The Effect of Clonidine on Tubular Obstruction in Postischemic Acute Renal Failure in the Rabbit Demonstrated by Microradiography and Microdissection

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Acute renal failure was produced in vasopressin-pretreated rabbits by clamping the left renal pedicle for one hour and removing the opposite kidney. Treatment with clonidine, an antihypertensive drug that blunts the kidney's response to vasopressin, resulted in significantly higher creatinine clearance and urine flow rate in the first 6 hours after unclamping. Clonidine (30 μg/kg given intravenously 30 minutes before unclamping) also significantly lessened the number of hyaline casts in outer medullary tubules and inner medullary loops of Henle 6 hours after unclamping and reduced the number of abnormal tubular contours in microradiograms produced by infusing barium sulfate into the renal artery at sufficient pressure to rupture glomerular capillaries, causing an escape of contrast material into the tubules. The spaces consistently observed between the ends of barium columns and hyaline casts in microdissection studies and the great lengths of the hyaline casts suggest that hyaline casts obstruct the flow of tubular fluid. Clonidine treatment resulted in fewer, shorter, and thinner hyaline casts. These results indicate that tubular obstruction by hyaline casts plays an important role in early postischemic acute renal failure, and that clonidine's beneficial effect is due in part to a reduction in cast formation. (Am J Pathol 98:123-150, 1980)

RECENT STUDIES of acute renal failure after temporary renal artery occlusion in the rat have suggested that tubular obstruction by casts plays an important role in the initiation phase of acute renal failure.[1-7] In previous studies[8] we showed that acute renal failure produced by pedicle clamping in the rabbit results in histologic and physiologic changes similar to those in human acute renal failure of the "hypotensive" type.[9] A prominent histologic feature of this model is cast formation. We have shown that clonidine treatment in this model results in a reduction in the number of outer medullary tubular casts and lessens the severity of acute renal failure in dehydrated or vasopressin-pretreated animals.[10]

The beneficial effect of clonidine appears to be related to an interference with the kidney's response to vasopressin.[10,10a] To assess whether clonidine actually reduced tubular blockage, we prepared postischemic rabbit kidneys for microradiography using the technique described by

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Gade [11] in which a high pressure intraarterial infusion results in filling of the tubular system by contrast material. In addition, we performed microdissection of tubules and studied histologic changes in thick (100 μ) and thin paraffin sections to investigate the characteristics of the tubular casts in this model.

The results showed that in the postischemic kidney hyaline tubular casts, extending from the thin limbs of Henle to the inner medullary collecting ducts, produced extensive tubular blockage. Clonidine significantly reduced the tubular blockage by reducing the number, length, and thickness of hyaline casts.

Materials and Methods

Animals

Experiments were carried out on 3–4-kg female New Zealand white rabbits (Bunnyville Farms), which were allowed free access to tap water and Purina Rabbit Chow.

Experimental Procedure and Terminology

One day before study, all rabbits were given a long-acting vasopressin preparation (Pitressin Tannate [Parke-Davis] in oil) subcutaneously (1.0 U/kg). We have previously shown that clonidine is much more effective as a protective agent in vasopressin-pretreated animals than it is in untreated animals.[10a] Surgery was not performed in one group of animals, referred to hereafter as *normal* rabbits. The other rabbits were anesthetized with intravenous sodium pentobarbital (50 mg/kg). The abdomen was opened through a midline incision. The right kidney was removed, and the left renal artery and vein were clamped with a small rubber covered bulldog clamp. This maneuver will be referred to as *pedicle clamping*. The ureter was not clamped. The clamp was removed from the left renal artery and vein after 1 hour, and the surgical incision was closed. Thirty minutes before unclamping half of the rabbits (referred to as *clonidine-treated*) in each experimental group were given clonidine (30 μg/kg in 1 ml of saline) intravenously; the other half (referred to as *control* rabbits) received no drug treatment during pedicle clamping.

Creatinine Clearance and Urine Flow in the First Six Hours After Unclamping

In 6 clonidine-treated and 6 control rabbits the bladder was emptied after pedicle clamping and the urine produced during the 6 hours after unclamping was collected for determination of creatinine clearance and urine flow rate. Blood was collected from the marginal ear vein at the start of the collection period and from the inferior vena cava at the end of the collection period for creatinine determinations.[10]

Procedure for Perfusion Fixation and Barium Infusion

Perfusion fixation and barium infusion were performed as described by Gade [11] with several modifications. At 2 hours (10 rabbits) and 6 hours (16 rabbits) after the release of pedicle clamping, the abdominal incision was reopened. The abdominal aorta was exposed and catheterized with a PE90 polyethylene catheter with 10 side holes for the infusion of the glutaraldehyde solution. The catheter tip was placed opposite the left renal artery. A ligature was placed around the abdominal aorta 2 cm above the bifurcation of the iliac artery to prevent the escape of fixative into the extremities. A liter of fixative consisting of Ringers lactate solution to which 15 ml glutaraldehyde and 9 g mannitol were added was

infused into the abdominal aorta through the catheter by the use of a hand pump. Infusion pressure, monitored through a catheter that was inserted into the carotid artery, was 90–110 mm Hg. Immediately after fixative entered the aorta, the large and small intestines were cross-clamped to prevent loss of fixative, and the inferior vena cava was cut close to the renal vein to reduce venous pressure and allow an exit point for the perfusion solution.

At the end of the perfusion, the renal artery was clamped proximally and the kidney was removed and placed in 37 C normal saline. The renal artery was catheterized with an 18-gauge Angiocath and flushed with 30–50 ml of warm (37 C) normal saline to prevent premature hardening of the gelatin–barium mixture by the glutaraldehyde. Twenty to fifty milliliters of 20% Baroloid, a small-particle barium sulfate suspension (C. B. Fleet Co., Lynchburg, Va) and 5% gelatin in normal saline warmed to 37 C were stirred with the use of a commercial blender for 30 minutes and then infused into the renal artery with a Harvard pump at setting 4 (corresponding to a pressure of approximately 350 mm Hg) for 5–10 minutes. The perfused kidney was then placed in a 1.5% glutaraldehyde solution and kept overnight in the refrigerator.

Histologic and Radiographic Methods

Two 3-mm–thick coronal slices of the kidney taken through the mid-portion of the kidney were selected from each kidney. One slice was embedded in paraffin, cut at 5 μ, and stained with hematoxylin and eosin. Another slice was used for frozen sections. Two 100-μ–thick sections were cut in a cryostat. One was fixed on a standard coverslip and radiographed on Kodak High Resolution Plate using a Picker Hot Shot portable x-ray machine (50-kVP, 6-ma, 5-min exposures). Another 100-μ–thick section was stained with toluidine blue and observed under the light microscope.

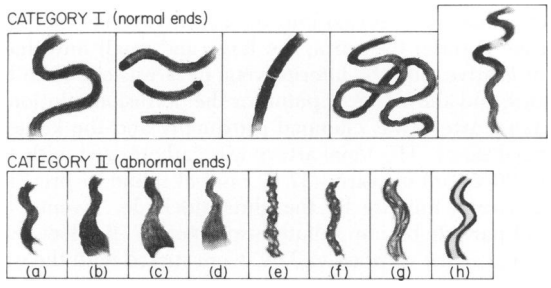
We excluded from study kidneys which were not well fixed by glutaraldehyde or not well filled by barium in the vascular system.

Microdissection Studies

Tubules were microdissected as described by Oliver et al.[12] Approximately a fourth of the glutaraldehyde-fixed kidney that was left after radiographic and histologic studies was macerated in concentrated HCl at room temperature for 24–26 hours. The macerated tissue was washed several times in tap water and dissected by the use of glass fiber needles under a binocular dissecting microscope. Unstained dissected tubules were observed and photographed with the use of both ordinary light microscopy and Nomarski differential interference phase contrast microscopy,[13] which improved the resolution of casts within tubules. The length of the casts was measured with the use of an ocular micrometer. Because of the fragility of the tubules, it was not possible to dissect out entire nephrons in continuity.

Microradiographic Studies

To search for evidence of tubular obstruction particular attention was paid to the appearance of the ends of the tubular barium columns. Tubular ends observed in five random cortical and outer medullary fields at a magnification of 100× were counted and categorized into two groups as follows: Category I tubular ends represented normal tubules that extended out of the plane of section at various angles. Category II tubular ends were abnormal tubular ends with features such as focal tubular widening, terminal concavity, irregular contour and lattice-like defects in tubular filling (Text-figure 1). Very faint tubular images were not counted. Because it was almost impossible to determine the particular part of the nephron being studied, we counted all tubular ends that were in the microscopic fields. The percentage of normal tubular ends (Category I) in the total was calculated. In most cases, the inner medulla did not have enough collecting tubules filled with



TEXT-FIGURE 1—Diagram showing "normal" (Category I) and "abnormal" (Category II) ends of barium columns in microradiographs.

barium to study meaningfully, and thus we did not count the tubular ends in the inner medulla.

Histologic Studies

Using 5-μ hematoxylin-and-eosin-stained sections, the number of tubular casts observed in 10 random cortical outer medullary and inner medullary fields at a magnification of 400× was determined for each animal without knowledge of drug treatments. It was difficult to distinguish distal tubules from collecting ducts in the outer medulla and the cortex of these perfusion-fixed kidneys. Therefore, casts in these segments were counted together. In the inner medulla, the casts of Henle's loops and collecting ducts were counted separately. The casts were divided into three types: hyaline casts, granular casts, and cellular casts.[14] Hyaline casts were homogeneous, structureless, and pink-staining. Granular casts were composed of irregular fine and coarse pink granular material mixed occasionally with cell debris and hyaline material. Cellular casts had many tubular cells with pyknotic nuclei surrounded by hyaline-cast-like homogeneous material. The Baroloid contrast material was very easy to distinguish from casts because of its characteristic appearance. It consisted of fine granular white refractile barium particles that were interspersed with pink-staining material representing gelatin and the sugar flavoring used in the Baroloid mixture.

Three histologic features that could not be precisely quantitated—outer medullary tubular necrosis, inner medullary collecting duct necrosis, and thickness of hyaline casts in inner medullary thin limbs relative to the size of the tubular lumen—were assessed by ranking. Two observers reviewed the slides independently without knowledge of the treatment given. After comparing the slides with one another, each observer placed them in rank order from least to greatest degree of change. The ranks assigned by the two observers were averaged, and these ranking data were used to seek correlations between lesions and differences between groups.

Statistical Treatment

Data were expressed as means ± standard error (SE). Statistical comparisons were made by the Student t test. Linear correlation coefficients were determined as described by Dixon and Massey.[15] For ranked data, the Spearman rank correlation test [16] was used to establish correlations, and the Wilcoxon two-sample test [17] was used to analyze differences between groups.

Results

Physiologic Studies

Urine flow rate during the first 6 hours after unclamping of the renal pedicle was significantly higher in the clonidine-treated group than in the

non-clonidine-treated control group (2.47 ± .22 ml/hr versus .87 ± .32 ml/hr; $P < 0.005$). Creatinine clearance was also significantly higher in clonidine-treated animals than in control animals (1.53 ± .52 ml/min versus .23 ± .13 ml/hr; $P < 0.05$), confirming the beneficial effect of clonidine on postischemic renal function that we reported previously.[10,10a]

Microradiographic Studies

In the kidneys studied, glomerular capillary rupture with extrusion of barium into Bowman's space and the proximal tubule occurred in 20–60% of glomeruli. Many nephrons were entirely filled with barium, and there was barium in the renal pelvis and ureter. In *normal* kidneys the tubules were well filled with contrast material. The barium columns within tubular lumens were homogeneous and smooth-contoured both in the cortex and in the outer medulla (Figures 1 and 2). Where a tubule passed out of the plane of section obliquely the end of the barium column was tapered or convex. The barium column appeared as a dense circle where tubules passed out of the plane of section at right angles ("Category I" ends, Text-figure 1).

At 2 or 6 hours after 1 hour of pedicle clamping, characteristic alterations in the appearance of the barium column were observed ("Category II" ends, Text-figure 1). Many columns ended in a concave fashion (Figures 3 and 4). The ends of other columns had a moth-eaten or lattice-like appearance (Figures 4 and 5). Irregular areas of dilatation and narrowing were seen (Figures 4 and 5). The extent of alteration in the appearance of the barium columns can be determined by calculating the percentage of "normal" (Category I) tubular ends observed in the various experimental groups. In normal kidneys 87.6 ± 4.1% of cortical tubular ends and 95.4 ± 0.7% of outer medullary tubular ends were of this type. In non-clonidine-treated control animals studied 2 hours after unclamping of the renal pedicle, this percentage dropped to 41.0 ± 13.6% ($P < 0.01$) and 32.0 ± 6.6% ($P < 0.001$) in cortex and outer medulla, respectively. Clonidine treatment during clamping did not significantly change this percentage in the cortex (36.3 ± 15.4%) or outer medulla (36.9 ± 19.1%) at 2 hours. At 6 hours after release of the clamp, the percentage of the normal tubular ends in the *cortex* was still significantly reduced in non-clonidine-treated animals (42.4 ± 11.0%; $P < 0.01$), and clonidine did not significantly alter the percentage (33.8 ± 8.2%). In the *outer medulla*, however, clonidine significantly increased the percentage of normal tubules (45.6 ± 6.0% with clonidine versus 27.6 ± 4.2% without; $P < 0.05$). Both of these figures were still significantly different from the value for normal rabbits ($P < 0.001$).

Histologic Studies

Proximal tubules in the *cortex* in both clonidine-treated and non-clonidine-treated rabbits were dilated 2 hours and 6 hours after removal of the pedicle clamp (Figure 6). Loosely arranged amorphous eosinophilic material and cellular debris was sometimes seen admixed with barium (Figure 6). This was not seen in normal kidneys (Figure 7). There were no casts in proximal convoluted tubules, even in kidneys with very large numbers of distal tubular casts. Hyaline casts and a few granular casts were found in the cortex in distal tubules or collecting ducts (Figure 8).

In contrast to the cortex, where tubular cells were well preserved in postischemic kidneys, necrotic tubular cells were frequently seen in the *outer medulla.* Patchy necrosis was seen in all nephron segments present in the outer medulla. There were many hyaline and granular casts and a few cellular casts in ascending limbs and collecting ducts. Hyaline cast material was not seen mixed with barium. Granular casts were frequently found mixed with barium (Figure 9A–C). The pars recta of the proximal tubules contained much cellular debris but not well-formed casts. Some tubules were represented only by basement membranes without tubular cells (Figure 10), and the specific nephron segments could not be identified.

In the *inner medulla* there were very many hyaline casts in the loops of Henle; some were present in collecting ducts, but very few in ducts of Bellini. Patchy collecting duct necrosis was seen and sloughed collecting duct cells with pyknotic nuclei were present in the lumen of some collecting ducts, which were themselves lined by normal epithelium (Figure 11). No necroses were seen in inner medullary loops of Henle. The collecting duct necrosis appeared more prominent in the rabbits used in this study than it was in non-vasopressin-pretreated animals studied previously.[8]

In clonidine-treated rabbits killed at 6 hours hyaline casts tended to be thinner both in the outer medulla (Figure 12) and in the inner medullary thin limbs (Figure 13), compared with non-clonidine-treated rabbits (Figure 11); the difference (assessed by ranking) fell just short of statistical significance ($0.1 > P > 0.05$).

Figures 14 and 15 show typical 100-μ frozen sections from the 6-hour groups without and with clonidine treatment, respectively. Very long hyaline casts were found in the kidneys of both rabbits, but in the clonidine-treated rabbit (Figure 15) there were spaces between cast edges and tubular walls that were not present in the untreated rabbit.

Tables 1 and 2 show the number of three kinds of casts observed in paraffin sections in cortex, outer medulla, and inner medulla at 2 and 6 hours. There were no significant differences between the clonidine-treated

Table 1—Number of Hyaline, Granular, and Cellular Casts in Cortex, Outer Medulla, and Inner Medulla at 2 Hours and 6 Hours After Unclamping With or Without Clonidine

	2 Hours vehicle	Signif-icance	2 Hours clonidine	6 Hours vehicle	Signif-icance	6 Hours clonidine
Hyaline casts						
Cortex	20.8 ± 6.8	NS*	38.2 ± 9.7	31.8 ± 6.8	NS	22.6 ± 3.4
Outer medulla	45.6 ± 11.3	NS	46.6 ± 15.3	68.6 ± 9.8	$P < 0.05$	42.4 ± 5.8
Inner medulla						
Loop of Henle	593.6 ± 178.5	NS	562.6 ± 153.5	941.0 ± 227.9	$P < 0.05$	340.0 ± 97.4
Collecting duct	47.8 ± 25.1	NS	45.8 ± 20.9	35.3 ± 14.5	NS	12.4 ± 5.0
Granular casts						
Cortex	1.2 ± 0.4	NS	0.6 ± 0.4	3.1 ± 0.7	NS	1.8 ± 0.6
Outer medulla	3.4 ± 1.3	NS	4.0 ± 0.9	21.9 ± 9.5	NS	6.9 ± 1.6
Inner medulla						
Loop of Henle	4.8 ± 3.4	NS	8.6 ± 6.6	7.5 ± 2.6	NS	9.9 ± 4.7
Collecting duct	0.4 ± 0.4	NS	0.6 ± 0.4	6.3 ± 3.0	NS	2.0 ± 1.5
Cellular casts						
Cortex	0.2 ± 0.2	NS	0.2 ± 0.2	0.0 ± 0.0	NS	0.0 ± 0.0
Outer medulla	0.6 ± 0.4	NS	2.4 ± 2.2	1.4 ± 1.1	NS	0.1 ± 0.1
Inner medulla						
Loop of Henle	0.0 ± 0.0	NS	0.0 ± 0.0	0.5 ± 0.5	NS	0.0 ± 0.0
Collecting duct	9.6 ± 5.9	NS	1.8 ± 1.4	10.6 ± 6.7	NS	4.3 ± 2.7

* NS = not significant.

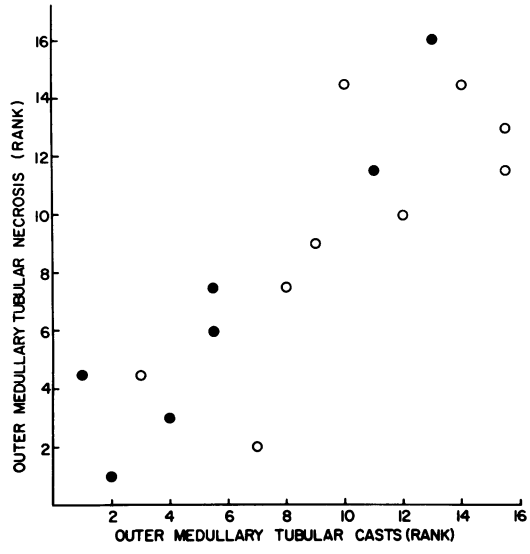
Table 2—Correlation Between Number of Hyaline Casts and Granular Casts

	Cortex	Outer medulla	Henle's thin limb in inner medulla
2 Hours after unclamping (n = 5)			
r	−0.45	0.42	−0.47
P	NS*	NS	NS
2 Hours after unclamping with clonidine treatment (n = 5)			
r	0.54	0.76	0.99
P	NS	NS	<0.01
6 Hours after unclamping (n = 8)			
r	0.87	0.30	0.82
P	<0.05	NS	<0.05
6 Hours after unclamping with clonidine treatment (n = 8)			
r	0.08	0.29	0.58
P	NS	NS	NS

* NS = not significant.

and non-clonidine-treated groups at 2 hours after unclamping. But at 6 hours the clonidine-treated group had significantly fewer hyaline casts in the outer medullary tubules and in the inner medullary loops of Henle. Six hours after unclamping there were significant correlations between the number of hyaline casts and granular casts in the cortex and the number in inner medullary loops of Henle. These correlations were eliminated by clonidine treatment.

There were no significant differences between the clonidine-treated and untreated groups 2 or 6 hours after unclamping with respect to outer medullary tubular necrosis, inner medullary collecting duct necrosis, or relative thickness of hyaline casts in inner medullary thin limbs. The inter-observer correlation coefficients for these three lesions were + 0.38, + 0.51, and + 0.74, respectively. There was a highly significant correlation (Text-figure 2) between outer medullary tubular necrosis and outer medullary casts in the 2 6-hour groups ($r = + 0.84$, $P < 0.001$ for combined ranking of Observers 1 and 2; $r = + 0.73$, $P < 0.01$ for Observer 1; $r = 0.66$, $P < 0.01$ for Observer 2). When correlations in the clonidine-treated and untreated 6-hour groups were examined separately, it was found that there was a significant positive correlation in the untreated group between collecting duct necrosis and number of thin limb casts ($r = +0.85$, $P < 0.05$) (Text-figure 3). This correlation could not be demonstrated in the clonidine treated group ($r = -.29$, NS).



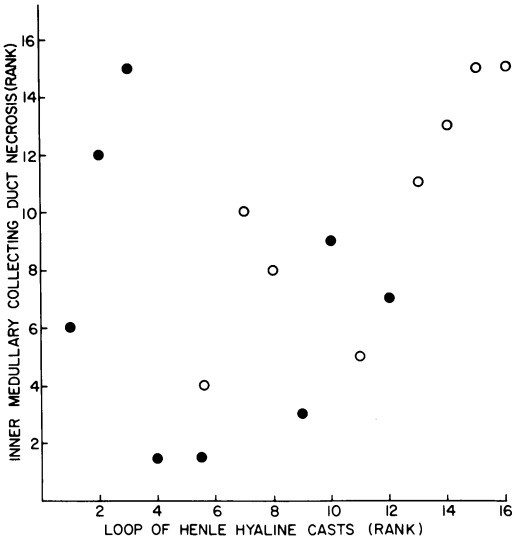
TEXT-FIGURE 2—Plot showing relationship between outer medullary tubular necrosis and outer medullary tubular casts in animals killed 6 hours after pedicle unclamping. (r = .84, $P < 0.001$) ● = Clonidine-treated rabbits; ○ = Non-clonidine-treated rabbits.

Microdissection Studies

In *normal kidneys*, all parts of the nephron had identifiable lumens that appeared either empty or filled with barium. Under the microscope, barium columns in unstained preparations appeared black in all portions of the nephron except the collecting duct, where they appeared brown, probably because of dilution of barium by tubular fluid. In distal tubules, connecting part of collecting ducts and main collecting ducts, the barium columns were sometimes interrupted. This breaking up of the barium column in the distal nephron may be the cause of the "abnormal" (Category II) ends observed occasionally in normal kidneys (see page 127). In *post-ischemic kidneys*, at both 2 and 6 hours after the ischemic insult, many nephrons without barium contained hyaline casts, granular casts, cellular casts, cellular debris, and small pieces of granular material. Thick hyaline casts were translucent yellow or gold colored. Thinner hyaline casts in the loop of Henle or collecting ducts sometimes appeared translucent and colorless. Granular casts were yellow or light brown in color. There were no casts but much cellular debris in proximal tubules, particularly in the pars recta.

Hyaline casts were frequently found in Henle's thin limb, distal tubules, connecting tubules, and connecting ducts. Most of them appeared to be continuous from Henle's loop to the inner medullary collecting ducts. Hyaline casts sometimes expanded the tubular lumen and deformed the tubules into irregular shapes.

The hyaline casts were variable in appearance, and many were very long. The longest one we could measure was 14 mm. This cast may have



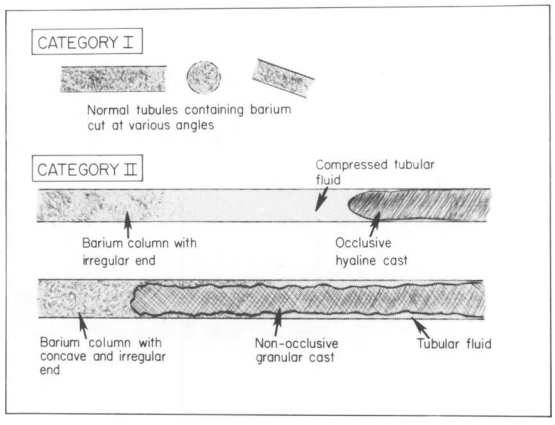
TEXT-FIGURE 3—Plot showing relationship between inner medullary collecting duct necrosis and loop of Henle hyaline casts in animals killed 6 hours after pedicle unclamping. A significant correlation exists only in the non-clonidine-treated rabbits ($r = .85$, $P < 0.05$). ● = Clonidine-treated rabbits; ○ = Non-clonidine-treated rabbits.

been longer than actually measured, because it appeared to have been broken at both ends.

Hyaline casts were usually interconnected at points of branching of collecting ducts and formed single casts at the main collecting duct (Figures 16 and 17).

In non-clonidine-treated rabbits hyaline casts were not in contact with a barium column (Figures 18, 19, and 20), probably because such casts completely blocked the tubules and created high intratubular pressure, which halted the flow of the barium column (Text-figure 4). The distance between the end of the barium column and the hyaline cast varied from 0.7 mm to 17.1 mm.

In kidneys of clonidine-treated rabbits hyaline casts tended to be thin (Figure 17) and not as long as in kidneys of non-clonidine-treated rabbits. In tubules containing these thin casts, there were spaces between the cast and the tubular wall. Casts in clonidine-treated rabbits were frequently limited to the distal tubules and the early part of the collecting duct, and there were many apparently empty Henle's thin limbs and deep collecting ducts. Barium columns were occasionally in contact with these thinner casts, suggesting that some of them did not completely occlude the tubule (Figure 21). In the clonidine-treated group 14.9% of the hyaline casts were thin and apparently nonocclusive. None of the hyaline casts observed in the non-clonidine-treated group were of this type. The difference between the two groups is significant using chi-square analysis ($P < 0.01$).



TEXT-FIGURE 4—Diagram showing explanation of barium column irregularities and spaces between barium columns and hyaline casts.

Granular casts were found frequently in kidneys from both clonidine-treated and non-clonidine-treated rabbits. These granular casts were not as long as hyaline casts; they neither expanded nor deformed the tubules, although they appeared to fill the lumen. They were usually found just behind, just beyond, or mixed with hyaline casts (Figure 18 and 19). When they preceded hyaline casts, they frequently were in contact with the barium column, the end of which was frequently concave or irregular (Figures 9 and 19).

Table 3 shows the quantitative microdissection data from the 6-hour clonidine and 6-hour non-clonidine groups. In all nephron segments from the thin limb to the outer medullary collecting duct there were significantly fewer hyaline casts in the clonidine-treated group.

Discussion

The microradiographic technique of Gade [11] demonstrates tubular patency and anatomy in a fashion that previously was possible only through use of the more difficult and complicated micropipette technique described by Beeuwkes.[18] Gade's method is preferable for statistical analysis because it allows one to visualize a large number of tubules in the same kidney.

We encountered several problems with this method, however. First, especially in normal kidneys, too many nephrons were filled with barium and overlapped each other, making it quite difficult to observe individual nephrons even in 200-μ-thick sections, the thinnest section Gade used.[11] We found that the best section thickness was 100 μ, but in 100-μ sections it is impossible to observe entire nephrons in continuity. Therefore, it is quite difficult to distinguish different nephron segments. In the medulla,

Table 3—Percentage of Tubular Segments Containing Hyaline Casts

Rabbit	Loop of Henle		Distal convolution	Connecting piece	Collecting duct	
	Thin limb	Thick limb			Early	Deep
No clonidine						
1279	93.8	91.7	96.4	92.3	89.5	88.2
1219	50.0	12.5	72.2	50.0	26.7	11.1
1212	72.7	84.6	92.0	84.6	83.3	77.7
1272	100.0	90.9	100.0	100.0	100.0	100.0
1328	90.0	92.3	96.4	100.0	94.1	91.7
Mean	81.3 ± 9.0	84.4 ± 5.6	91.4 ± 5.0	85.4 ± 9.3	78.7 ± 13.3	73.8 ± 16.1
Clonidine						
1281	21.4	42.9	52.4	10.0	0.0	—
1332	50.0	29.4	52.4	52.9	46.7	40.0
1217	83.3	73.7	57.1	63.2	62.5	28.6
1331	36.4	33.3	70.8	69.2	52.2	41.2
1296	38.9	56.2	43.8	37.5	16.7	0.0
Mean	46.0 ± 10.4	47.1 ± 8.1	55.3 ± 4.3	46.6 ± 10.6	35.6 ± 11.7	27.5 ± 9.6
Significance	$P < 0.05$	$P < 0.01$	$P < 0.001$	$P < 0.05$	$P < 0.05$	NS

* NS = not significant.

in addition, it is difficult to distinguish Henle's hairpin loops from the vasa recta. Second, we noticed considerable pressure artifacts (Figures 6 and 7). Small arteries, arterioles, and glomerular tufts that contained contrast material were often extremely expanded, probably because of the very high pressures used. This was less noticeable in radiographic plates but was quite obvious in regular histologic sections. Tubules seemed to show no pressure artifacts, but it is difficult to be certain that they did not exist. Third, as Gade reported, red blood cells frequently remained in the kidney following perfusion fixation and flushing with saline. This resulted in poor capillary filling by contrast material. Fourth, contrast material mixed with tubular fluid and gradually became diluted in the distal part of the nephron. This process resulted in discontinuities and irregularities in the tubular barium column even in normal kidneys.

In the present study, using a combination of microradiography, microdissection, and paraffin section histology, we were able to demonstrate tubular dilatation, very long and thick hyaline casts, deformed barium columns within tubules, and lack of contacts between hyaline casts and barium columns. These findings strongly suggest that many tubules were completely obstructed.

Complete blockage of the tubules by a hyaline cast prevents the barium column from reaching the cast. The intratubular barium columns frequently intermingle with granular cast material. This indicates that, unlike hyaline casts, granular casts do not produce complete obstruction. The tubular fluid that originally separates the barium column from the granular cast is able to pass through or around the cast, allowing the barium column and granular cast to come into contact. Tubular leakage at the site of the granular cast would also account for this finding. However, we observed barium passing through ruptured tubule walls only very occasionally.

Microdissection studies of acute renal failure in man have usually shown rather short granular casts and very long (2–3 cm) hyaline casts.[12] Long hyaline casts are seen in human urinary sediments,[14] but these casts are not as long as those observed within the kidney in our experimental studies or in Oliver's microdissection studies in man.[12] It appears that long hyaline casts tend to break up to form shorter casts and that it is these shorter casts that find their way into the urine. We found many long hyaline casts within tubules 6 hours after unclamping. Most appeared to be continuous from the loop of Henle to the collecting duct in mid to deep inner medulla. This length increases the likelihood that such casts block tubules completely. Additionally, long casts will damage tubular cells along a greater length of the tubule. In the human kidney, one collecting

duct has 7–8 branches, each of which has 9–11 attached nephrons.[19] This means that total blockage of a few collecting ducts may interfere with the function of hundreds of nephrons.

The strong correlation between outer medullary necrosis and outer medullary casts demonstrated in histologic studies (Table 2) does not establish whether obstruction by casts causes tubular necrosis in a manner analogous to the way in which ureteral obstruction causes tubular necrosis [20] or whether, alternatively, tubular necrosis promotes the formation of hyaline casts by producing partial obstruction and stasis of tubular fluid.

The number of granular casts was significantly correlated with the number of hyaline casts (Table 1), and the granular casts were frequently found just before, just beyond, or between hyaline casts. These results suggest that granular casts may promote hyaline cast formation. Clonidine appears to inhibit the formation of hyaline casts but has no significant effect on granular casts. Slow tubular fluid flow caused by the presence of granular casts would encourage precipitation of Tamm-Horsfall protein from the tubular fluid to form hyaline casts in distal tubules. While the initial immature hyaline cast is moving into the collecting duct, new cast material will form behind it, eventually producing a very long cast. The presence of many well-formed hyaline casts in Henle's thin limb is hard to interpret, because Tamm-Horsfall protein is believed to be secreted only from Henle's ascending thick limb, distal convoluted tubules, and collecting ducts.[21,22] It is possible that Tamm-Horsfall protein diffuses upstream to Henle's thin limb from distal tubules. Other possibilities are that the loop of Henle epithelium in the rabbit has the ability to produce Tamm-Horsfall protein or that other substances such as the procoagulant recently described by Matsuda et al [23] may contribute to the formation of thin limb hyaline casts.

In our previous report [10] we showed outer medullary microvascular damage and disturbance of medullary blood flow in postischemic acute renal failure. This outer medullary perfusion disturbance probably contributes to outer medullary tubular cell damage leading to desquamation of cell components and partial tubular obstruction.

Donohoe et al [5] described casts in proximal tubules in rat after temporary renal ischemia. We did not find well-formed casts in proximal tubules in the rabbit. We found instead amorphous eosinophilic material and cellular debris. Our studies were carried out 2–6 hours after the ischemic insult. At this time, debris from dead tubular cells still contains nuclei and is loosely scattered in the tubular lumen, bearing little resemblance to a granular cast. Later, when the dead tubular cells lose their nuclei and the cellular debris becomes tightly packed in the tubular lumen, it is not pos-

sible to distinguish this debris from a granular cast. Indeed, in the post-ischemic rat kidney most granular casts probably come from necrotic tubular cells. In the rabbit, where tubular necrosis in the cortex is almost totally absent following 1 hour of ischemia,[8] the origin of the granular cast is more obscure. It is likely that in the rabbit, as in man, granular casts may form through mechanisms that do not involve tubular necrosis.[14]

In a previous study [10] we reported that clonidine has a beneficial effect on acute renal failure following 1 hour of pedicle clamping. Clonidine increased outer medullary blood flow, decreased hyaline cast formation, and reduced ischemic microvascular injury in the outer medulla. In this study, we confirmed that clonidine decreases the number of hyaline casts. In addition to these findings, we found that clonidine significantly decreased the number of abnormal (Category II) tubular barium column ends at 6 hours after unclamping. Text-figure 4 shows the way in which we believe these abnormal tubular contours come about. In totally blocked tubules the barium column halts prematurely and often has an irregular end. Barium columns in contact with granular casts also have ragged or concave ends (Text-figures 1 and 4). In microdissection studies, we found that clonidine treatment made hyaline casts thinner and shorter. The mechanism by which clonidine inhibits hyaline cast formation is not clear. Clonidine treatment tends to preserve the outer medullary blood flow,[10] and it is possible that this lessens outer medullary tubular damage and subsequent cast formation. However, it seems unlikely that clonidine's main effect is on tubular necrosis, since no differences in necrosis could be demonstrated in the treated and untreated groups at 6 hours, when there was a significant difference in the number of outer medullary and inner medullary casts. Clonidine also blunts the kidney's response to vasopressin, thus leading to formation of a less concentrated tubular fluid and decreasing the propensity for hyaline casts to form. The higher rate of tubular fluid flow increases the likelihood that those casts that do form will be flushed out of the kidney into the urine before they become large enough to obstruct the tubule.

In the present study we demonstrated that creatinine clearance 6 hours after pedicle unclamping in the rabbit was significantly higher in clonidine-treated animals. The main factor that contributed to this significant difference was not a striking dissimilarity in serum creatinine in the two groups, but rather a great disparity in urine flow rate with near anuria (.87 ml/hr) in the non-clonidine-treated rabbits. Mason et al [24] have recently pointed out that a marked oliguria must be present if tubular obstruction is proposed as the main factor leading to renal failure in a given model of acute renal failure.

Finally, it must be emphasized that the present study is not in conflict with previous work by Oken et al [25,26] showing that *granular* casts formed from necrotic debris (the principal type of cast found in the models Oken used) can be easily dislodged and are unlikely to produce tubular obstruction. In the rabbit model used in the present study [8] and in "hypotensive" acute renal failure in man [9] there is virtually no tubular necrosis in the renal cortex, and the principal type of cast found is the *hyaline* cast. The great length of these hyaline casts [12] and their interconnections at points of coalescence of the collecting ducts make it very unlikely that such casts can be easily expelled once they form.

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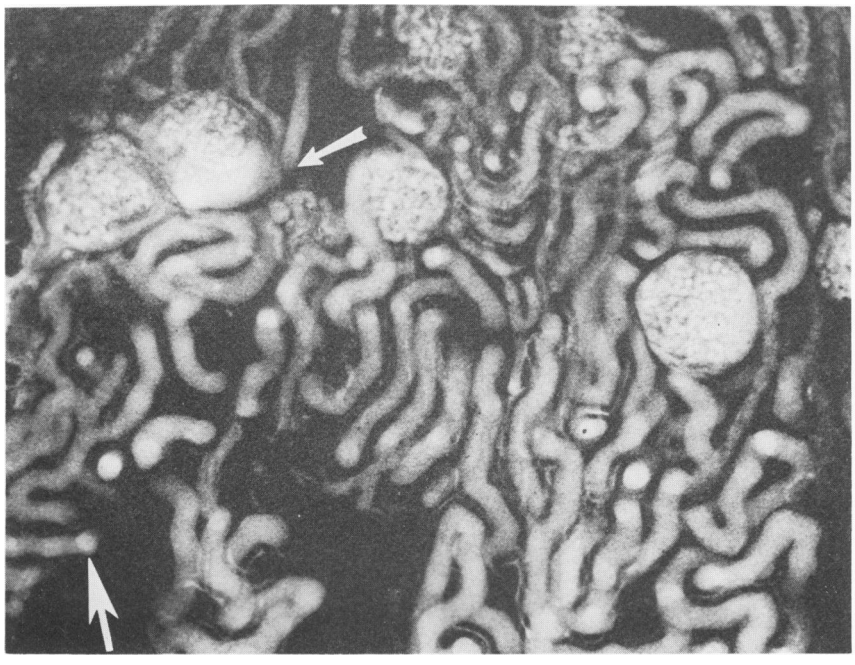
Figure 1—Cortical field from microradiograph from a normal rabbit kidney. Bowman's spaces and proximal tubules are well filled with barium. Barium columns within tubules are smooth contoured and have convex (*small arrow*) or dense circular (*large arrow*) ends of the Category I type (see Text-figure 1). (×65)

Figure 2—Outer medullary field from normal rabbit kidney. Many straight tubules are seen well filled with barium. It is not possible to distinguish the different nephron segments. (×65)



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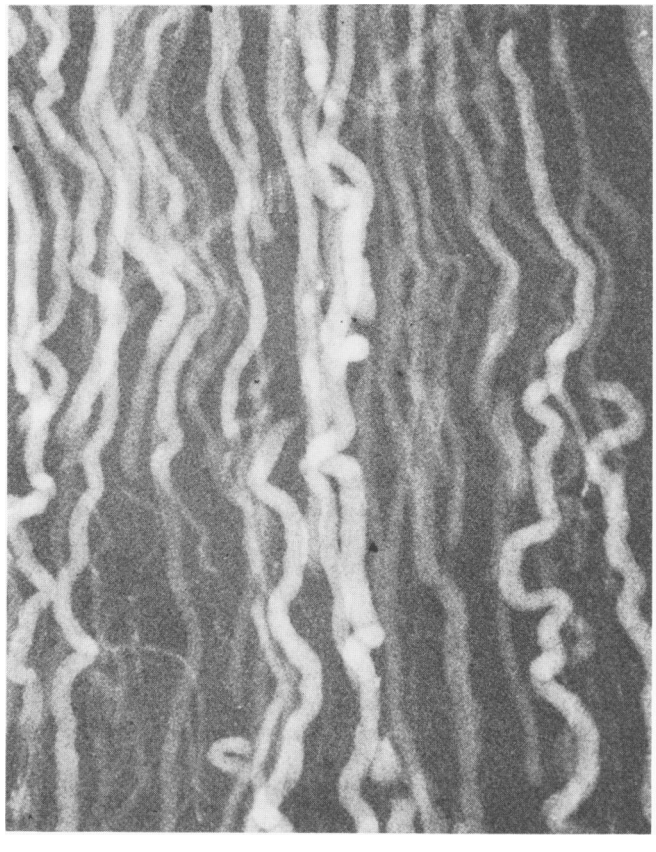

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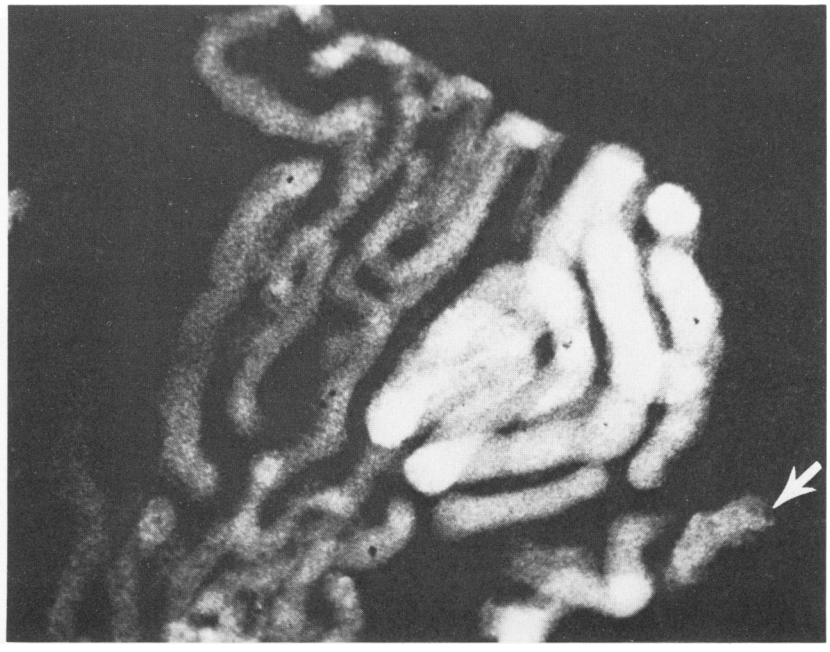

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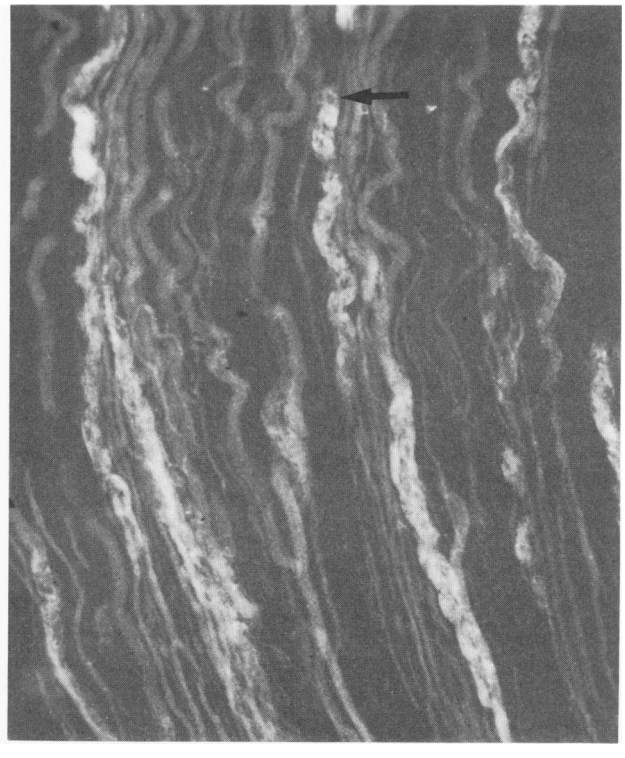

Figure 3—Superficial cortical field from a rabbit killed 6 hours after unclamping. The barium column in the tubular system to the left appears abnormally thin. The short column to the far right has a concave end (*arrow*). (×185) **Figure 4**—Outer medullary field from a rabbit killed 6 hours after unclamping. There are many irregularly expanded tubular barium columns with a moth-eaten appearance (*arrow*). (×65)

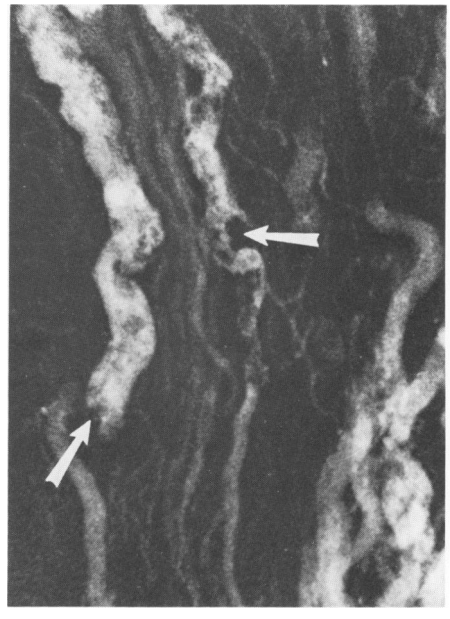

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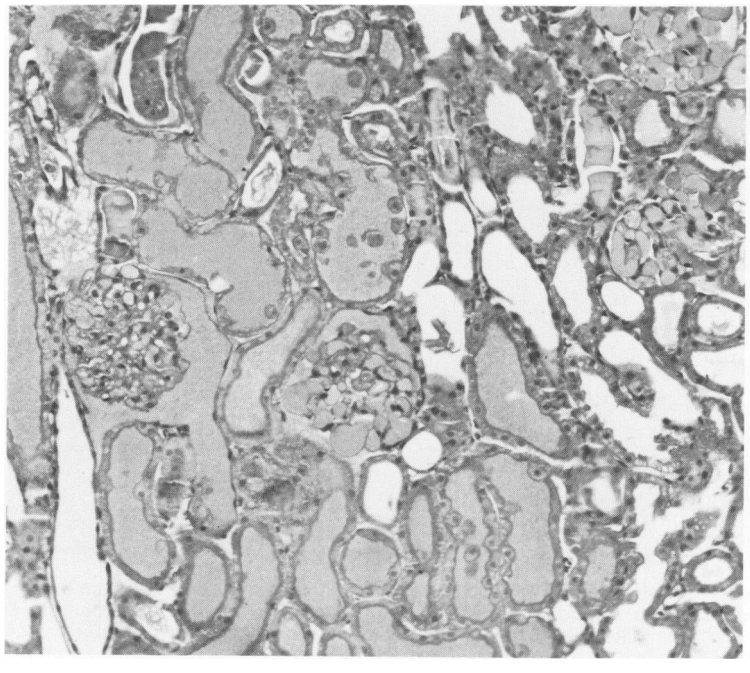
Figure 5—Outer medullary field from a rabbit killed 6 hours after unclamping. The two irregularly expanded tubular barium columns on the right show patchy barium filling and concave ends (*arrows*). (×115) **Figure 6**—Paraffin section from a rabbit killed 6 hours after unclamping. Proximal tubules are expanded and show desquamation of tubular cells and cell fragments into the barium-filled lumen. Distal tubules do not contain barium. The high pressure of the barium infusion has caused marked dilatation of some of the capillary loops in the glomerulus in the middle of the field. (H&E, ×170)



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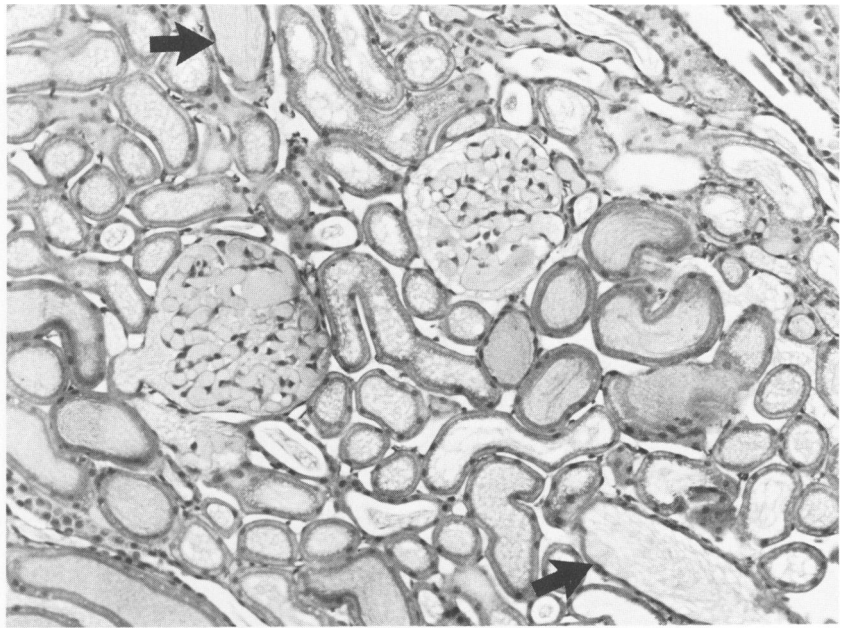

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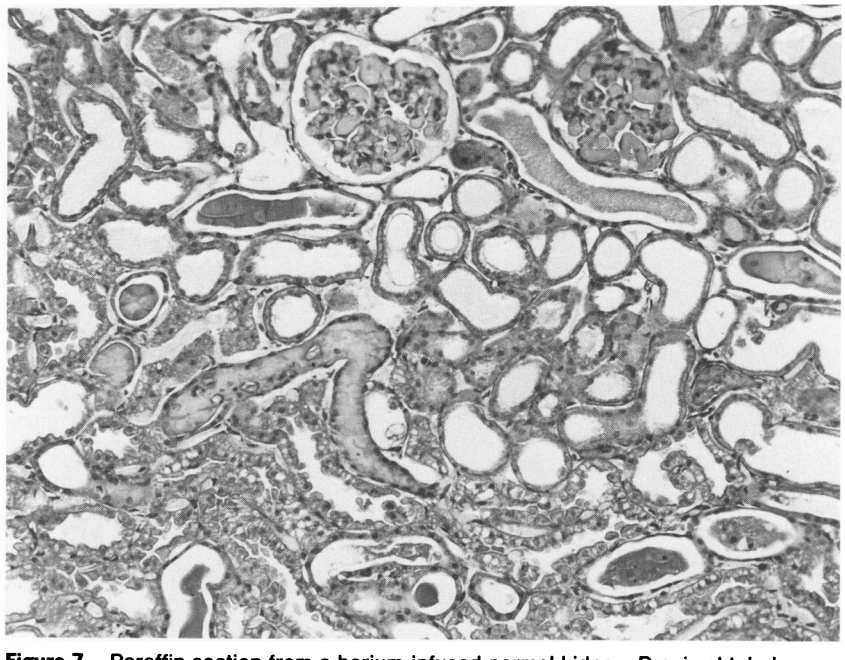
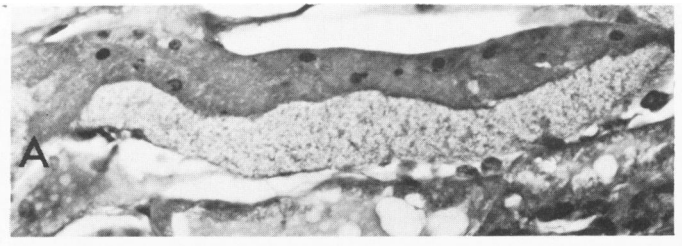
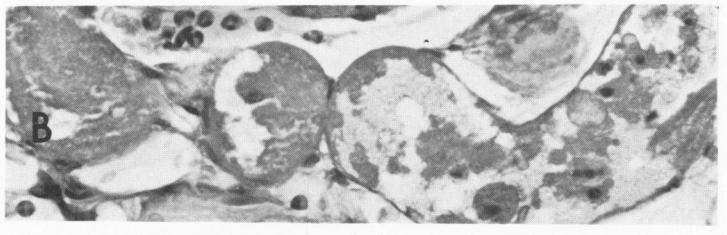


Figure 7—Paraffin section from a barium-infused normal kidney. Proximal tubules are of normal caliber and contain identifiable brush borders (not seen in Figure 6). Distal tubules contain diluted barium. Arteries (*arrows*) and glomerular capillaries are dilated by the high infusion pressure. (H&E, ×170) **Figure 8**—Hyaline casts and occasional granular casts in distal tubules and collecting ducts in an animal killed 6 hours after unclamping. In this field glomerular capillary ruptures have not occurred, and there is no barium in Bowman's spaces or in tubules. (H&E, ×170)





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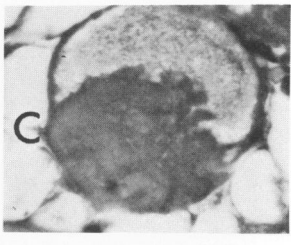
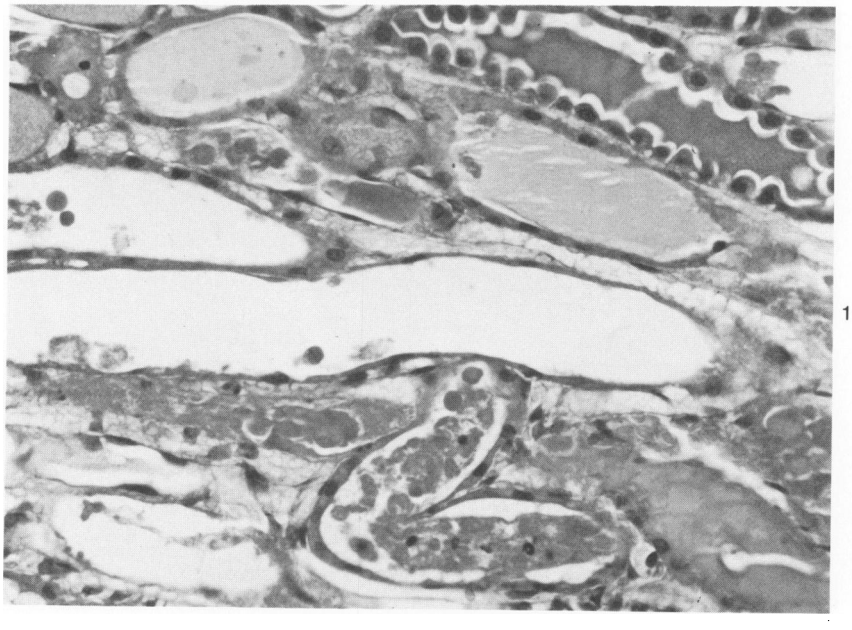


Figure 9—Outer medullary fields from animals killed 6 hours after unclamping. Contacts between pale-staining coarsely particulate barium mixture and eosinophilic granular cast material are seen. Similar contacts between barium and *hyaline* casts were not observed in paraffin sections, although contacts between *thin* hyaline casts and barium columns were occasionally found in clonidine-treated animals by microdissection (see Figure 21). (H&E, ×400)
Figure 10—Outer medullary tubule represented only by a bare basement membrane 6 hours after pedicle unclamping. (H&E, ×400)



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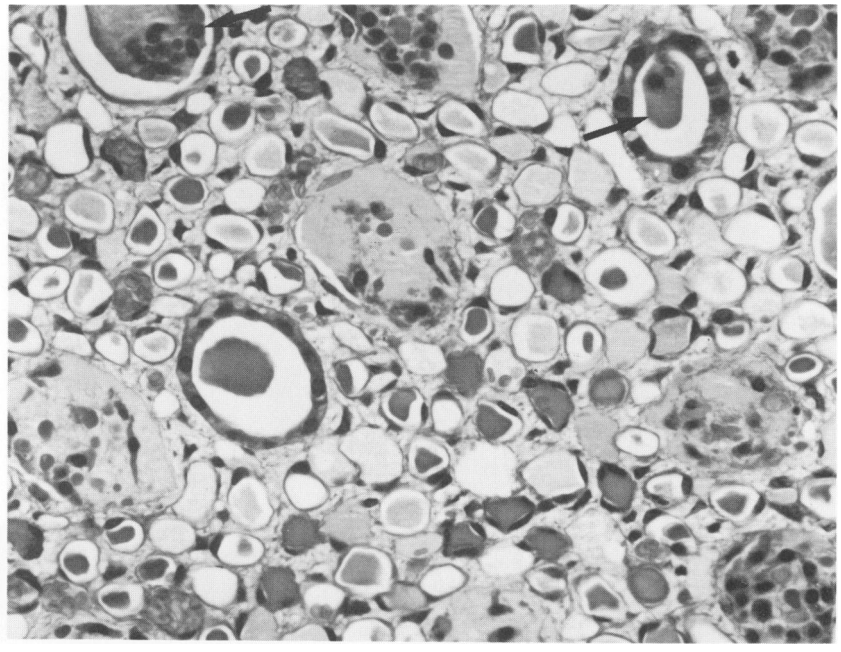

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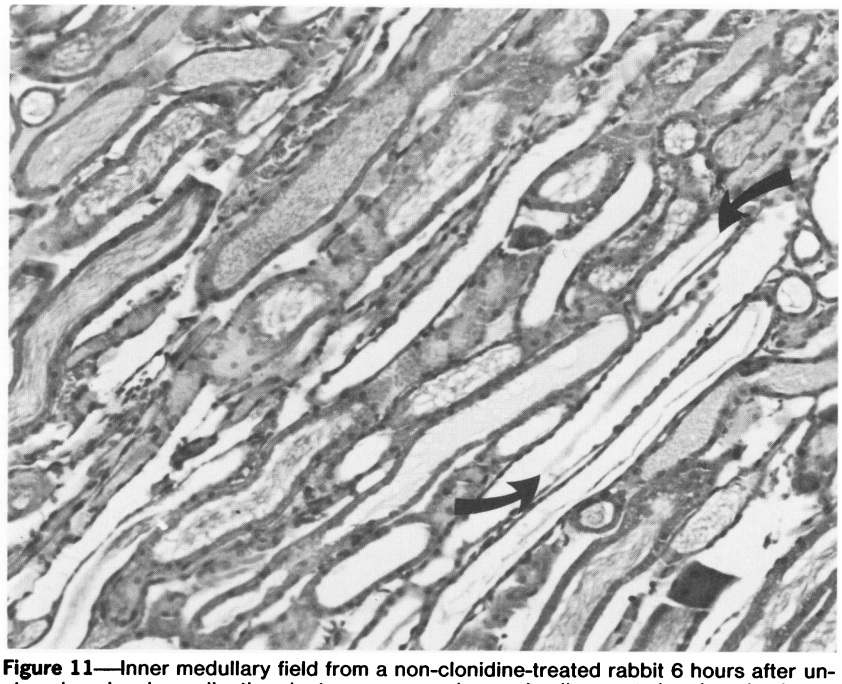
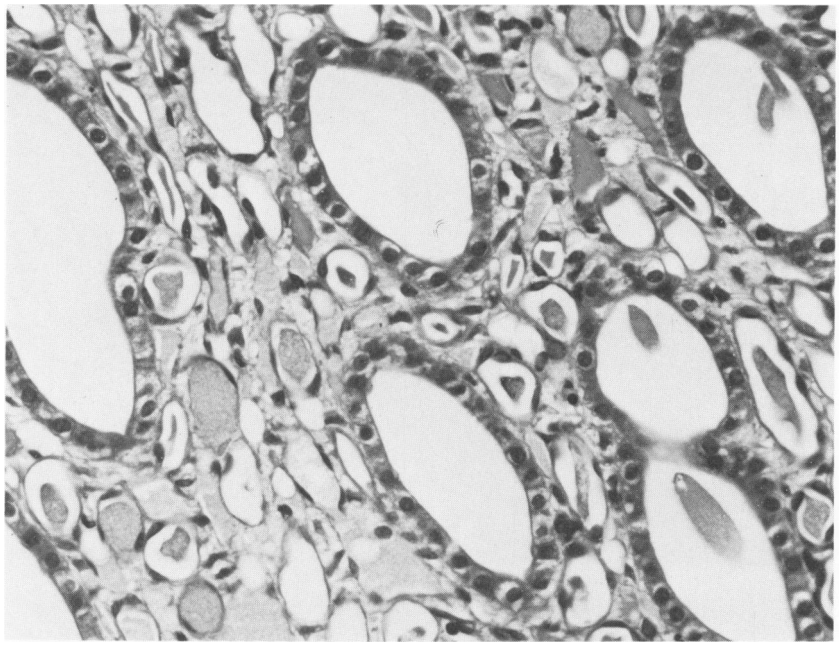


Figure 11—Inner medullary field from a non-clonidine-treated rabbit 6 hours after unclamping showing collecting duct necroses and many hyaline casts in otherwise intact thin limbs. Two collecting ducts that are not themselves necrotic contain cellular casts suggesting necrosis further upstream in the same nephron (*arrows*). (H&E, ×400) **Figure 12**—Outer medullary field from clonidine-treated animal killed 6 hours after unclamping. Very thin hyaline casts are seen (*arrows*). Other tubular segments are well filled by barium, and there is little evidence of tubular damage. (H&E, ×170)



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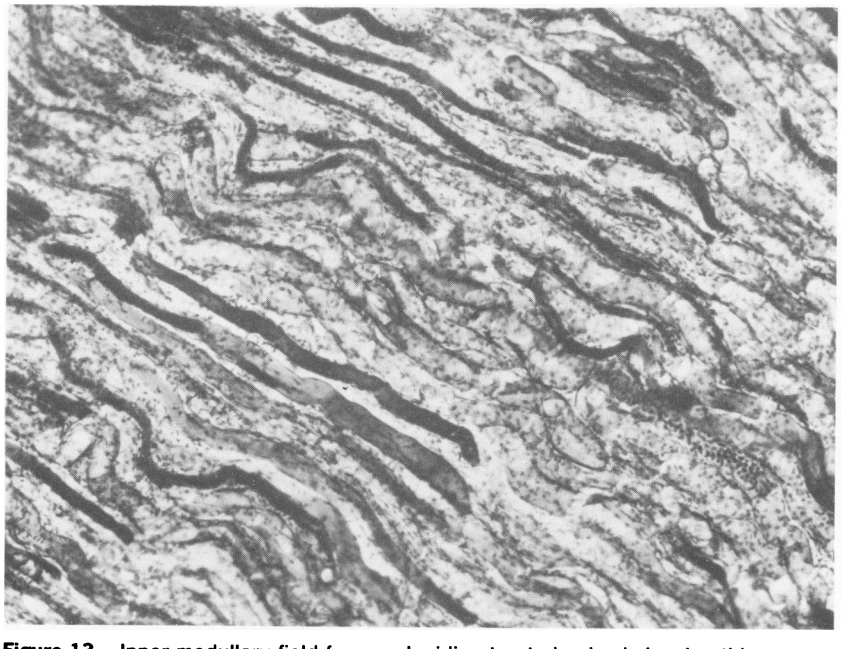

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Figure 13—Inner medullary field from a clonidine-treated animal showing thin, apparently non-occlusive hyaline casts in loops of Henle and collecting ducts. (H&E, ×400) **Figure 14**—Toluidine-blue–stained 100-μ frozen section from a non-clonidine-treated rabbit killed 6 hours after unclamping. In this outer medullary field many long, thick hyaline casts can been seen. (×80)

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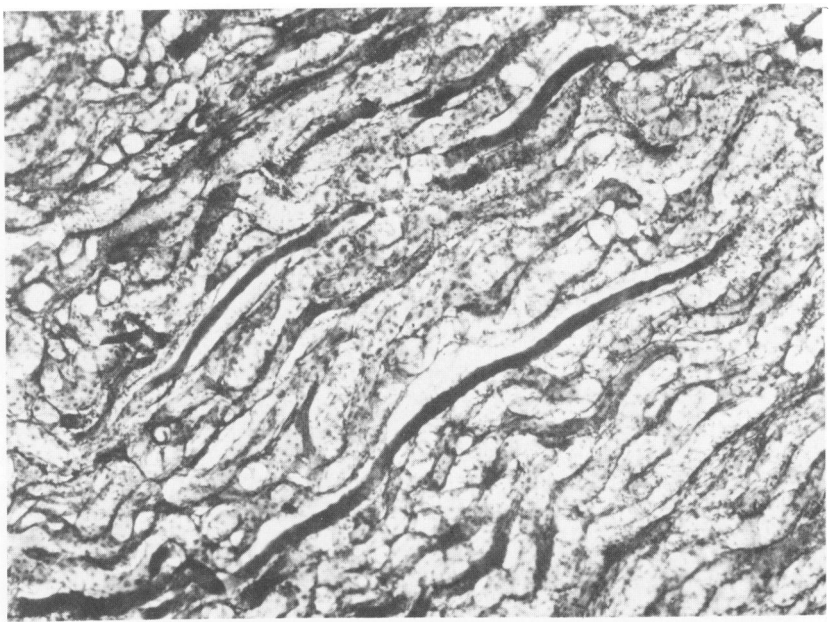

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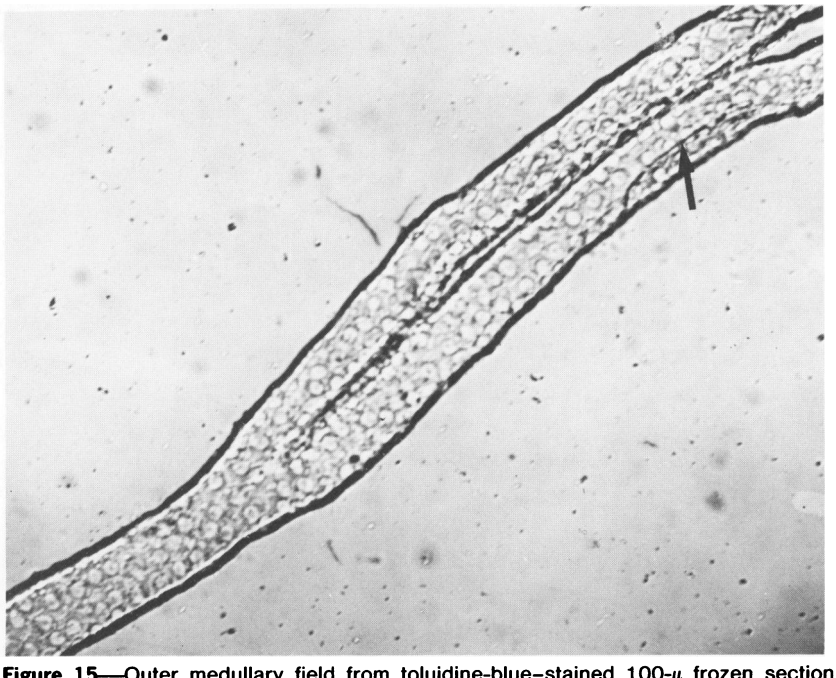

Figure 15—Outer medullary field from toluidine-blue–stained 100-μ frozen section from a clonidine-treated rabbit killed 6 hours after unclamping. The hyaline casts appear thinner than those in Figure 14, and spaces can be seen between cast edges and the tubular wall in several areas (*arrows*) (×80) **Figure 16**—Hyaline casts in two microdissected collecting tubule branches joining to become one cast as the branches merge in a non-clonidine-treated rabbit killed 6 hours after unclamping. The edge of the cast can be seen at the *arrow*. (Unstained, phase contrast, ×210)

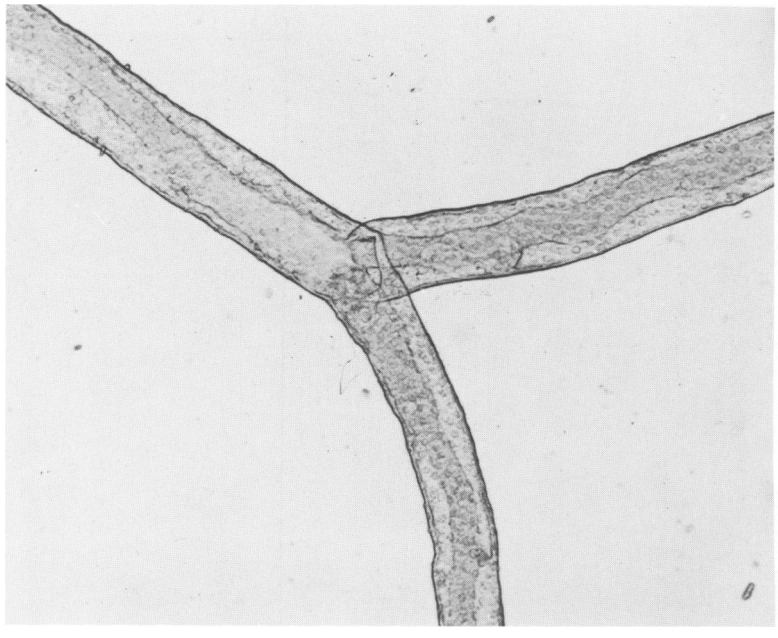

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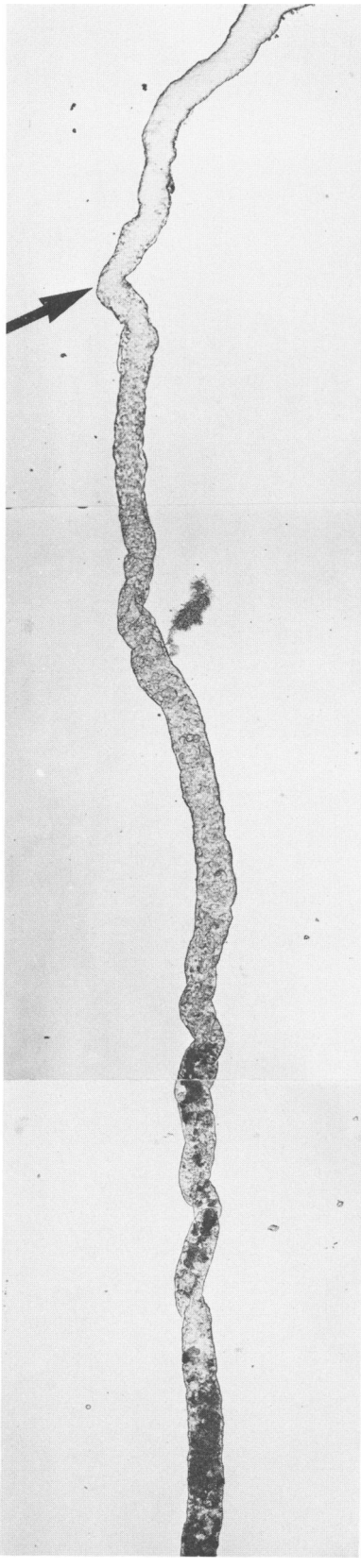
Figure 17—Thin hyaline casts in two microdissected collecting tubule branches joining to become one cast as the branches merge. The casts do not appear to obstruct the tubular lumen. From a clonidine-treated rabbit. (Unstained, ×170) **Figure 18**—Microdissected distal tubule showing black-appearing contrast material in contact with a granular cast, which in turn is in contact with a hyaline cast at the *arrow.* (Unstained, ×120)



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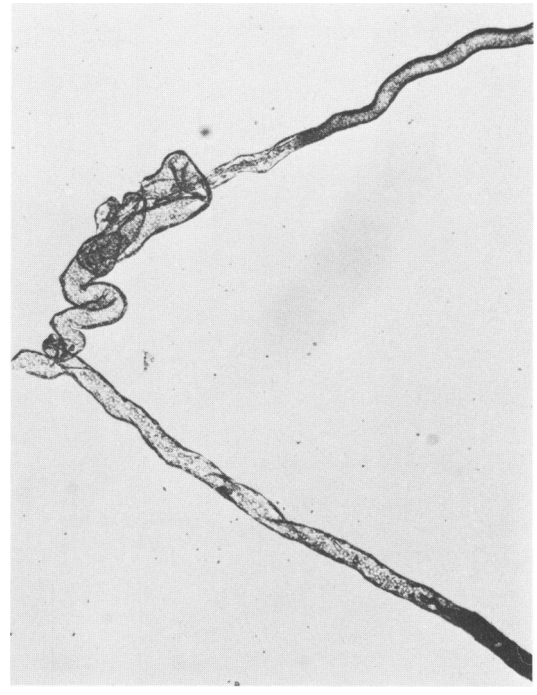

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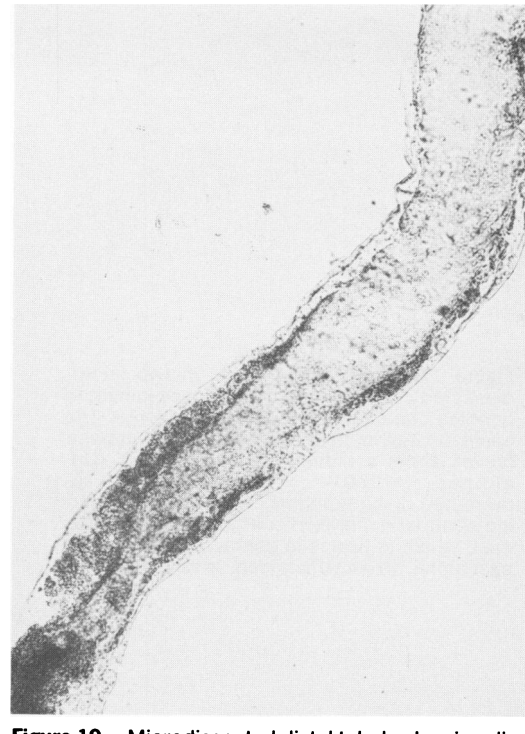

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Figure 19—Microdissected distal tubule showing disrupted barium column intermixed with cellular debris and granular cast material. The granular cast merges with a homogeneous-appearing hyaline cast at the *arrow.* (Unstained, ×120) **Figure 20**—Microdissected thick ascending limb, distal convoluted tubule, and connecting piece (early collecting duct). The barium column ends in the thick ascending limb, the distal convoluted tubule is empty, and a hyaline cast is present in the connecting piece. (Unstained, ×70) **Figure 21**—Microdissected collecting duct from a clonidine-treated rabbit showing the barium column in contact with a relatively thin hyaline cast. (Unstained, ×300)