Experimental Ischemic Renal Arterial Necrosis with Resolution

Boyd E. Terry, MD, David B. Jones, MD, and C. Barber Mueller, MD

WHILE RENAL ARTERIAL NECROSIS has been observed in a variety of clinical states—ie, malignant nephrosclerosis,[1,2] periarteritis nodosa,[3,4] bilateral renal cortical necrosis and others,[5,6] the mechanisms of production are not entirely clear. Experimentally, arterial necrosis has been produced by bacterial toxins,[7] hypertension,[8–12] hypersensitivity,[13] and in a variety of other ways including acute ischemia,[14,15] which is the subject of the present investigation.

The present study was carried out as part of a project to compare the renal morphology and pathophysiology produced by renal ischemia with the changes in structure and function produced by acute tubular obstruction following intratubular precipitation of globin.[16,17] Focal arterial and arteriolar necrosis was a uniform development in the rat following 75 minutes of bilateral complete renal arterial occlusion. The development and healing of these necrotic arteries was studied by light and electron microscopy. A remarkable susceptibility of arterial smooth muscle to hypoxia is emphasized by this work. It is possible that this susceptibility to ischemia following occlusion or spasm may be of importance in the development of some forms of arterial necrosis not explained in other ways.

Methods

Seventy-five white rats of the Sprague-Dawley strain, weighing 200–250 g were used. Under intraperitoneal Nembutal 50 mg/kg anesthesia, the renal arteries were isolated through two dorsal paraspinal incisions without damage to other hilar structures. A 4–0 Merscelene loop was placed around each renal artery excluding the vein, and this loop was pulled into a flanged segment of PE-150 polyethylene tubing, thus occluding each artery. The occlusion was maintained for 75 min. Fol-

From the Department of Surgery, University of Missouri Medical Center, Columbia, the Department of Pathology, State University of New York, Upstate Medical Center, Syracuse, NY, the Department of Surgery, McMaster University, Hamilton, Ontario, Canada.

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Address for reprint requests: Dr. David B. Jones, Department of Pathology, State University of New York, Upstate Medical Center, 766 Irving Ave., Syracuse, NY 13210.

lowing release of the snare, direct observation confirmed prompt reestablishment of circulation. Animals were sacrificed at 5 min, at 4 hr, and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 14, and 24 days. A randomized serial sacrifice scheme was used to give maximal meaning to serial findings, since mortality associated with this model exceeded 50%. Control rats recieved a sham operation in which the same procedures were carried out, but without sustained arterial occlusion.

Ten additional groups of 6 rats each were prepared. Each group consisted of rats prepared in the following manner: (1) a sham operation, (2) a standard 75-min occlusion, (3) standard occlusion following fasting and water deprivation for 24 hr, (4) standard occlusion following fasting and water deprivation for 24 hr plus 0.6 cc of 50% mannitol intravenously prior to occlusion, (5) standard occlusion given 5% body weight water load by gastric tube, (6) standard occlusion given 5% body weight water load by gastric tube plus 100 mouse units of antidiuretic hormone intramuscularly. Since gross, microscopic, and electron microscopic morphology of the injured kidney was not modified by these treatments, the observations on the rats from these studies are considered with the standard preparations.

Animals dying spontaneously were promptly necropsied, organs were fixed in 10% formalin, and tissues were prepared for light microscopy. The kidneys of sacrificed animals were fixed in 4% glutaraldehyde in phosphate buffer at pH 7.3. Paraffin sections were stained by hematoxylin and eosin, periodic acid Shiff, Masson's trichrome, and Verhoeff elastic counterstained with Van Gieson stains. In 54 of the sacrificed animals glutaraldehyde-fixed kidney tissue was treated with 1% osmic acid and embedded in Epon 812.[18] From numerous micron sections stained with an alkaline thionin, toluidine blue, methylene blue stain mixture areas were chosen for thin sectioning for electron microscopy. Sections were cut by glass or diamond knives, mounted on bare copper grids, stained with uranyl acetate and lead citrate,[19] and examined under an RCA EMU3G electron microscope.

Observations

Gross Changes

With application of the renal arterial snares the kidney became pale and lost turgor. After release of the occlusion, prompt recirculation developed and the kidneys became deep red and swollen in a few seconds. The appearance at sacrifice depended on the duration of the experiment. Within the first three days the congested, swollen appearance persisted. Occasional rats showed focal, pale, infarcted zones by

the third day. By the seventh day, the kidneys had resumed a generally normal size and appearance, but focal or occasionally massive infarction was seen.

Light and Electron Microscopic Changes—Summary of Tubular Changes

Four hours after release of the snares the tubules showed marked cloudy swelling. The electron microscope revealed widespread swelling of mitochondria, nuclear chromatin clumping, ballooning of nuclear envelope, and other changes seen in badly damaged and dying cells. By 24 hr the majority of the tubules, both proximal and distal, showed acidophilic necrotic changes with sloughing of tubular cells into the lumen. The juxtamedullary region seemed often to be more severely involved than other portions of the kidney. In 3 days mitoses were frequently seen in surviving tubular cells, and in 5 days these cells had generally repopulated the tubular lumina. Extensive dystrophic calcification of the necrotic tubular cells was frequently seen. By 7 days the tubules were returning to a pattern approaching the normal, although foci of atrophic tubules were commonly present. In some cases, one kidney was severely atrophic while the other had regenerated to a near-normal morphology. The changes up to 25 days were those of progressive return to a near-normal structure in most tubules with islands of atrophic tubules remaining.

Arterial Lesions

Necrotic arterial lesions were encountered in all kidneys examined from 4 to 96 hr following recirculation. These necrotic lesions involved short segments of arteries and were present in 10–40% of arteries in a given microscopic section. Within 4 hr following the reestablishment of arterial flow, necrosis could be seen in the smooth muscle cells in patchy areas in the renal arteries, arcuate arteries, interlobular arteries, and in afferent arterioles that developed acidophilic necrosis. The arcuate and interlobular arteries appeared to be the most susceptible. In addition to the necrosis seen in smooth muscle, extensive diapedesis of red cells into the necrotic media developed, but only slight adventitial diapedesis occurred (Fig 1, 2, 4). Fibrin deposits formed in the necrotic media. Fibrin was identified by characteristic staining with Masson's trichrome stain and by the characteristic morphology and periodicity under the electron microscope. Even in the areas of severe necrosis, islands of individual surviving smooth cells were characteristically seen. At 4 hr the endothelium was generally intact, but small areas of denuded intima were often seen (Fig 2).

At 24 hr the endothelium was generally intact, and by 48 hr the endothelium showed a partial return toward normal appearance (Fig 4). As early as 24 hr the surviving smooth muscle cells showed projections of swollen processes into the necrotic debris of the media.

Mitoses were seen in smooth muscle cells by 72 hr (Fig 3, 5). The surviving muscle cells usually showed swollen cytoplasm, poorly formed myofibrils and phagosomes containing osmophilic debris. The evidence of the proliferative response of the smooth muscle cells was manifest not only by mitoses, but also by nuclear enlargement, bizarre nuclear forms and binucleation.

The number of necrotic arterial lesions rapidly decreased through the fourth and fifth day (Fig 6) so that they were rarely seen by the sixth day. The exception to this was the occasional larger lesions seen in the renal artery. Some of these lesions were still unhealed by the eighth day. Occasionally tearing of the elastica interna of larger arteries occurred with accumulation of fibrin in large amounts in the media at this point (Fig 7).

As surviving smooth muscle cells proliferated and migrated they displaced the necrotic debris and repopulated the connective tissue skeleton of the media. Even in the later stages of healing, remnants of necrotic osmophilic debris were seen between regenerating smooth muscle cells.

The regeneration of smooth muscle cells appeared to be mildly excessive: The walls of the arteries were thicker and contained more smooth muscle cells than did control arteries. These cells were irregular in shape, orientation, and size. There was often an apparent moderate increase in intercellular connective matrix. A few lymphocytes, histiocytes, and fibroblasts accumulated in the adventitia of many of the injured arteries. These cells first became apparent by the fourth day.

The regeneration of the arteries proceeded to a progressively more normal morphology. Although they showed a somewhat disorganized media (Fig 8, 9) by ninth day it was difficult to distinguish the injured arteries from control arteries. Some arteries at the twenty-fourth day showed apparent increase in connective tissue matrix between smooth muscle cells (Fig 10).

Glomerular Changes

During the acute phase of injury the glomeruli showed a pink-staining, protein-rich fluid in Bowman's space. By 48 hr this pink fluid had disappeared. Except for glomeruli located in or adjacent to areas of total infarction, the only glomerular change was an apparent thickening

of basement membranes as seen with electron microscopy, in some glomeruli by 25 days (Fig 11).

Discussion

Renal artery necrosis with diapedesis of red cells has been repeatedly seen in human malignant hypertension,[1,2] and periarteritis nodosa,[3,4] and has been produced experimentally by hypertension,[8,9,11,12] vasopressin,[10] hypersensitive necrosis,[13] mechanical distension,[20] and temporary renal vascular occlusion. While the mechanisms for the production of the arterial necrosis and diapedesis of red cells seem understandable in the hypersensitivity diseases and perhaps in the extreme hyperdistension of Byrom and Dodson,[20] it is not entirely clear why hypertension will so commonly produce these changes. Sheehan and Davis[14,15] in a series of studies of temporary unilateral occlusion of the renal pedicles of rabbits for 1–3 hr, were able to regularly produce medial necrosis, and medial arterial diapedesis of arcuate and interlobular arteries and arterioles. They found that larger arteries appeared to be spared in the rabbit so treated. In their experiments 90 min of occlusion produced occasional necrotic arterial lesions, but only with 2 hr of ischemia did the lesion become common. These vascular lesions healed with medial regeneration and intimal proliferation with narrowing of the lumen. In the experiments we have described in the rat, we noted certain differences: the frequent involvement of major branches of the renal artery; almost complete regeneration by the seventh day post-occlusion; the absence of endothelial or intimal proliferation in arteries; and remarkable tubular regeneration to a near-normal morphology in many of the rats. These differences may best be explained by species differences, shorter periods of ischemia, and bilaterality of the ischemia in the present experiments.

It is particularly interesting that we can uniformly produce arterial vascular lesions in a bilateral injury with such remarkable resolution but of so mild a degree that excellent tubular regeneration can occur.

We were impressed by a considerable resemblance of these hypoxic arterial lesions to those seen in arteries of experimental malignant hypertension.[11,12] In each case, smooth muscle necrosis and diapedesis of red cells is seen, with a relatively intact endothelium. In both hypertensive and hypoxic lesions fibrin may be identified amid the necrotic debris. It is of interest that in spite of ischemic injury and patchy desquamation of endothelium in the first 24 hr, thrombosis of the arteries did not usually occur. We feel that the present experiments

support the hypothesis that ischemia may be important as a cause of arterial necrosis in malignant hypertension.

Summary

White rats, having bilateral renal arterial occlusion for 75 min, uniformly developed in 4–24 hr focal arterial necrosis with deposition of fibrin and diapedesis of red cells into the arterial media. The endothelium survived, although damaged, and thrombosis was not seen. Mitoses appeared in surviving smooth muscle cells by 72 hr. By proliferation and migration the smooth muscle cells repopulated the damaged areas so that a near-normal appearance was attained by the ninth day. It is suggested that this susceptibility of smooth muscle to hypoxia may be an important cause of arterial necrosis in diseases such as malignant hypertension.

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[Illustrations follow]

Legends for Figures

Fig 1. *(upper left)* Arcuate artery seen 4 hr post ischemia shows smooth muscle necrosis, diapedesis of red cells into media and persistent endothelium. H & E. × 384.

Fig 2. *(bottom)* Low power electron micrograph at 4 hr after ischemia shows necrosis with occasional surviving muscle cells (*m*) in an arcuate artery which is giving off a branch. Surviving but damaged endothelium may be seen internal to the elastica (*e*). Extravasated red cells (*r*) can be seen in media and adventitia. Fibrin (*f*) may be seen in media. × 1500.

Fig 3. *(upper right)* Interlobular artery at 72 hr post ischemia shows disorganized structure and mitotic figure. H & E. × 640.

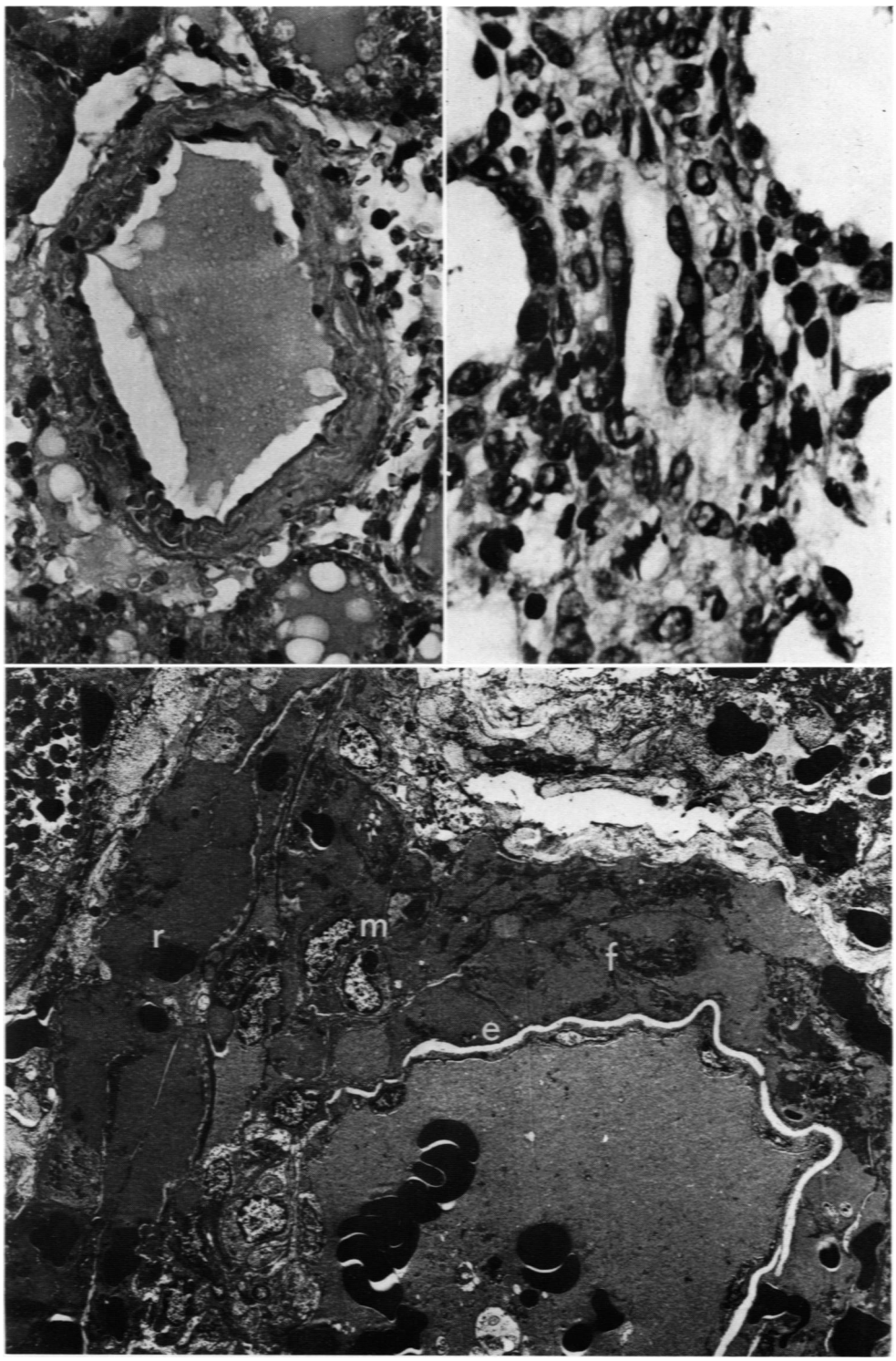
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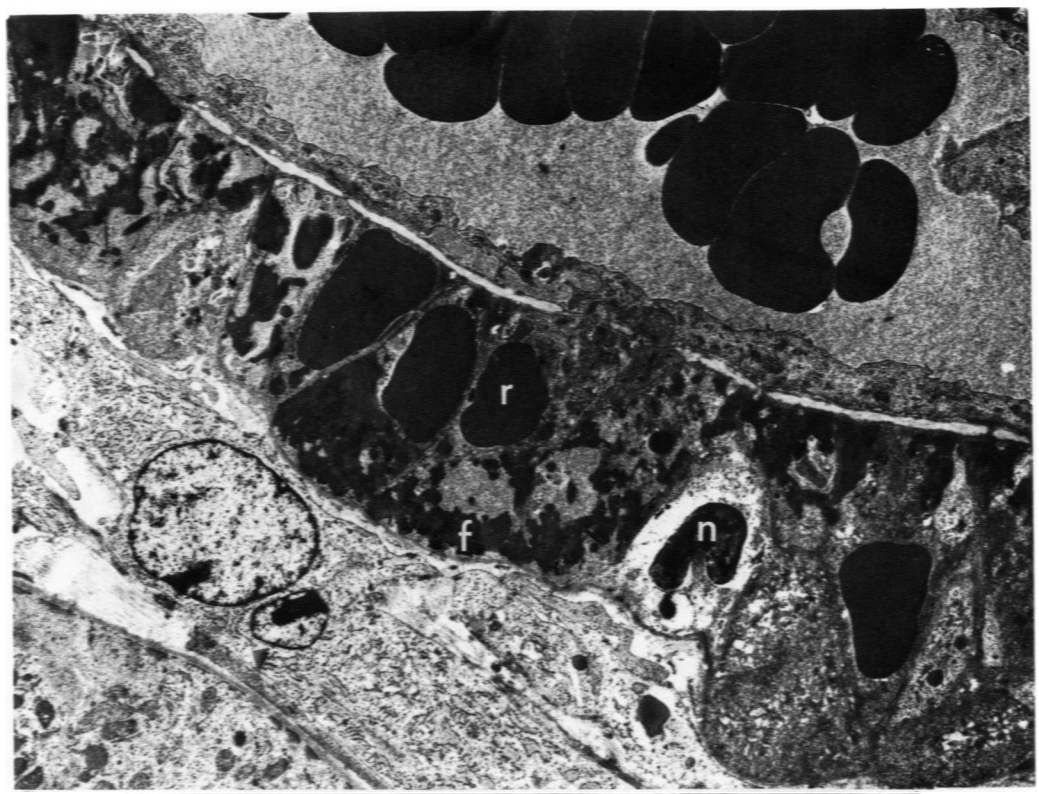
Fig 4. *(upper)* Interlobular artery at 48 hr still shows necrosis of media with fibrin *(f)*. extravasated red cells *(r)*, a rare neutrophile *(n)*, and intact but abnormal endothelium. × 5000.

Fig 5. *(lower)* Media of interlobular artery 72 hr post ischemia shows smooth muscle in mitosis amid necrotic debris *(d)*. Note pseudopodial extensions of the cytoplasm into debris. × 3400.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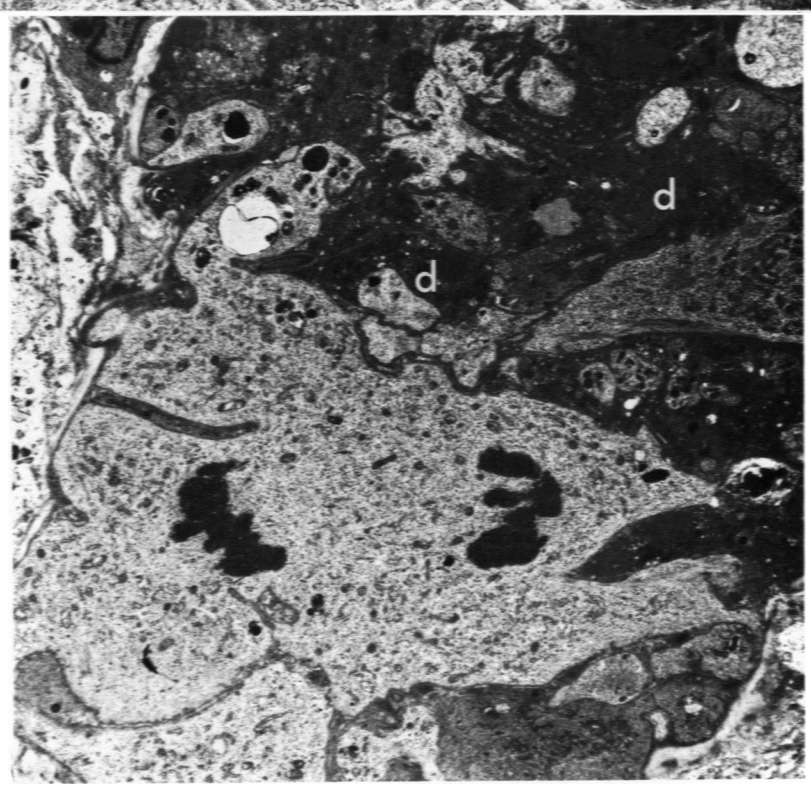
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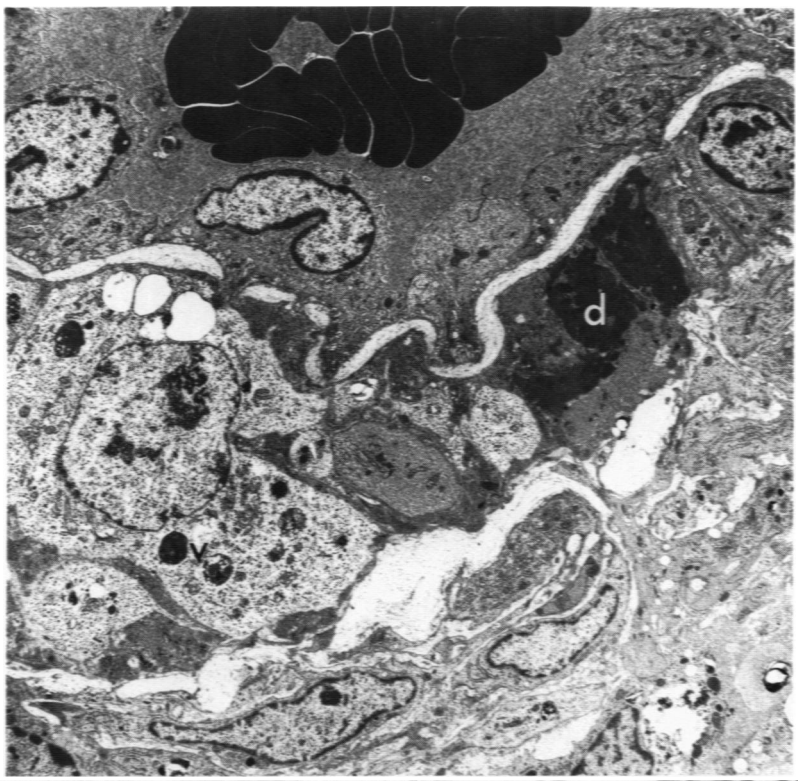
Fig 6. *(upper)* Arcuate artery 5 days post ischemia shows residual necrotic debris *(d)*, bizarre regenerating smooth muscle cells containing various lysosomal vesicles (v) and perivascular collar connective tissue cells. × 3400.

Fig 7. *(lower left)* Arcuate artery at 5 days shows uncommon finding of rupture of internal elastica in zone of necrosis with extravasation of fibrin into necrotic media. Verhoeff's elastic stain. × 96.

Fig 8. *(lower right)* Arcuate artery at 8 days post ischemia shows the somewhat disorganized cellular media of regenerating artery. H & E. × 200.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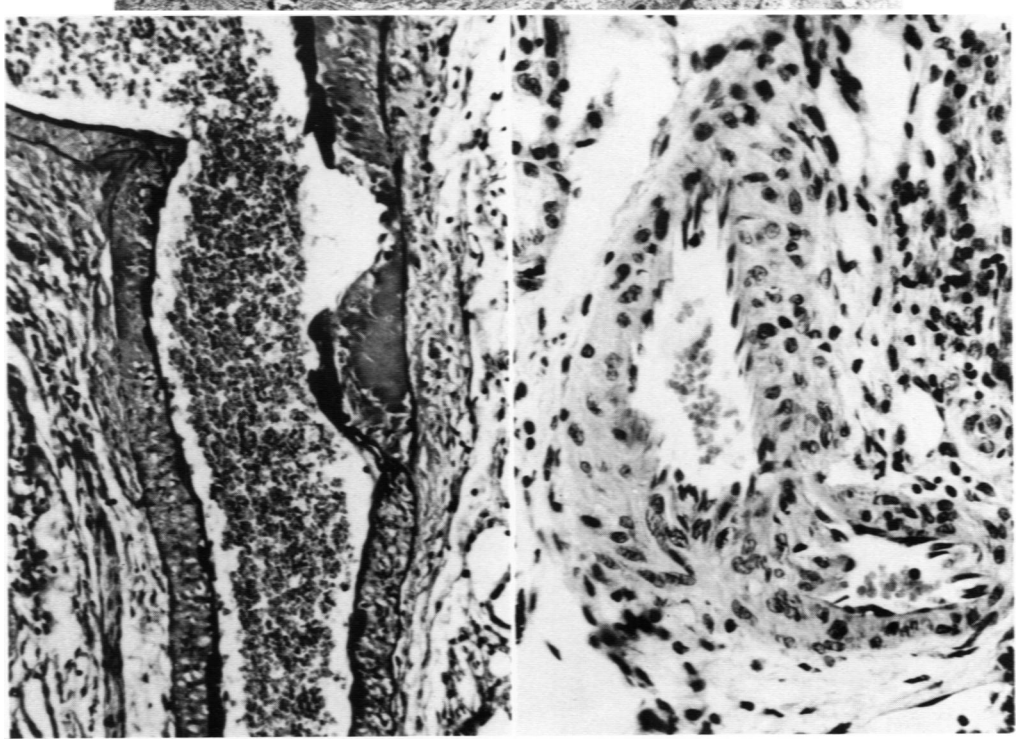
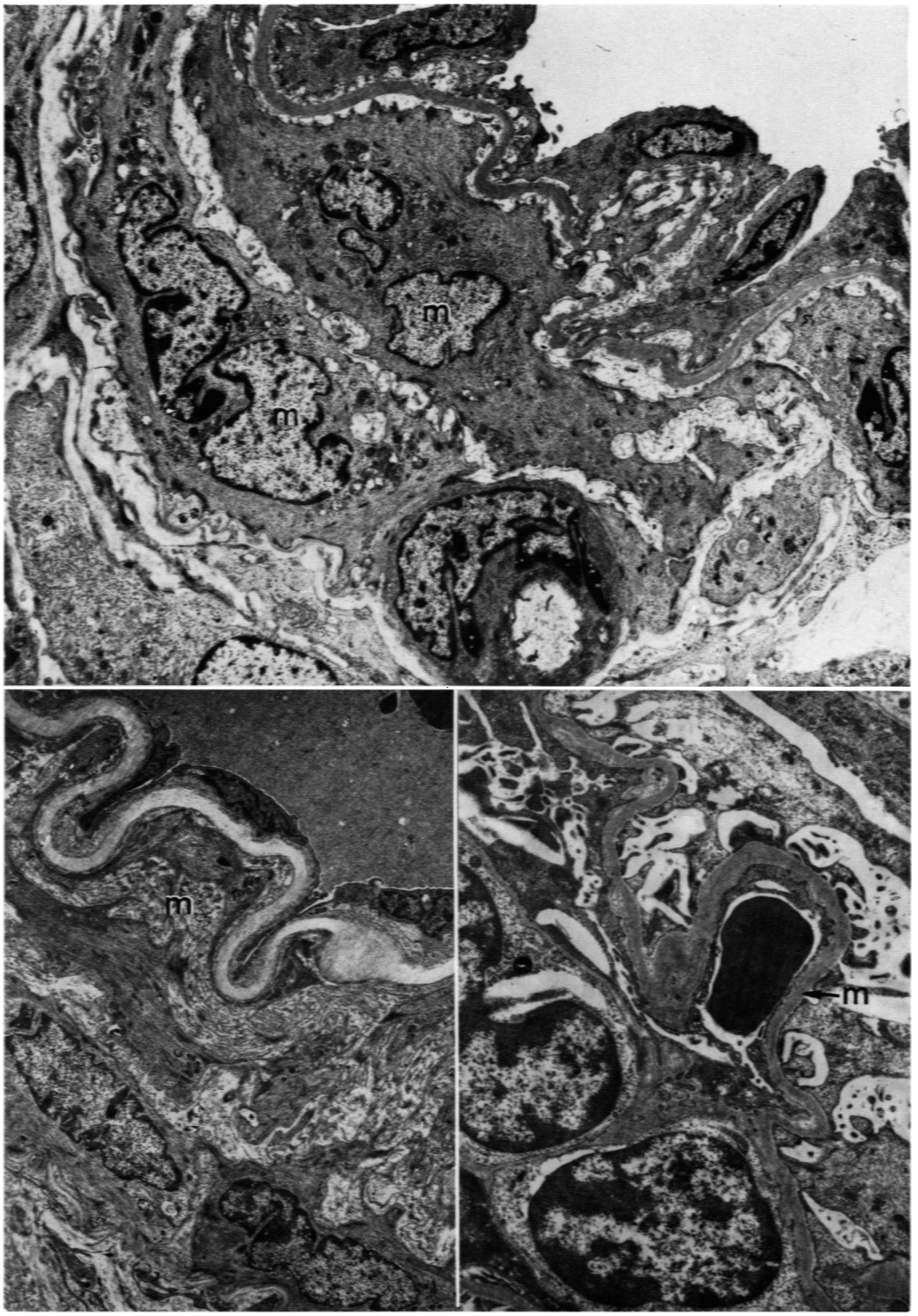


Fig 9. *(upper)* Low power electron micrograph shows large, somewhat bizarre smooth muscle cells *(m)* in a healed artery at 8 days. × 5000.

Fig 10. *(lower left)* At 24 days there appeared to be moderate increase in connective tissue matrix *(m)* between smooth muscle cells. × 3400.

Fig 11. *(lower right)* Some glomeruli at 24 days post ischemia showed moderate basement membrane *(m)* thickening. × 3400.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