Renal Parenchymal Tolerance to Artery Occlusion:

A Time and Damage Study in Rats Developing Collateral Circulation

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ACUTE RENAL ARTERY OCCLUSION occurs in man from trauma and embolization. In order to evaluate the role of surgical intervention in these cases it is important to define the contribution of collateral blood vessels to renal blood flow. This study will describe observations of collateral blood flow, both immediate and long-term in the rat following acute renal artery occlusion, as demonstrated by microangiography. It will, in addition, describe the accompanying histologic changes in the kidney.

Methods and Materials

Sprague-Dawley 6-week-old female rats were fed a standard laboratory diet, supplemented by antibiotics for 3 days following operation. Young rats were chosen to take advantage of the stimulatory effect of growth hormone upon collateralization.[9]

Group I. In 45 rats the left renal artery was divided, followed by Micropaque perfusion studies at intervals varying from 1 hour to 97 days, to demonstrate patterns of collateral blood flow to the kidney.

Group II. In 10 rats the left renal artery was divided, and the right kidney was simultaneously removed, to determine if pre-existing collaterals were sufficient to sustain life.

Group III. In six animals the left renal artery was temporarily ligated for intervals of 20 minutes to 4 hours, after which the ligature was released, and flow to the kidney reestablished for 10 minutes, to demonstrate the earliest or most sensitive site of ischemic injury. Micropaque studies were then performed.

Microangiographic Technic

Micropaque is a finely divided barium sulfate suspension of 4 to 10 microns particle size.[10] Selective regional perfusion was performed by injecting micropaque into the aorta via a polyethylene catheter just below the renal arteries with the aorta ligated above the celiac axis and below the level of insertion of the catheter. 200 units of USP Heparin were injected prior to perfusion to prevent thrombus formation. A 15% Micropaque and saline suspension was perfused at a pressure of 100 mm. Hg for 20 minutes; pressure was kept constant with a mercury release bath. The inferior vena cava was partially divided to provide an egress for the perfusate. Animals were then prepared for histologic examination by immersion in 10% formalin for 24 hours; perfusion was continued at 3 feet of gravity pressure. Radiographs of the specimens were then made using a Faxitron 805 x-ray unit with a Beryllium window and a 0.5 mm. focal spot. The factors for radiography were 30 KV, 3 mA, and 4 minutes. One mm paraffin imbedded sections of the left kidney were made and further radiographs taken at factors of 30 KV, 3 mA and 1 minute. Stereoscopic pair x-rays were analyzed in a Stereorealist viewer providing 8 to 10 times magnification. Further radiographs of selected specimens were taken using high resolution plates pro-

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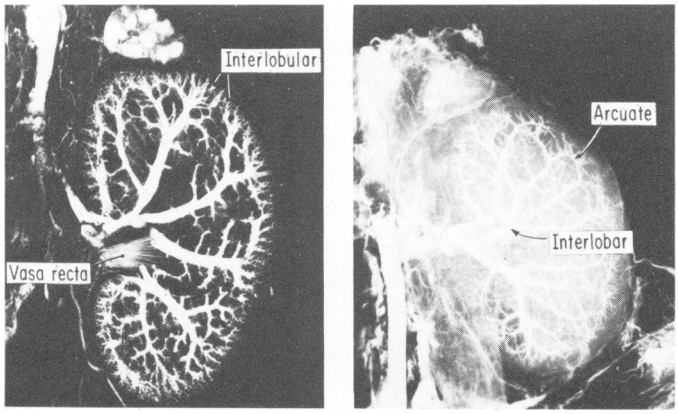


FIG. 1a (left). Micropaque perfusion of normal rat kidney (1 mm section) showing interlobar, interlobular and arcuate vessels. Glomeruli are well filled but can not be appreciated at this magnification. Vasa recta are well filled. 1b (right). Micropaque perfusion of whole rat kidney 2 hours after division of the renal artery. Micropaque fills the arcuate vessels and a few interlobular vessels.

viding magnification capabilities of 300 ×. Representative histologic sections were stained with hematoxalin and eosin.

Results

Group I. Division of Main Renal Artery

a) 1-2 hours. Micropaque perfusion filled the major interlobar, arcuate, interlobular arteries, and a few afferent arterioles, showing that pre-existing collaterals are present and patent immediately when the main renal artery is occluded. However, histologic examination showed early degeneration of tubules, and nonfilling of glomeruli and vasa recta. Figure 1a shows perfusion of a normal rat kidney; Figure 1b shows a rat kidney perfused 2 hours after ligation of its renal artery.

b) 3–4 hours. Micropaque filling stopped at the arcuate vessels. Histology showed further tubular necrosis and early glomerular damage (Fig. 2).

c) 7–24 hours. Micropaque perfusion showed nonfilling of the intrarenal vessels. The preexisting collaterals noted at 1 to 4 hours became dilated and tortuous. Histology showed progressive damage of cortex and medulla, except for the cortex nearest to the hilum of the kidney. Thus, although collaterals are present, they can not sustain the kidney; there is stasis in the vessels, followed by thrombosis, and then renal necrosis. Figure 3a & b show the microvascular anatomy and histology of a kidney from this group.

d) 5–12 days. Micropaque perfusion showed filling of interlobar vessels but not their branches; collaterals increased progressively in size, number and tortuosity. Histology showed progressive renal destruction except for the parahilar cortex (Fig. 4a & b).

e) 16–97 days. The kidney became progressively calcified, decreasing to one third of its original size. Micropaque perfusion showed continued filling of interlobar arteries, but nothing beyond, *i.e.*, no filling of arcuate, interlobular or glomerular arteries. Collat-

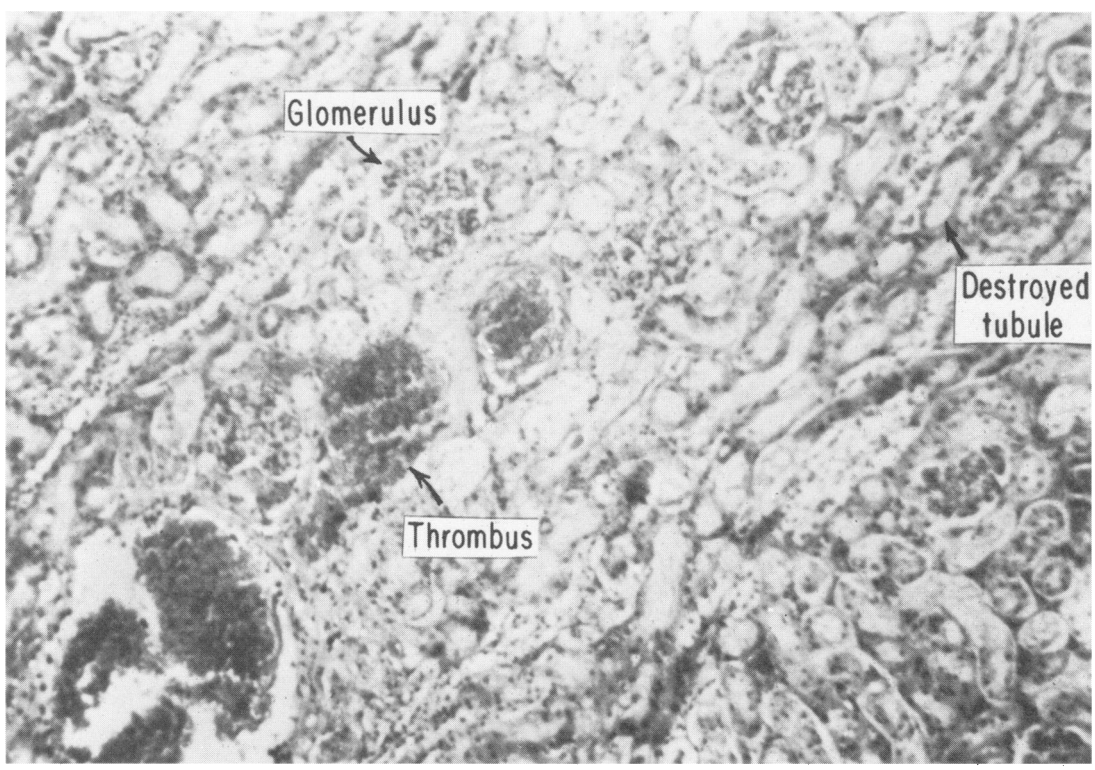


FIG. 2. 4 hours after division of the renal artery the cortex shows stasis, tubular destruction & non filling of the glomeruli with micropaque (100 ×)

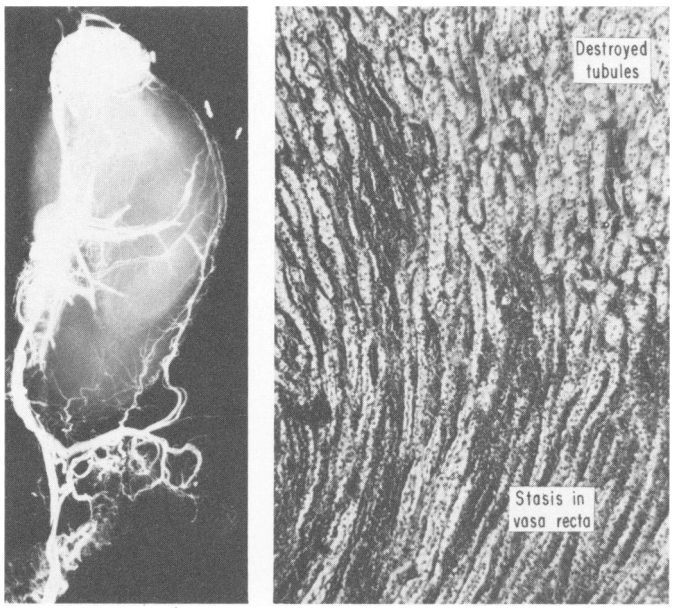


FIG. 3a (left). Micropaque perfusion of the rat kidney 24 hours after division. There is essentially no filling beyond the interlobar arteries. The collaterals are dilated and tortuous. 3b (right). Histologic sections show stasis in the vasa recta and tubular cellular destruction.

erals further increased in number, size, and tortuosity, but were ineffective to preserve the kidney (Fig. 5a & b).

Group II. Division of the Left Renal Artery and Right Nephrectomy

All of these rats died within 48 hours, proving that collaterals can not support a kidney after acute ligation of its main renal artery.

Group III. Temporary Occlusion

a) When the artery was occluded for less than 90 minutes, followed by 10 minutes of arterial reflow, micropaque perfusion showed filling of the glomeruli and vasa recta. Histology, however, showed tubular cellular damage as early as 40 minutes (Fig. 6).

b) When the artery was occluded for more than 90 minutes, followed by 10 minutes of reflow, micropaque did not fill vessels beyond the interlobular arteries. Histology showed stasis in the vasa recta and more tubular cellular damage. The glomeruli filled poorly except in the parahilar cortex (Fig. 7a & b).

Discussion

Collaterals to the kidney, come from six major sources[1, 4,5,7,8,11] (Fig. 8): 1) *hilar,* from the aorta across the stump of the divided renal artery; 2) *adrenal,* to the superior capsule and distal renal artery; 3) *ovarian,* to the inferior capsule and the distal renal stump; 4) *ureteric,* to the inferior capsule and to the distal renal artery stump; 5) *lumbar,* to the posterior capsule and parahilar area and; 6) *inferior phrenic,* to the posterior and lateral capsule. In these experiments, the ureteric and ovarian collaterals showed the most striking enlargement with some vessels becoming nearly as large as the original renal artery.

The micropaque studies showed three phases of collateral revascularization: first, there is immediate filling via pre-existing vessels; second, there occurs dilatation and elongation of pre-existing vessels; finally, there is formation of new vessels. Perfusion studies at 1 to 4 hours after renal artery division fill the kidney but the collaterals are essentially no larger than seen on perfusion studies of a normal kideny. At 16–24 hours the pre-existing collaterals dilate, elongate, and become more tortuous when compared with the normal collateral anatomy. Neovascularization, as seen as a network of small, tortuous vessels at the hilum and around the capsule of the kidney, began between 1 and

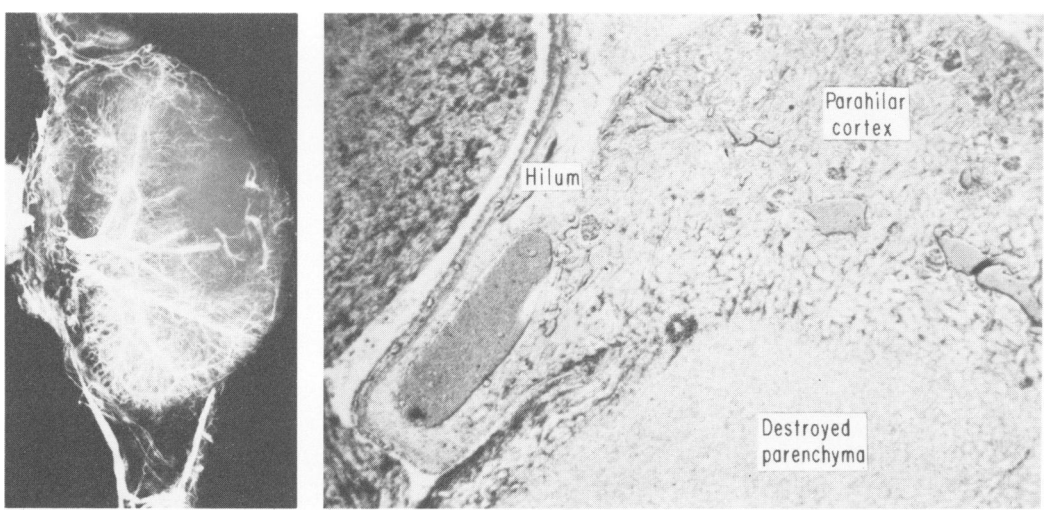


FIG. 4a (left). Micropaque perfusion at 5 days shows neovascularization of the collaterals and "pruning" of the interlobar vessels. 4b (right). Histologic sections demonstrate relative sparing of the parahilar cortex.

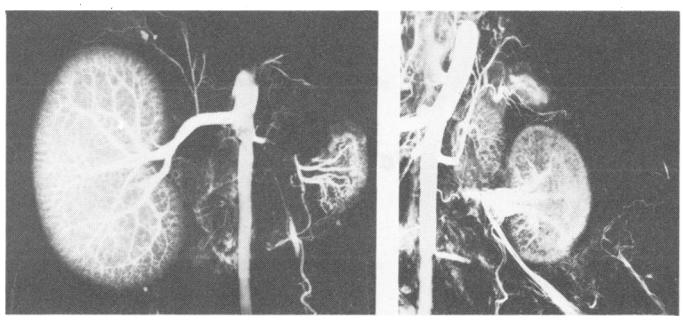
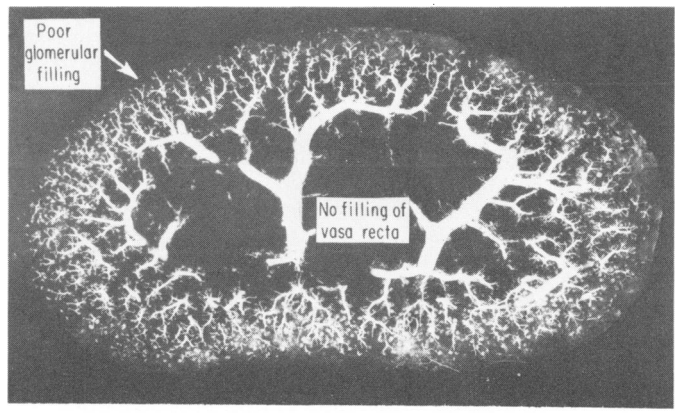


FIG. 5a (left). The left kidney at 97 days after division of the renal artery is markedly atrophic & calcified. The interlobar arteries fill well. 5b (right). Large collaterals develop around the ureter and from the ovary.



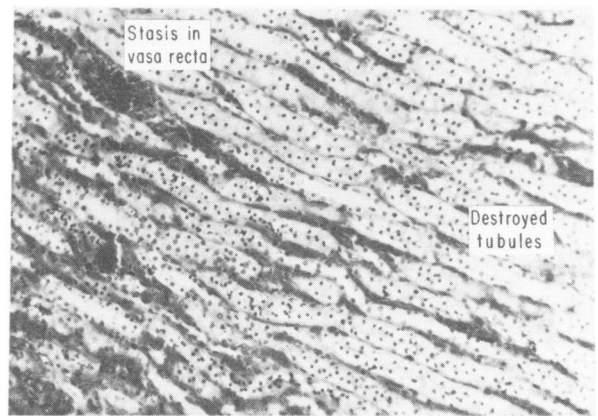


FIG. 7a (top). After 4 hours of temporary occlusion micropaque perfusion (1 mm section) shows very poor glomerular filling and non-filling of the vasa recta. 7b (bottom). The medullary area shows destroyed tubules and stasis in the vasa recta.

5 days and progressively increased to the end of the experiment, 97 days. Weibel[12] demonstrated by electron microscopy that neovascularization occurs in the rat lungs at 5 days after division of the main pulmonary artery. The delay in the development of these new vessels suggests that there may be a humoral mechanism involved similar to that which has been demonstrated by Folkman *et al.*,[16] to be responsible for the development of tumor vessels.

Immediate injury, as demonstrated by reflow studies, appears to be reflex, with shutdown of the efferent arterioles and vasa recta. This was pointed out by Diethelm and Wilson,[3] using carbon particle injections, and confirmed by the present micropaque studies. Heparin failed to reduce the damage, indicating that thrombosis was probably not important in this post glomerular obstruction of early renal ischemia.[3] Constriction of the vasa recta and efferent arterioles produces back pressure and stasis,[2] but flow from the collaterals is never sufficient to reopen the efferent arterioles; therefore, thrombosis occurs. The thrombus eventually un-

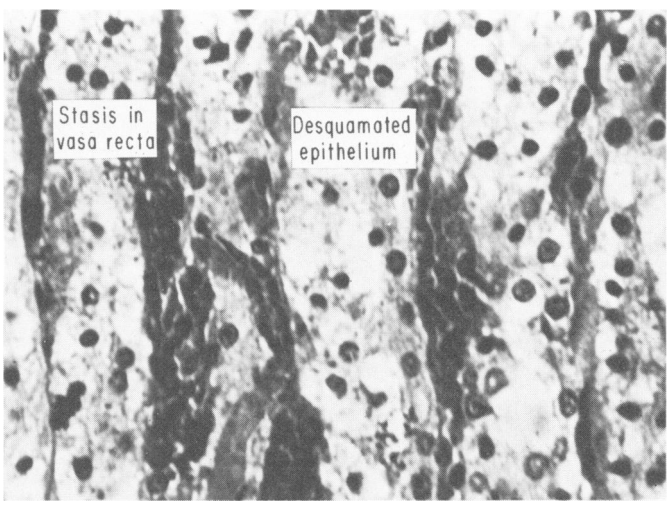


FIG. 6. After 40 minutes of temporary artery occlusion the medullary area shows widespread stasis in the vasa recta and desquamation of tubular epithelium (400 ×)

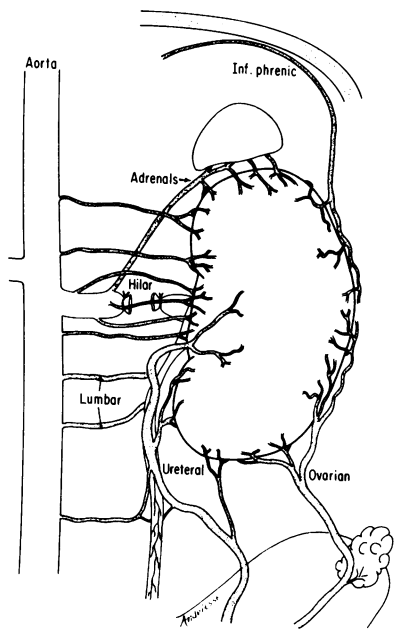


FIG. 8. Diagram of major collaterals to the rat kidney, after 30 days. Many of these major collaterals are often seen to go directly to the distal renal artery stump.

dergoes lysis leading to collateral refilling of the interlobar vessels, as noted in the later phases of the study.

Preservation of the parahilar cortex was observed from the first hour to the 97th day. As the rest of the kidney progressively atrophied, this area appeared viable, probably related to the concentration of collaterals around the hilum. There were no associated collecting tubules, however, and so, this small area of cortical preservation would likely not represent preservation of renal function.

Summary

Collaterals, consisting of pre-existing vessels and new vessels, contribute significantly to the blood supply of the ischemic rat kidney. They appear to spare preferentially the parahilar area of the kidney, but can not protect the kidney from necrosis when the renal artery is acutely divided. These studies suggest that salvage of a kidney after acute, complete renal artery occlusion or interruption must depend on re-establishment of its main arterial flow in a very short time, possibly within 90 minutes.

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