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Vascular Permeability of the Renal Medullary Vessels in the Mouse and Rat

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The vascular permeability of the vessels of the renal medulla of normal rats and mice and potassium-deficient rats was studied by using ferritin, horseradish peroxidase and carbon particles as markers. Fenestrated endothelia are more permeable to ferritin and horseradish peroxidase than nonfenestrated endothelia. The ease with which the tracers are able to pass from the vascular lumina through the endothelia into the interstitium is directly related to the size of the tracers (horseradish peroxidase permeates most readily, followed by ferritin and carbon particles, in this order), suggesting the presence of a molecular sieving mechanism for the passage of substances through the capillary walls of the renal medulla. At the initial stage after injection of horseradish peroxidase and ferritin, these tracers pass from the medullary vascular lumina into the interstitium, not from the tubular lumina into the interstitium. It appears that the passage of ferritin and horseradish peroxidase through the vascular wall is enhanced in potassium-deficient rats (Am J Pathol 71:155–166, 1973).

THE RENAL MEDULLA plays an important role in urinary concentration.[1–4] It has delicate and complex capillary and tubular arrangements that are embedded in ground substance and are closely and intimately attended by medullary interstitial cells. As the thin loops of Henle descend toward the tip of the papilla they form a hairpin-like sharp turn and ascend toward the cortex, thereby forming multiple fine arcades. This structural arrangement of the loops is ideal for the operation of a countercurrent multiplier system.[5–7] A

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similar function, namely, a countercurrent multiplier system, has been suggested to exist in the medullary vascular bundles.[8] The capillaries in the medulla, particularly those in the papilla, form arcades just like the thin loops of Henle.[9,10] For the loop of Henle to have a countercurrent multiplier system, the ascending and the descending limbs must be differentially permeable to certain substances such as water, sodium and urea. Similarly, if there indeed is a vascular countercurrent multiplier system, a difference in vascular permeability should exist between the ascending vasa recta, which is lined with fenestrated endothelia, and the descending vasa recta, which is lined with nonfenestrated endothelia. Therefore, any difference in permeability between the fenestrated and the nonfenestrated endothelia may become important in understanding the mechanism of urine concentration both in normal and in pathologic conditions. We have attempted to study the permeability of the ascending and descending portions of these vessels using as tracers, ferritin, carbon particles and horseradish peroxidase; the findings are reported below.

Materials and Methods

Studies of Renal Medullary Vascular Permeability in Rats and Mice Using Ferritin as a Marker

Horse spleen ferritin obtained from General Biochemicals, Chagrin Falls, Ohio, was freed of cadmium by dialysis for 48 hours against 0.1 M EDTA (disodium salt) solution in 0.7 M phosphate buffer (pH 7.2) and then for 24 hours in the phosphate buffer alone, as reported by Farquhar and Palade.[11] Young adult male albino Wistar rats were anesthetized either by inhalation of ether or by intraperitoneal injection of pentobarbital, and 1 ml of the dialyzed solution containing from 43.2 mg/ml to 84 mg/ml of ferritin was injected into the right femoral vein. The time intervals from the completion of the injection to the removal of the left kidney and the number of rats used are shown in Table 1. The medulla was divided into the outer and inner medulla and cut into small pieces, which were then fixed in ice-cold veronal-buffered 1% osmium tetroxide

Table 1—Time Intervals Between Completion of Injection of Ferritin and Removal of Left Kidney in Male Albino Wistar Mice

Time after injection	No. of rats
30 sec	8
1 min	6
2 min	3
5 min	4
17 min	1
90 min	1
19 hr	2

Table 2—Time Intervals Between Completion of Injection of Ferritin and Removal of Left Kidney in Male Albino Swiss Webster Mice

Time after injection	No. of mice
15 sec	2
30 sec	6
1 min	2
19 hr	5

for 1 hour, dehydrated and embedded in the Number 1 Epon-Araldite mixture of Mollenhauer.[12] Thin sections were cut using the Porter-Blum (MT-2) microtome, doubly stained with uranyl acetate and lead citrate and examined under the Zeiss EM-9A electron microscope. The right kidney was fixed in 10% neutral formalin and embedded in paraffin. Sections were cut at the thickness of 6 μ, stained with Gomori's iron reaction method and examined by light microscopy.

Similarly, young adult male albino Swiss Webster mice were injected into the tail vein with 0.3 to 0.5 ml of the dialyzed solution containing 43.2 mg/ml to 65.0 mg/ml of ferritin. The intervals between the completion of the injection and the removal of the left kidney and the number of mice used in the experiment are shown in Table 2. The kidney was divided into the outer and the inner medulla, processed, embedded and studied by electron microscopy as described above. The right kidney was fixed in 10% neutral formalin and was treated for study by light microscopy as described above.

Studies of the Vascular Permeability of Mouse Renal Medulla Using Horseradish Peroxidase as a Marker

Young adult albino male mice (Swiss Webster strain) were anesthetized by inhalation of a 1:1 mixture of carbon dioxide and oxygen or by intraperitoneal injection of pentobarbital, and 0.1 to 0.5 ml. of Ringer's solution containing 10 mg/ml of horseradish peroxidase (Worthington Biochemical Corporation, Freehold, NJ) was injected into the tail vein. The time intervals between the completion of the injection and the removal of the left kidney and the number of mice used for the experiment are shown in Table 3. The kidney was divided into the outer and the inner medulla, and placed in a fixative containing 4% formaldehyde and 5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2) at room temperature for 5 hours, following which the tissue was washed overnight in 0.1 M cacodylate buffer (pH 7.2). Frozen sections were then cut on the cryostat at 40 μ and the sections were incubated for 3 to 10 minutes at room temperature in the Graham-Karnovsky medium.[13,14] Following the incubation, the sections were washed in three changes of distilled water and postfixed for 1 hour in veronal-buffered 1% osmium tetroxide. The sections were dehy-

Table 3—Time Intervals Between Completion of Injection of Horseradish Peroxidase and Removal of Left Kidney in Male Albino Swiss Mice

Time after injection	No. of mice
10 sec	1
30 sec	3
1 min	1
5 min	3

drated, embedded, sectioned and studied by electron microscopy as reported above.

Vascular Permeability of Renal Medulla of Potassium-Deficient Rats to Horseradish Peroxidase, Ferritin, and Colloidal Carbon

Young adult male rats (Wistar Furth strain) were fed a potassium-deficient diet obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.[15] On the seventh and fourteenth day of the experiment, 3 and 2 rats, respectively, were injected intravenously with 1 ml of Ringer's solution containing 5 to 10 mg of the horseradish peroxidase and were killed 20 seconds after the completion of the injection. The corresponding control rats were pair-fed a diet supplemented with potassium salts and were similarly injected with horseradish peroxidase and sacrificed. The renal medullae of all of these rats were studied by electron microscopy as described previously.

In addition, male Wistar Furth rats fed the potassium-deficient diet were injected intravenously with 1 ml of the dialyzed solution containing 43.2 to 50 mg/ml of ferritin and were killed 20 seconds after the completion of injection; 2 potassium-deficient rats were treated in this fashion on the seventh day of the experiment and 2 on the fourteenth day. Corresponding pair-fed control rats were similarly injected with the ferritin and sacrificed. The vascular permeability of the renal medullary vessels was studied by electron microscopy as described previously.

Furthermore, colloidal carbon (Pelikan) was dialyzed against 0.9% NaCl solution for 24 hours, and 1 ml of it was injected intravenously into 3 rats fed the potassium-deficient diet for 7 days. The pair-fed control rats were similarly injected with the colloidal carbon solution. All of these rats were killed at 5 minutes after the completion of the injection, and the vascular permeability was studied by electron microscopy.

Results

Vascular Permeability of Rat and Mouse Renal Medulla to Ferritin

The result of our observations in rats and mice were similar. The renal medullary tissue removed 30 seconds after the injection appeared the most suitable for study of the vascular permeability, since it showed an early stage of passage of the injected tracer material. By light microscopy, an iron reaction product of blue color was seen in the renal medullary capillary lumina at 30 seconds. By electron microscopy, the ferritin particles were readily seen as electron-dense particles in the medullary capillary lumina but not in any of the tubular lumina. Some of these particles were found to have reached the interstitial tissue at 30 seconds after the injection, presumably after crossing the fenestrated endothelia of the capillaries (Figures 1 and 2). The particles in the interstitium must have come from the vascular lumina and not from the tubular lumina, because they were present in the former but not observable in the latter. The ferritin particles apparently crossed the fenestrae without leaving any demonstrable morphologic alteration in the membrane lining the fenestrae. Occasionally, cytoplasmic vacuoles (presumably pinocytotic vesicles)

which contained some ferritin particles, were seen in fenestrated endothelia, but these apparent pinocytotic vesicles were few in number and did not appear to be the major route of passage of ferritin into interstitium from the capillary lumina. Since we could not see any route of passage of ferritin through the nonfenestrated endothelia other than these cytoplasmic vacuoles or pinocytotic vesicles, it appeared that the major route of passage of ferritin was through the fenestrated endothelia, not the nonfenestrated endothelia. Studies on the dynamic process of the passage of the tracer material were attempted by examining the tissues removed at the various time intervals after the injection. However, a certain difference in degree of permeability from capillary to capillary and the promptness of the ferritin to pass the capillary walls to reach the renal medullary interstitium in a matter of 30 seconds after the injection made such a study subject to considerable inaccuracy. Therefore, this matter will be left for further future study. Nineteen hours after the injection, the ferritin particles were seen in membrane-bound structures of the cytoplasm of the interstitial cell. This finding confirms the phagocytic nature of the renal medullary interstitial cells which have been reported by Morrison and Schneeberger-Keeley [15] and also by Shimamura.[16] The localization of ferritin in the interstitial cells was detected only in the outer medulla, and not in the inner medulla.

The renal medulla was divided into the outer and the inner medulla in an attempt to find out the difference of capillary permeability in these two different sites. However, whether the capillary walls of the fenestrated type in the outer medulla were more or less permeable than those vessels of the inner medulla having a similar type of endothelium was hard to ascertain by our electron microscopic study.

Vascular Permeability of Mouse Renal Medulla to Horseradish Peroxidase

The horseradish peroxidase injected into the tail vein of the mouse crossed the fenestrated endothelia from the capillary lumen into the interstitium as early as 30 seconds after the injection (Figure 3). In some areas the horseradish peroxidase was already entering the lateral intercellular space of the tubular epithelia. The capillary and tubular basement membranes, respectively, were readily permeable to horseradish peroxidase and did not appear to act as any significant barrier. Since we could not observe any horseradish peroxidase activity in any of the tubular lumina, the reaction product of horseradish peroxidase observed at the interstitium and sometimes at the lateral intercellular space of tubular epithelia most likely must have come from

the capillary lumina. In contrast to the fenestrated endothelia, the nonfenestrated endothelia were much less permeable to horseradish peroxidase (Figure 4). In the present study, we are not certain whether there was any difference in vascular permeability between the capillaries of the outer and the inner medulla.

Vascular Permeability of Renal Medulla of Potassium-Deficient Rats to Horseradish Peroxidase, Ferritin and Colloidal Carbon

The passage of horseradish peroxidase from the capillary lumina into the renal interstitium through the fenestrae was prompt and was observable with ease at 20 seconds after the completion of the injection. The horseradish peroxidase was already seen entering into the lateral intercellular space of epithelia lining loops of Henle and collecting tubules and even extended to a point immediately adjacent to the tubular lumina (Figure 5). The passage of ferritin from the vascular lumina into the interstitium through the fenestrae, however, was observable only in some places at 20 seconds after the injection, but it was not seen at most of the sites. The passage of ferritin particles and horseradish peroxidase from the capillary lumina into the interstitium through the nonfenestrated endothelia was not detectable as early as 20 seconds after the injection. The junctional sites of endothelia were specially examined for permeability to these tracer substances; it appears that the tight junctions stayed quite nonpermeable to these, though we must admit that our data on this are still insufficient. Since the tubular lumina did not contain any tracer substances such as horseradish peroxidase or feritin as early as 20 seconds after the injection, the tracers observed in the interstitial space must have come from the capillary lumina. Examination of the control rats revealed that both the ferritin and horseradish peroxidase were much harder to observe in the interstitium at 20 seconds after the injection. Since these tracer substances were easily seen in the vascular lumina and hardly identifiable in the interstitium, it seems to suggest that the capillary walls of these control rats were not as permeable as those of the potassium-deficient rats. This apparent increase of vascular permeability observed in potassium-deficient rats was more overt when horseradish peroxidase was used as a tracer, but was not so clear when ferritin particles were used. The rats fed the potassium-deficient diet for 7 days and the other group fed the same diet for 14 days all had numerous characteristic granules of potassium deficiency and we could not detect any progressive increase of vascular permeability to ferritin and to horseradish peroxidase in the latter animals.

The passage of carbon particles through the capillary wall of the potassium-deficient rats was extremely slow, showing no difference from that of the control rats. We have no convincing data which show that the particles can pass through the capillary walls even at 5 minutes after the injection, both in the normal and the experimental rats.

Discussion

Most of the passage of ferritin and horseradish peroxidase occurs through the fenestrated endothelia. Fenestrated endothelia are much more permeable to these tracer substances than nonfenestrated endothelia of the renal medulla of rats and mice, and this finding is in accord with those reported by others.[17,18] Horseradish peroxidase is a low molecular weight protein and an ideal tracer substance; its use clearly demonstrated the extension of peroxidase activity through the fenestrae from the vascular lumen into the adjacent interstitium. For at least 30 seconds after the injection of tracer substances such as ferritin into normal rats and ferritin and horseradish peroxidase into normal mice, these tracers were not observed in tubular lumina; they were seen only in the capillary lumina and in the interstitium, which strongly suggests that they reached the interstitium from the medullary vascular lumina by passing through the capillary walls and that they did not originate from the tubular lumina. The ferritin and the horseradish peroxidase injected into the potassium-deficient rats also yielded a similar result. The vascular permeability of potassium-deficient rats, however, was apparently increased, at least to horseradish peroxidase and possibly to ferritin particles also. How this finding explains the loss of urine concentrating mechanism which has been known to take place in potassium-deficient rats [15] is a difficult problem to answer, but it may well be that the increased vascular permeability interferes with the creation of the medullary concentration gradient necessary for urinary concentration.

Passage of horseradish peroxidase through the endothelial fenestrae takes place readily, in comparison to the passage of ferritin particles. The latter have a higher molecular weight than the former. Carbon particles, the largest of all tracer substances used in this study, were the least permeable through the vascular walls. The finding raises the possibility of a molecular sieving effect in the capillary walls of the renal medulla both in normal and potassium-deficient rats. Admitting that the present kind of experiments only brings tenuous evidence for a vascular countercurrent multiplier system, the existence of a

difference in permeability between the fenestrated and the nonfenestrated endothelia is consistent with the idea that a countercurrent multiplier mechanism does exist in the renal medullary vascular bundles. In addition, the possible existence of a molecular sieving mechanism of the medullary vascular walls and the rapidity of passage of small molecular weight substances [18] such as ferritin and horseradish peroxidase from the medullary capillary lumina into the interstitium are worthy of note. Furthermore, the accumulation of intravenously administered ferritin particles in the renal medullary interstitial cells observed in this study confirms the existence of the phagocytic activity of renal medullary interstitial cells that has been previously reported.[15,16]

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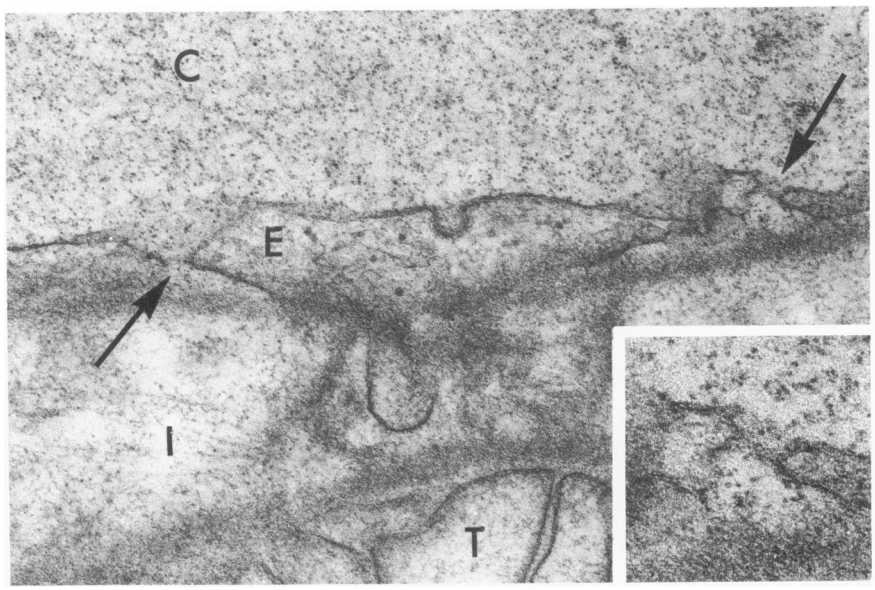
[Illustrations follow]

Legends for Figures

Fig 1—Rat inner renal medulla 30 seconds after the injection of 64 mg of ferritin reveals numerous ferritin particles in the capillary lumen. Some of these ferritin particles give the appearance of crossing the endothelium through the fenestrae from the capillary lumen into the interstitium (*arrow*). The rat was anesthetized with pentobarbital. *C* = capillary lumen, *E* = capillary endothelium, *I* = interstitium, *T* = tubular epithelium (× 52,000). **Inset**—Higher magnification of a part of the endothelium illustrating ferritin particles both on the luminal and interstitial sides of the fenestra (× 102,000).

Fig 2—Mouse outer renal medulla 30 seconds after injection of 21.5 mg of ferritin reveals ferritin particles apparently crossing through the endothelial fenestrea from the capillary lumen into the interstitium (*arrow*). The mouse was anesthetized with pentobarbital. *C* = capillary lumen, *E* = endothelium, *I* = interstitial cell (× 57,000).

Fig 3—Mouse inner renal medulla 30 seconds after injection of 4 mg of horseradish peroxidase into the tail vein shows extension of the horseradish peroxidase activity from the capillary lumen into the interstitium through the fenestrae (*arrow*). The capillary lumen (*C*) is filled with an intense reaction product of horseradish peroxidase. The mouse was anesthetized with pentobarbital. *E* = endothelium, *I* = interstitium, *T* = tubular epithelium (× 19,000).



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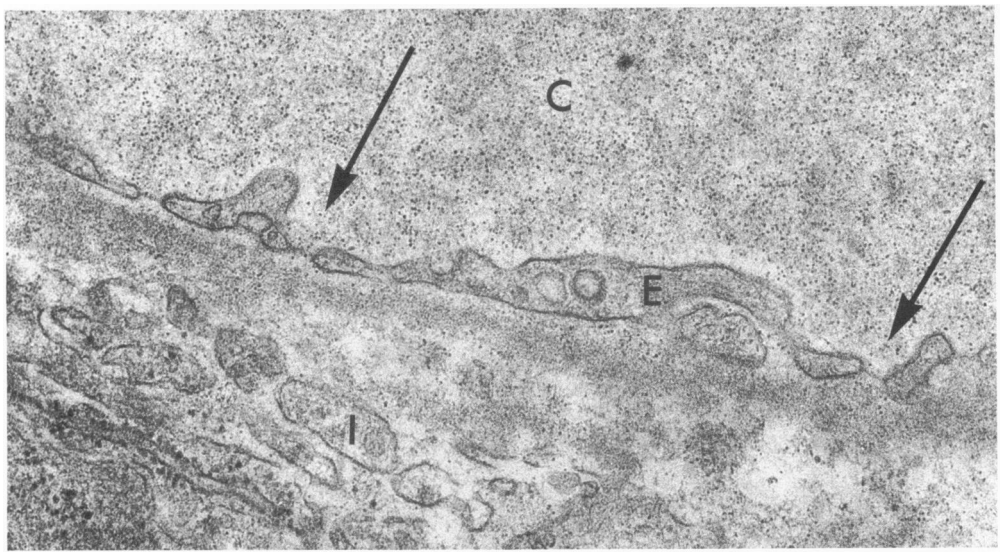

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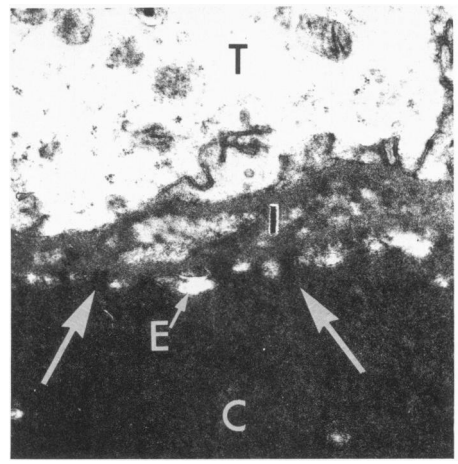

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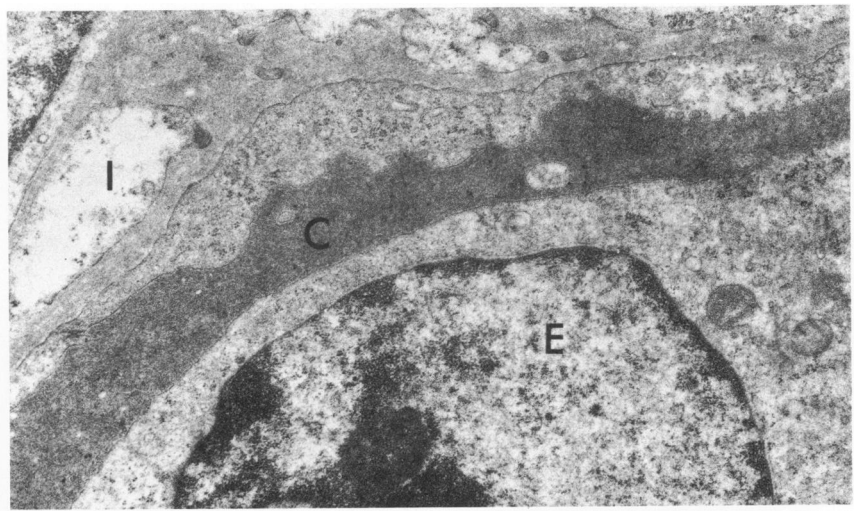

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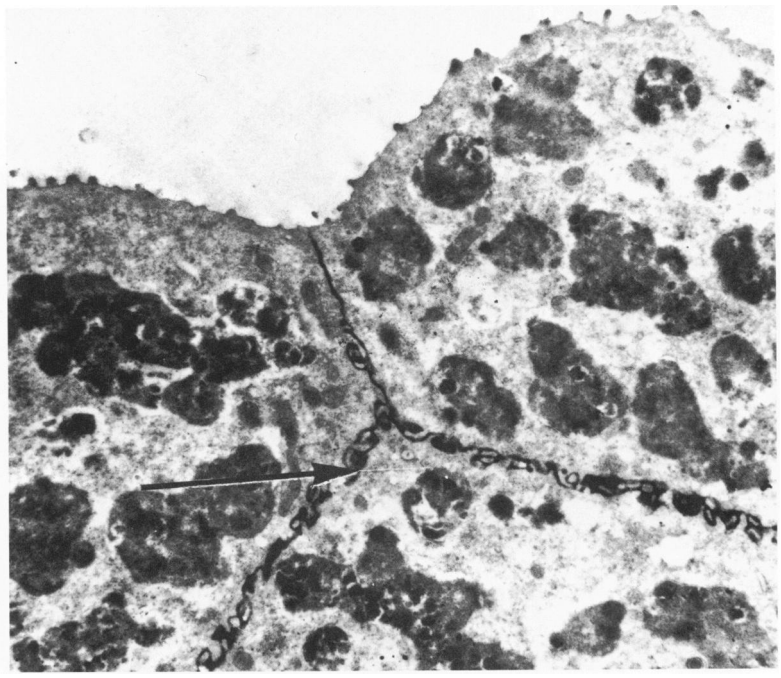

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Fig 4—Mouse outer renal medulla 30 seconds after injection of 3 mg of horseradish peroxidase into the tail vein shows the enzyme activity in the capillary lumen, but no demonstrable enzyme activity is seen in the adjacent interstitium. The mouse was anesthetized with inhalation of 1:1 mixture of CO_2 and oxygen. *C*=capillary lumen, *E*=endothelium, *I*=interstitial cell (× 29,700). **Fig 5**—Rat inner renal medulla 20 seconds after injection of 10 mg of horseradish peroxidase into the tail vein shows the enzyme activity within the lateral intercellular space of collecting tubular epithelia (*arrow*). Notice that the activity extends immediately adjacent to the tubular lumen. The latter contains no demonstrable enzyme activity. The collecting tubular epithelia are loaded with cytoplasmic granules characteristic of potassium-deficient rats. This electron micrograph is from a rat that was on potassium-deficient diet for 2 weeks (× 7800).