rats it produces acute renal failure characterized by tubular casts and oliguria. The globin is identifiable within vesicles and channels in the cytoplasm of the proximal tubules, through which it passes from lumen to basal side with no apparent serious effect on the cells. When a very minimal amount of globin is taken up by cells of the distal limb of Henle's loop or distal tubules (lower nephron), a markedly deleterious effect is apparent and the cells die within a short time. The mixture of cell debris and precipitated globin forms plugs within the confines of the basement membranes of the former distal limbs and distal tubules. After a number of lower nephrons are plugged a disruption of proximal tubules is found, which apparently results from the effect of back pressure in the obstructed nephrons.

We suggest that any amount in excess of a low threshold of globin, either alone or combined with heme or related material, has a toxic effect on lower nephron cells. Once initiated, the toxic effect is not reversible and the resulting plug of debris and precipitate will occlude the lumen. If a sufficient number of nephrons are made non-functional the animal becomes anuric; otherwise it is oliguric. A high rate of urine flow will protect against the excess absorption of material and thus against acute renal failure.

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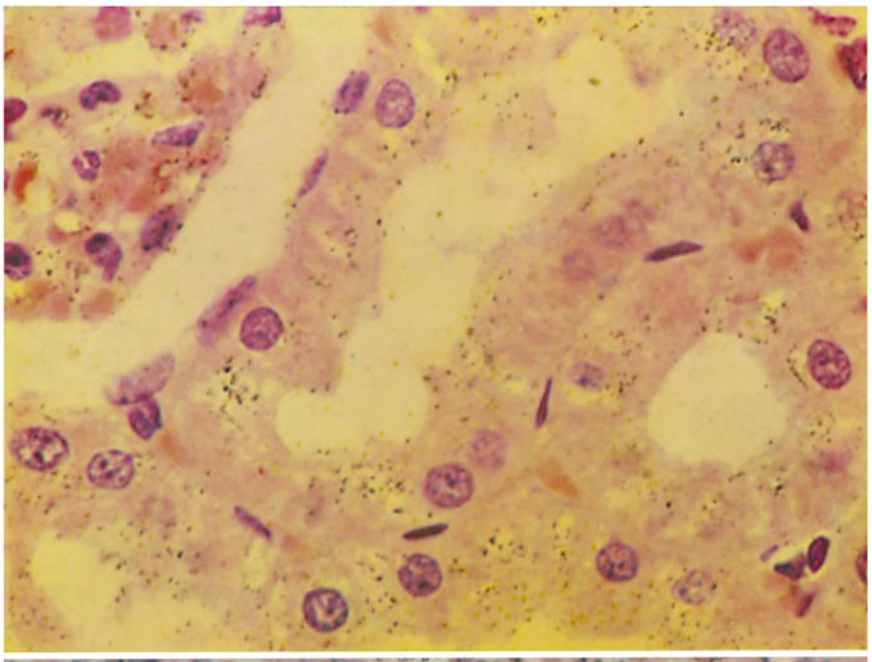
EXPLANATION OF PLATES

PLATE 117

FIG. 1. Proximal tubules of rat injected 2 hours before fixation with radioactive globin. Stained with hematoxylin and chromatrope. Emulsion exposed for 81 days. The radioactivity is scattered throughout the proximal tubule cells. Oil immersion. × 1000.

FIG. 2. Light micrograph of distal tubules and ascending loop of Henle. Treatment as in Fig. 1. The lumens are filled with precipitated globin whose radioactivity results in many exposed silver grains. × 600.



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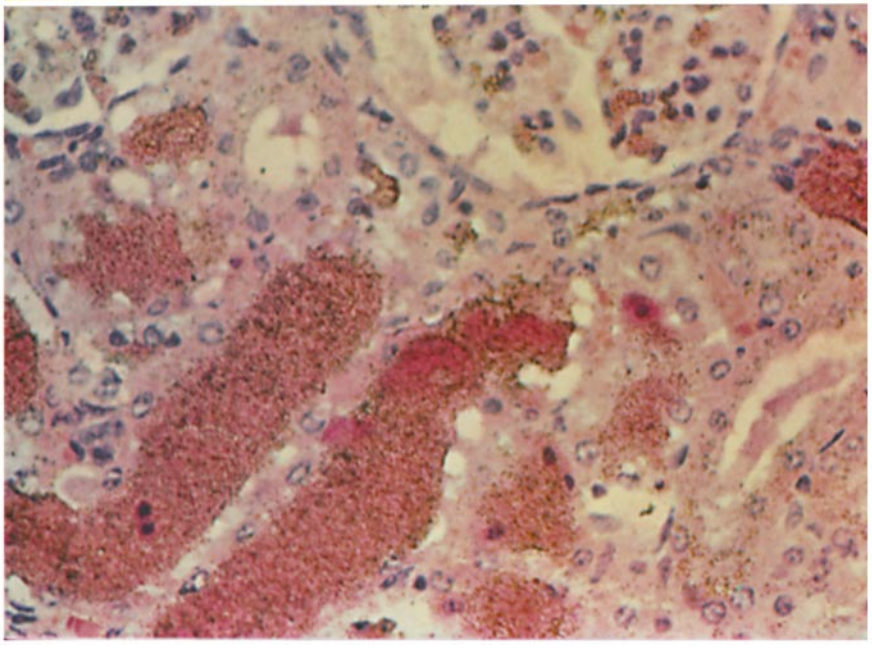


2

(Menefee *et al.*: Acute renal failure. II)

PLATE 118

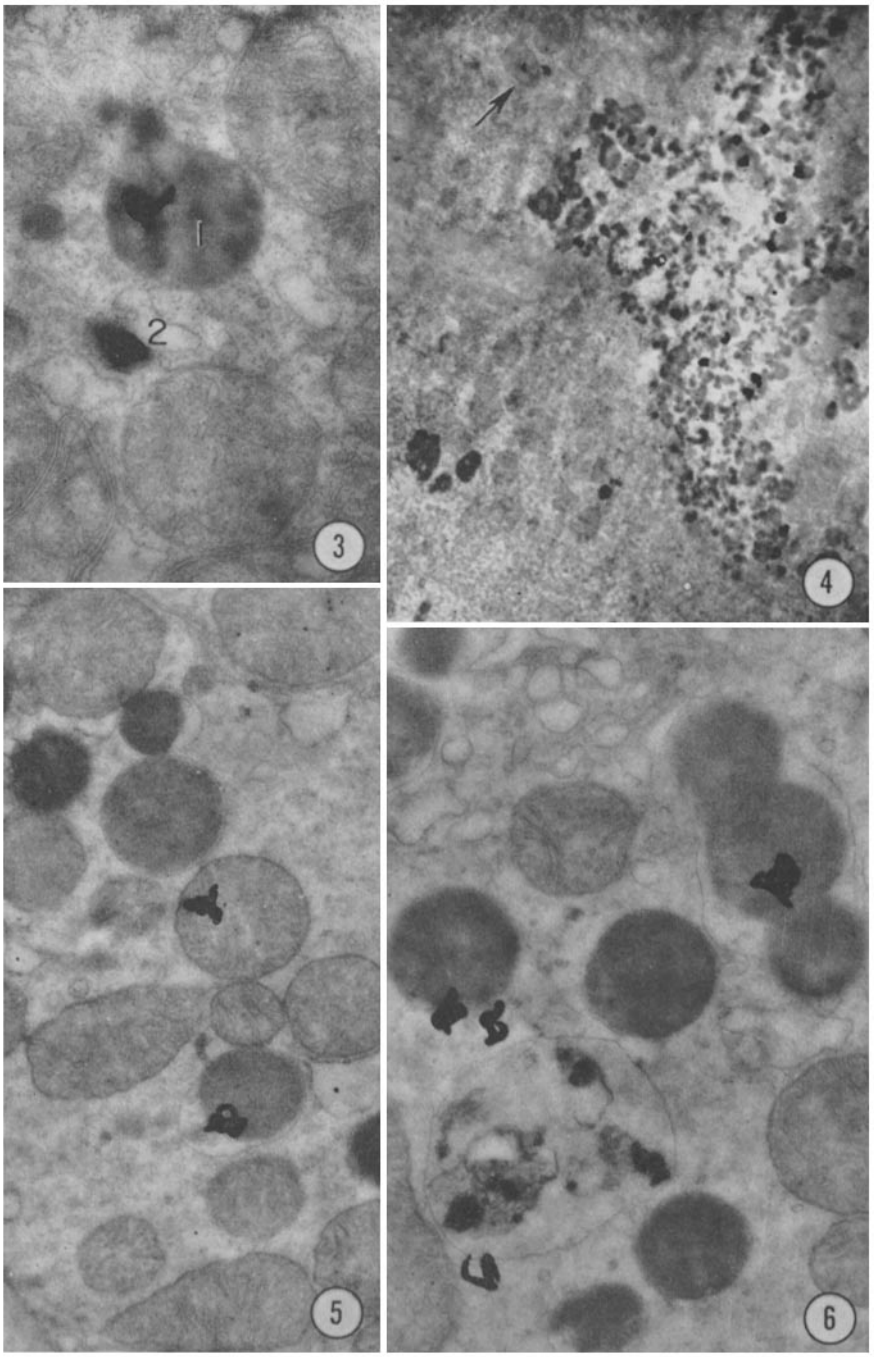
FIGS. 3 to 6. Electron microscopic autoradiographs of rat kidney fixed 2 hours after injection of radioactive globin.

FIG. 3. Portion of a proximal tubule showing globin completely filling a membrane-bounded vesicle, *1* and partially filling an irregular vesicle, *2*. × 23,000.

FIG. 4. Aggregate of globin filling the lumen of a distal tubule with considerable radicactivity in evidence. A few globin droplets are in the cytoplasm, and a mitochondrion (arrow) has a grain associated with it. × 3000.

FIG. 5. Mitochondrion manifesting radioactivity in a proximal tubule. × 23,000.

FIG. 6. Several aggregates of globin showing radioactivity in the proximal tubule. × 14,000.



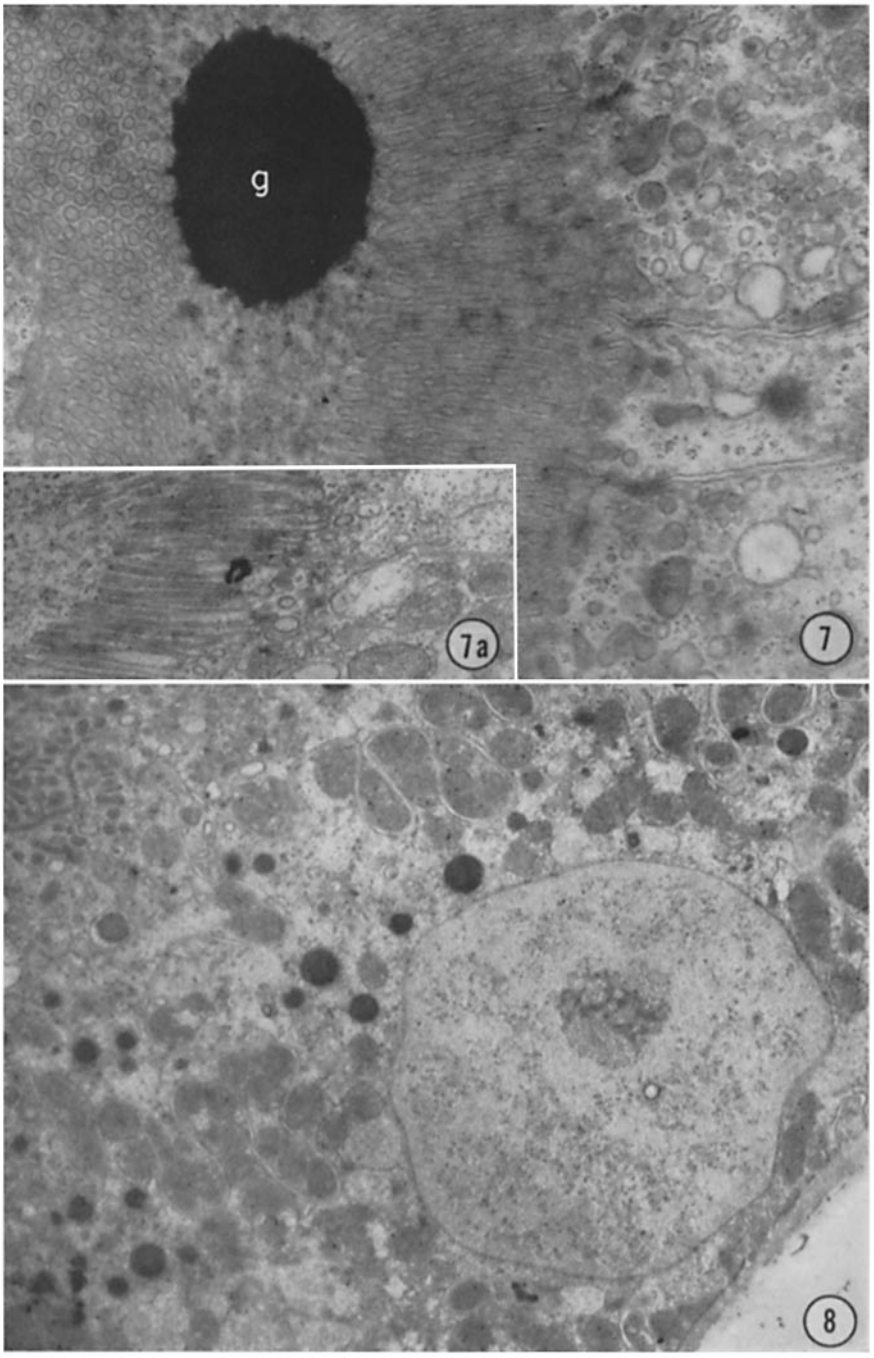
(Menefee *et al.*: Acute renal failure. II)

PLATE 119

FIG. 7. Portion of proximal tubule and lumen 5 minutes after injecting globin intravenously. A large dense globin aggregate (*g*) and a less dense granular density are present in the lumen and the density extends between the microvilli and into extensions leading into the cytoplasm. The difference in appearance between globin droplets and lipid is illustrated in Fig. 12. × 16,000.

FIG. 7 *a*. Autoradiograph showing granular precipitate in the lumen similar to that of Fig. 7. An exposed photographic grain is seen near the granular material within an expansion at the base of the brush border. × 11,000.

FIG. 8. Proximal tubule 10 minutes after globin injection. The globin contained within cytoplasmic vesicles is mostly on the lumen side of the cells. The basement membrane is on the right. × 8000.



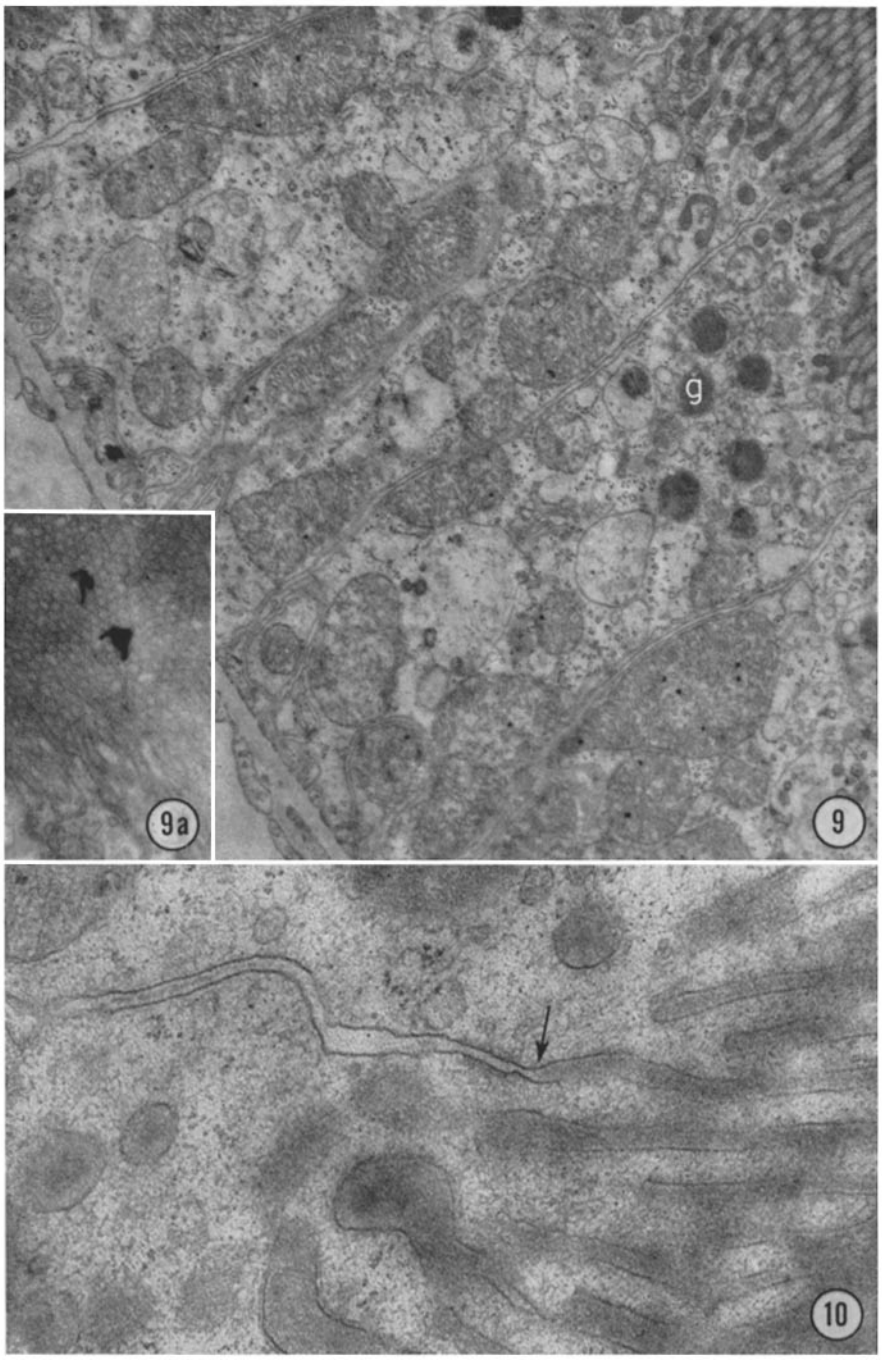
(Menefee *et al.*: Acute renal failure. II)

PLATE 120

FIG. 9. Proximal tubule 10 minutes after globin injection. An aggregate of globin is seen within a closely applied membrane at *g*. To the left of that aggregate is another membrane-bounded vesicle with a relatively small amount of globin. Also present are other profiles of vesicles located at the base of the microvilli. × 12,000.

FIG. 9 *a*. Autoradiograph of an area showing diffuse density between microvilli and at their base similar to that of Fig. 9. Two photographic grains are seen over the brush border suggesting that the diffuse density is actually globin. × 12,000.

FIG. 10. Base of microvilli in the region of the tight junction (arrow) between two proximal tubule cells on the lumen side. The density within the lumen between the microvilli is seen to extend to their base and into dilatations in two places. It is also seen to extend as far as the region of the seal but no further between cells. × 54,000.

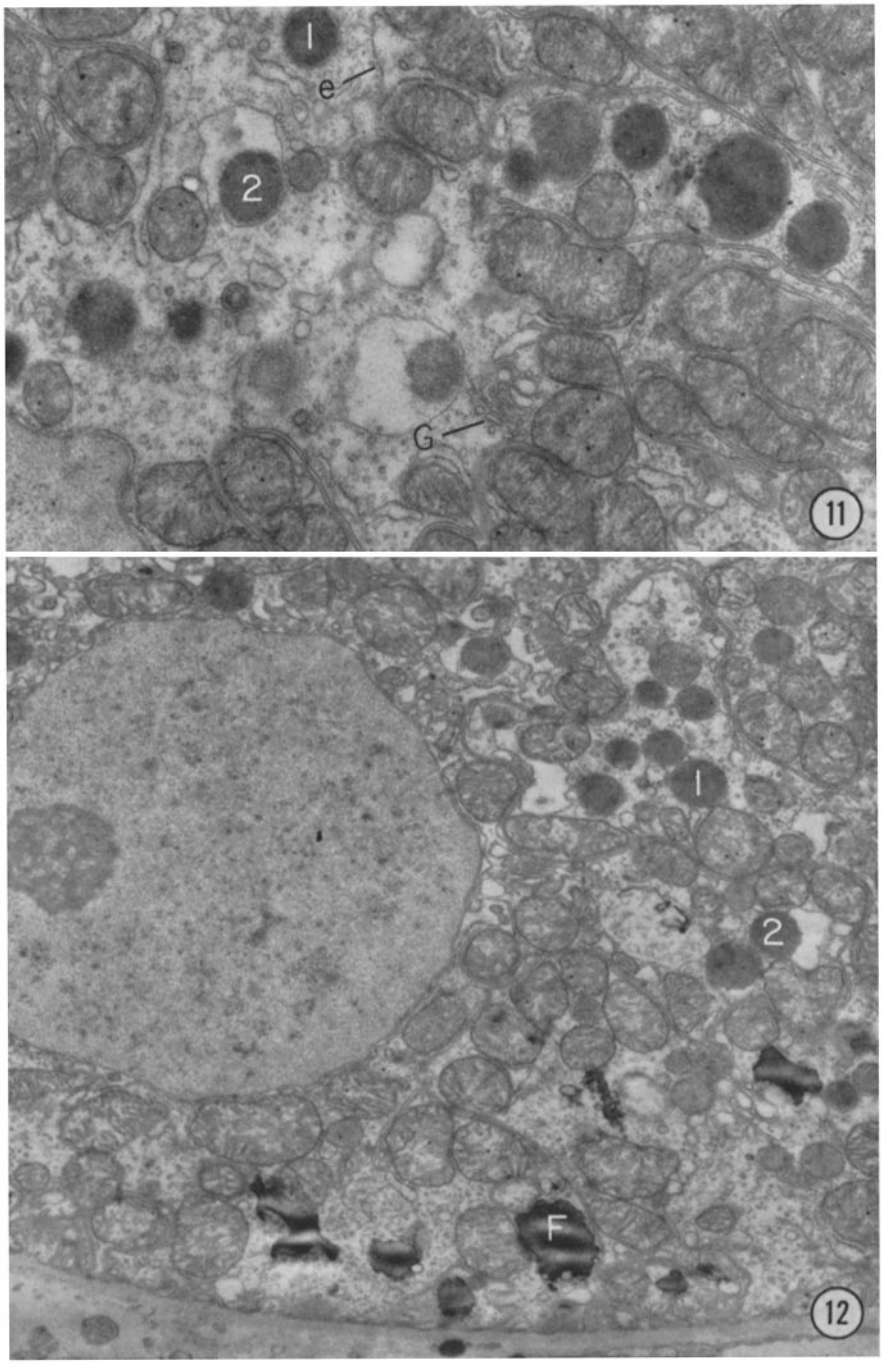


(Menefee *et al.*: Acute renal failure. II)

PLATE 121

FIG. 11. Proximal tubule 30 minutes after globin injection. Globin is seen within what appears to be sections of a system of channels. At *1* the membrane is closely applied to the globin and at *2* the globin aggregate is within a section of irregular outline and does not fill the enclosed space. The membranes enclosing globin do not have associated granules although other granular membranes are present within the cell (*e*). The Golgi apparatus (*G*) is seen in close relation to a part of the membrane system within which globin is enclosed. × 14,000.

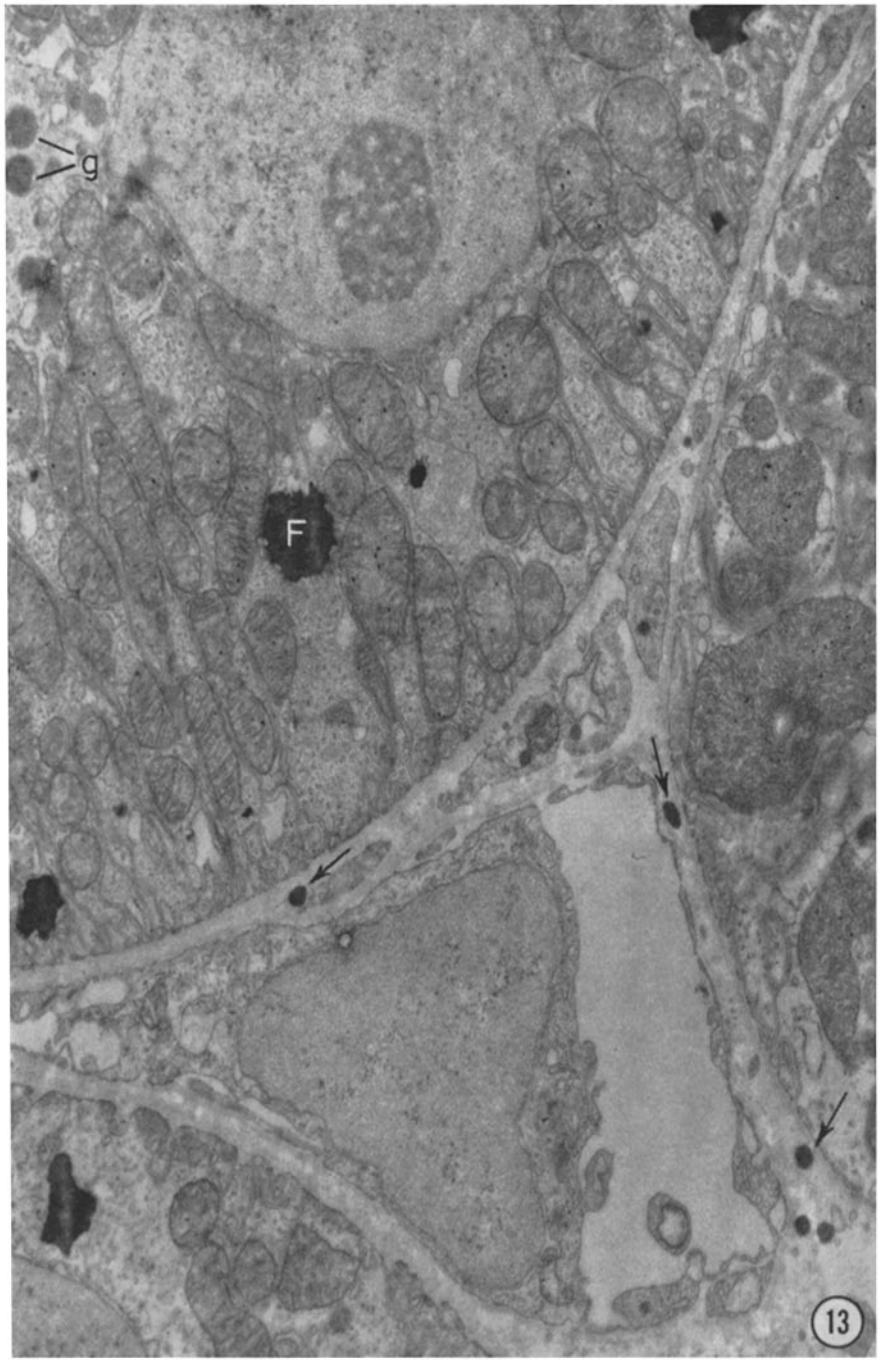
FIG. 12. Base of proximal tubule 30 minutes after globin injection. Globin within a closely applied membrane is seen at *1* and within an irregular membrane at *2*. The difference between the appearance of lipid (*F*) and globin in these preparations is apparent. The lipid is generally electron-opaque and tends to compress in cutting, whereas the globin is homogeneous, less electron-opaque, and does not compress as easily. × 9000.



(Menefee *et al.*: Acute renal failure. II)

PLATE 122

FIG. 13. Proximal tubules 30 minutes after globin injection. Globin is contained within the cells (*g*) and may be contrasted to lipid (*F*) in the same cell. Some globin is also seen within the tubular basement membranes (arrows). A capillary is in the lower center of the micrograph and it seems unlikely that the globin in the basement membrane at the left would have come from that capillary because of the intervening nucleus and fibroblast process. The globin does not accumulate in the extracellular areas. × 13,000.

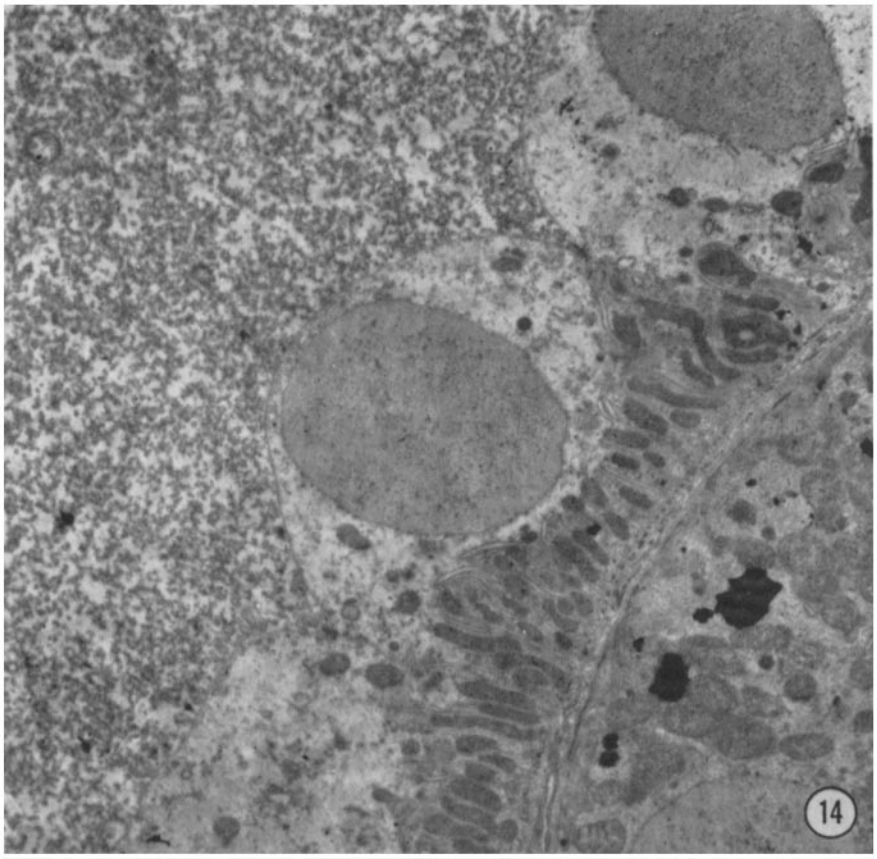


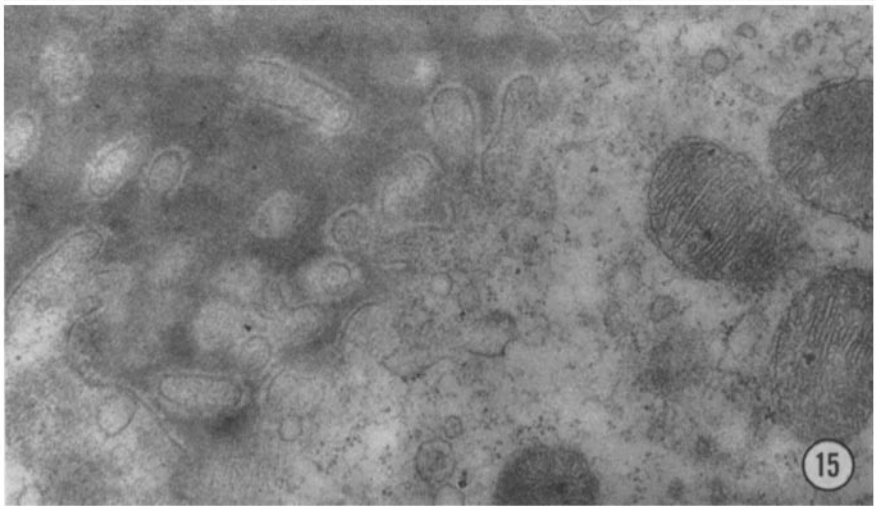
(Menefee *et al.*: Acute renal failure. II)

PLATE 123

FIG. 14. Distal tubule 5 minutes after injecting globin. The lumen is filled with precipitate, presumably globin. × 5000.

FIG. 15. Lumen side of distal tubule 10 minutes after injection of globin. The lumen is filled with globin. Even the small number of pinocytotic vesicles containing globin in this micrograph is unusual for the distal tubule. × 34,000.





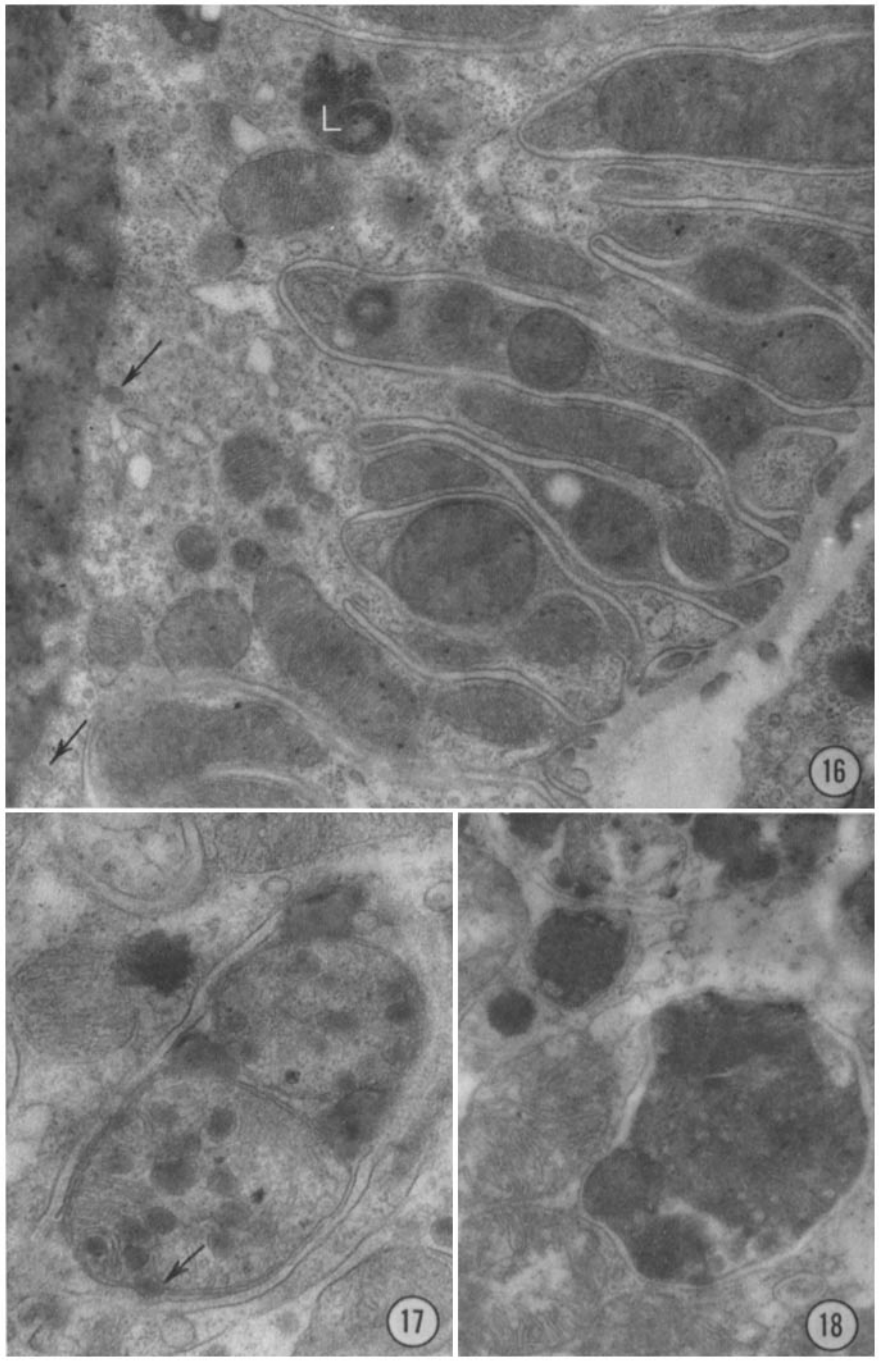
(Menefee *et al.*: Acute renal failure. II)

PLATE 124

FIG. 16. Distal tubule 25 minutes after globin injection. A few pinocytotic vesicles are present on the lumen side (arrows) and an occasional globin aggregate is seen within the cytoplasm. A lamellar body probably derived from a mitochondrion, is at *L.* × 22,000.

FIG. 17. Mitochondrion of a distal tubule cell 25 minutes after globin injection. It has taken up a considerable amount of globin. At one point (arrow) globin is seen between the outer and inner membranes. × 40,000.

FIG. 18. Distal tubule 1 hour after globin injection. Both normal and pathological mitochondria are present. Globin aggregates are seen both within and without mitochondria. × 22,000.

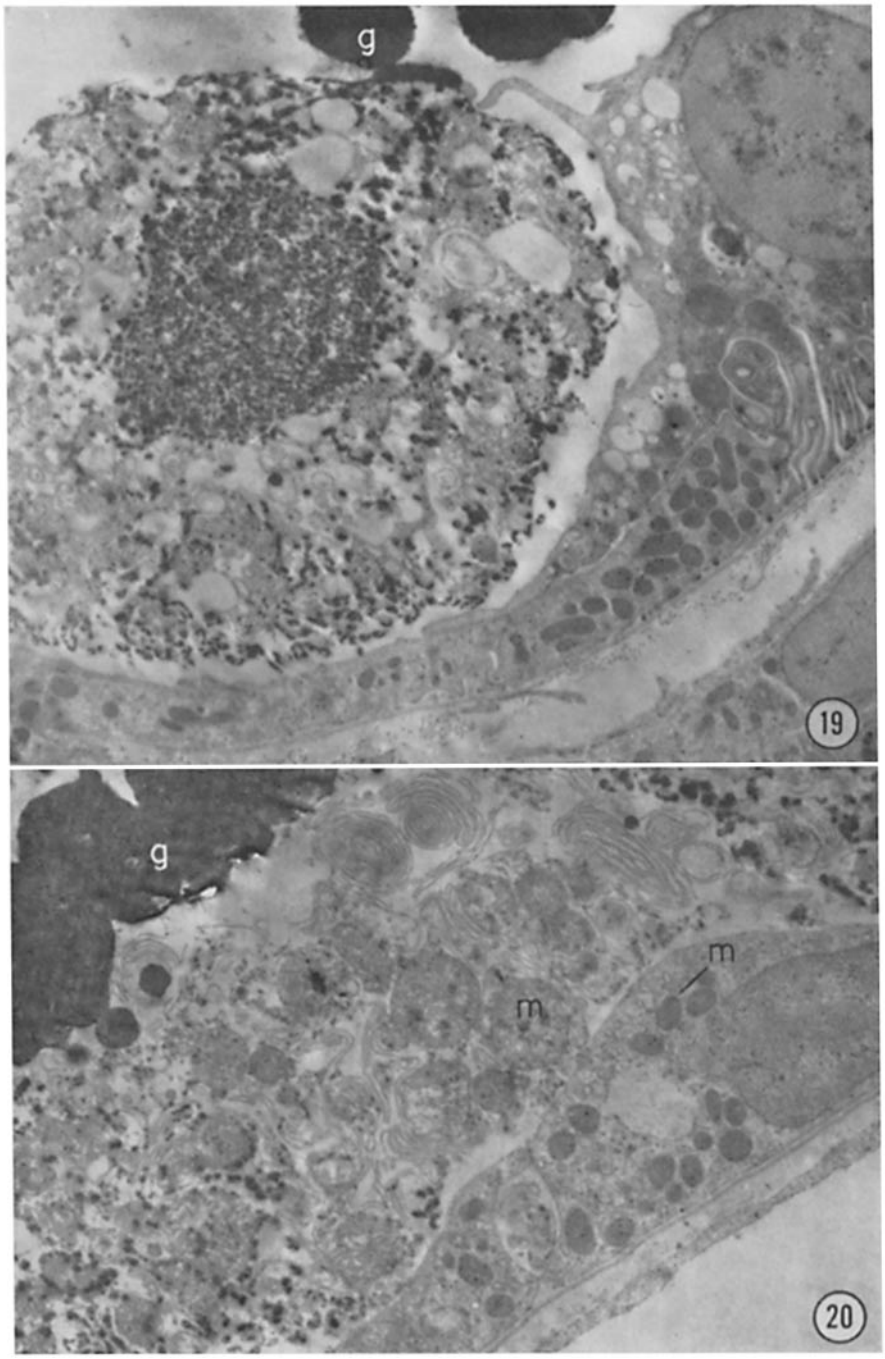


(Menefee *et al.*: Acute renal failure. II)

PLATE 125

FIG. 19. Thick loop of Henle 3 hours after globin injection. One cell has taken up a considerable quantity of globin and is degenerating. Large globin aggregates are in the lumen (*g*) but the intracellular globin is all in small clumps. The other cells of the tubule do not appear to be affected. × 8000.

FIG. 20. Distal tubule 3 hours after globin injection. Aggregated globin (*g*) is in the tubule lumen. Just outside the lumen is a layer of cells which are degenerating with many myelin figures, small clumps of globin, and degenerating mitochondria. Next to the basement membrane is a layer of cells without any contained globin and apparently intact. The mitochondria (*m*) of the basal cells are much smaller and denser than those of the degenerating layer of cells. × 6000.

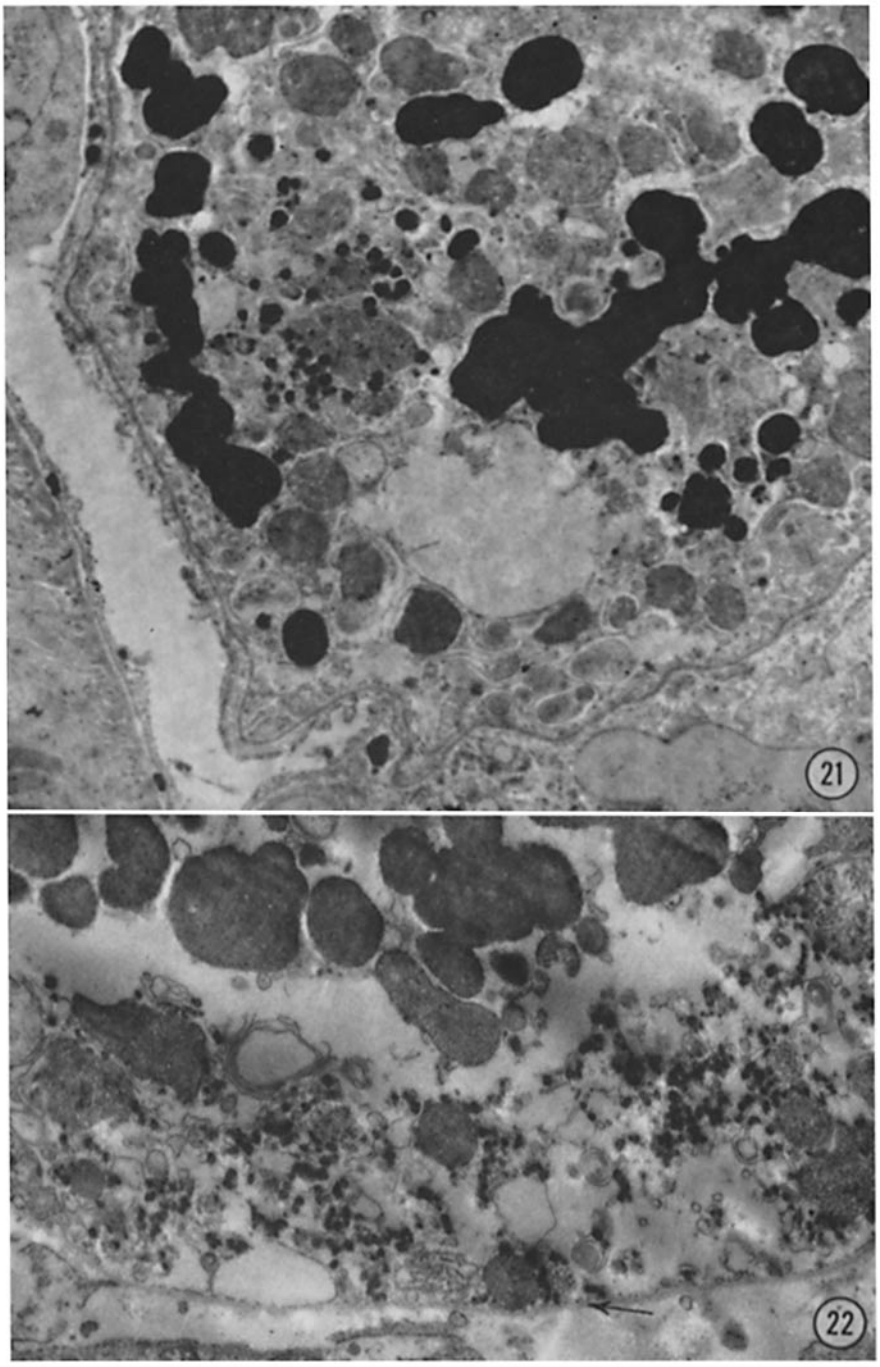


(Menefee *et al.*: Acute renal failure. II)

PLATE 126

FIG. 21. Distal tubule 1½ hours after globin injection. The intact basement membrane encloses a mass of degenerating cells and globin, although some remnant of cell structure is still present. × 6000.

FIG. 22. Distal tubule 6 hours after globin injection. The basement membrane (arrow) is still intact in many places but has begun to break up in others. The former tubule is now a mass of cell debris and globin. × 9000.

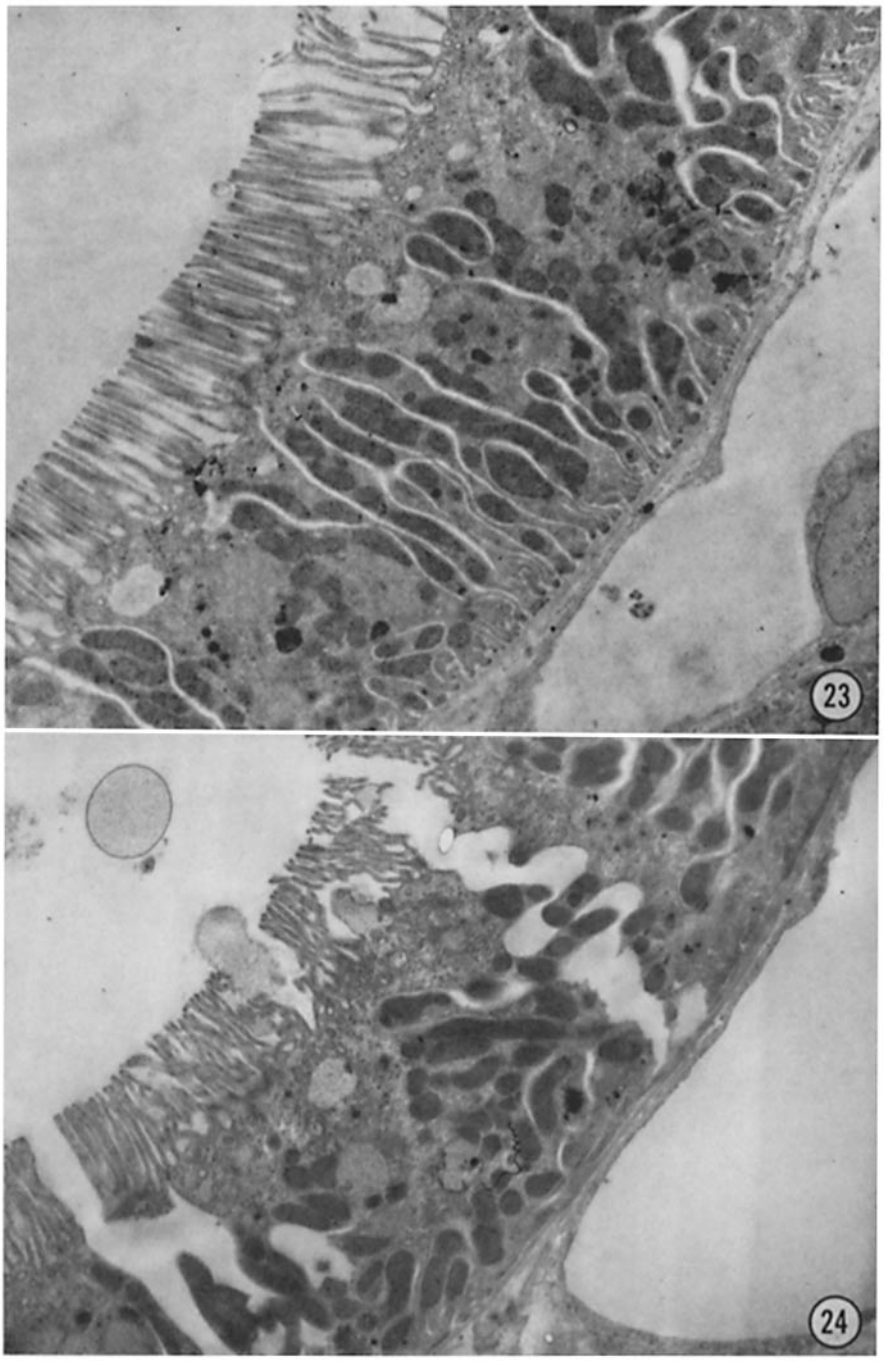


(Menefee *et al.*: Acute renal failure. II)

PLATE 127

FIG. 23. Proximal tubule 3 hours after globin injection. No globin remains in the lumen and very little is within the cells. × 6000.

FIG. 24. Proximal tubule 3 hours after globin injection. The cells are pulling apart and tearing. The points of attachment at the seal or at desmosomes do not pull apart but rather the cell tears somewhere near these. At other places the interdigitations are seen to simply slide apart. This is presumably because of increased intraluminal pressure caused by blockage in the lower nephron which would increase pressure in the proximal tubule. × 4000.



(Menefee *et al.*: Acute renal failure. II)