Gentamicin and Tobramycin Nephrotoxicity

A Morphologic and Functional Comparison in the Rat

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Fischer 344 rats were treated with tobramycin or gentamicin, 40 mg/kg/day, for up to 10 days or with tobramycin, 120 mg/kg/day, for up to 14 days. Serum creatinine and BUN at the time of sacrifice were determined, and kidney tissues were examined by light and electron microscopy. Rats receiving gentamicin demonstrated progressive renal proximal tubular necrosis which was nearly universal at the end of 10 days. Their BUN and creatinine levels rose progressively over the same period. Even at the higher dosage, tobramycin therapy resulted in only rare foci of proximal tubular necrosis and minimal elevation of BUN and creatinine. Although they occurred later and were substantially less severe, the ultrastructural changes induced by tobramycin were the same as those seen following gentamicin administration. These results indicate that the mechanism of tobramycin-induced renal injury is probably similar to that of gentamicin and that tobramycin is significantly less nephrotoxic in this experimental model. (Am J Pathol 93:137–152, 1978)

TOBRAMYCIN is a comparatively new aminoglycoside antibiotic derived from *Streptomyces tenebrarius*. Its antibacterial spectrum is similar to that of gentamicin, except that it is probably more effective against infections due to *Pseudomonas aeruginosa*.[1,2] The nephrotoxic potential of tobramycin has been comparable to or less than that of gentamicin at equivalent doses in both clinical [3,4] and experimental [5] trials. In this study we compared the changes in renal morphology and the levels of azotemia induced by gentamicin and tobramycin in rats. Susceptibility to the nephrotoxic effects of gentamicin is variable among mammalian species and among strains of rats.[6-8] The Fischer 344 rat was used in this study because its proved high sensitivity to the drug was thought likely to maximize differences between gentamicin and tobramycin.

Materials and Methods

Groups of adult male Fischer 344 rats weighing between 175 and 250 g (7 to 10 weeks old) were housed individually in cages, fed standard rat chow, and given free access to

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Supported in part by Grant GM-22928 from the National Institutes of Health and by grants from the Eli Lilly Company, the Veterans Administration Hospital, and the Oregon Heart Association.

Accepted for publication June 2, 1978.

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0002-9440/78/1010-0137$01.00

Table 1—Blood Urea Nitrogen (BUN), Serum Creatinine (Creat), Initial Weights, and Extent of Severe Tubular Injury of Treated and Control Animals

Days of treatment		Control	Gentamicin (40 mg/kg/day)	Tobramycin (40 mg/kg/day)	Tobramycin (120 mg/kg/day)
3	BUN*	18.9 ± 2.2 (4)†	22.2 ± 2.9 (3)	25.0 ± 1.4 (3)	23.5 ± 2.0 (4)
	Creat*	0.58 ± 0.20 (4)	0.59 ± 0.05 (3)	0.67 ± 0.08 (3)	0.80 ± 0.12 (4)
	Weight‡	201 (162-224)	196 (194-205)	261 (255-265)	159 (152-166)
	Injury§	0	0	0	0
7	BUN	18.9 ± 2.5 (4)	39.4 ± 14.5 (3)	23.9 ± 2.4 (3)	22.1 ± 1.8 (4)
	Creat	0.57 ± 0.05 (4)	1.42 ± 0.58 (3)	0.86 ± 0.03 (3)	0.75 ± 0.06 (4)
	Weight	200 (170-252)	201 (98-202)	251 (245-255)	162 (154-170)
	Injury	0	2	1	1
10	BUN	18.1 ± 2.0 (4)	127.6 ± 17.3 (3)	26.7 ± 6.5 (3)	24.7 ± 4.9 (4)
	Creat	0.54 ± 0.05 (3)‖	2.8 ± 0.75 (3)	0.74 ± 0.11 (3)	0.70 ± 0.12 (4)
	Weight	198 (164-252)	197 (195-202)	256 (235-282)	154 (146-170)
	Injury	0	3-4	1-2	2
14	BUN	20.1 ± 3.7 (2)			21.8 ± 4.4 (4)
	Creat	0.65 ± 0.10 (2)			0.73 ± 0.13 (4)
	Weight	168 (162 & 174)			168 (154-174)
	Injury	0			2 (regeneration)

* Expressed as mg/dl ± standard deviation
† No. of animals
‡ Expressed as mean weight and range in grams
§ Grades 0-4 based on criteria listed in Table 2
‖ One value lost due to technical error

water. The sodium content of the chow was 0.49% dry weight. The mean initial weight of animals in each treatment group is included in Table 1. The animals were kept in this environment for at least 3 days before the beginning of any experiment. A total of 24 rats were treated for up to 10 days with 40 mg/kg/day of either gentamicin or tobramycin administered in equally divided doses at 8 AM and 4 PM. The drugs were administered subcutaneously in a total volume of 0.5 ml using water as diluent. The rats were killed 1 hour after the last dose. In a separate trial, 16 animals were treated in the same manner with tobramycin, 120 mg/kg/day, for up to 14 days. Control animals were injected with 0.5 ml of saline twice a day. Three rats in each 40 mg/kg/day aminoglycoside treatment group and 2 control animals were killed after the 3rd, 7th, and 10th days of the experiment. In the high-dose tobramycin group, 4 treated and 2 control animals were killed after the 3rd, 7th, 10th, and 14th days.

At the time of sacrifice, animals were anesthetized with ether and opened with a ventral midline incision. Blood was obtained by cardiac puncture for the determination of blood urea nitrogen (BUN) and serum creatinine concentrations. The systemic vasculature was perfused through the left ventricle with 0.1 M phosphate buffer containing 1% glucose and 1% procaine until the kidneys blanched. One kidney was then removed and fixed in 10% formalin for light microscopy. The second kidney was fixed *in situ* by cardiac perfusion with 2% glutaraldehyde in 0.1 M phosphate buffer. All perfusion solutions were approximately 300 milliosmolar.

After glutaraldehyde perfusion, renal cortical tissues were cut into 1-cu-mm blocks, immersed for 2 hours in the same glutaraldehyde solution, and postfixed for 2 hours in 2% osmium tetroxide in 3% sucrose and 0.05 M cacodylate buffer, pH 7.4. Tissue blocks were rapidly dehydrated in graded ethanol solutions, embedded in Araldite 502 according to the method of Luft,[9] and sectioned with a diamond knife on a Porter-Blum MT2-B ultramicrotome or LKB ultrotome. Thin sections stained with uranyl acetate and lead citrate were examined at 60 kV in a Philips EM-200 microscope. One-micron-thick sections of Araldite-embedded tissue were also examined by light microscopy after toluidine blue staining.

Tissue from all animals of the 40 mg/kg/day aminoglycoside treatment groups and the corresponding control animals were examined by light and electron microscopy. In the high-dose tobramycin treatment group, renal tissues of all animals, including controls, were examined by light microscopy and tissues from 1 control and 1 treatment animal at each sacrifice interval were examined by electron microscopy.

BUN and serum creatinine were determined by standard laboratory methods.

Results

BUN and Creatinine Studies

The results of BUN and serum creatinine determinations at the time of sacrifice are shown in Table 1.

As expected, gentamicin was quite nephrotoxic. The BUNs and creatinines were all significantly elevated after 7 days of treatment, and the animals had become severely azotemic by the end of the 10th day. The BUN and creatinine levels varied considerably among the animals in the 7- and 10-day sacrifice groups.

Animals treated with 40 mg/kg of tobramycin per day showed evidence of mild nephrotoxicity, as indicated by modest but significant elevations of BUN and creatinine, which remained stable throughout the course of treatment.

Animals receiving tobramycin, 120 mg/kg/day, manifested similar chemical evidence of mild nephrotoxicity. The BUN and creatinine levels were not significantly different from those in the lower dose tobramycin treatment groups.

Morphologic Studies

The light and electron microscopic appearances of all control animals conform to those described by Rouiller [10] and Ericsson and Trump.[12]

General Description of Renal Pathology

The tubular injury induced by tobramycin was qualitatively the same as that caused by gentamicin but significantly less severe.

In each treatment group the earliest ultrastructural changes were detectable in most proximal tubule cells after 3 days. Most conspicuous was the increased number of membrane-bound cytoplasmic vacuoles; most were cytosomes with homogeneous electron-dense matrices, and some were cytosegresomes containing membrane fragments and remnants of identifiable cellular organelles such as mitochondria. These vacuolar structures, hereafter referred to collectively as cytosomes, were most numerous in the cells of the proximal tubules. Most contained one or more prominent aggregates of concentrically whorled membranes (myeloid bodies) (Figure 1). Similar structures were only rarely observed in proximal tubule cells in control tissues and were never as large or as clearly defined as those in the treated animals.

Later, when other changes of cell injury could usually be observed, cytosomes had become very numerous, sometimes virtually filling the cell. Some were quite large and irregular, appearing to have formed by coalescence of smaller vacuoles. Many contained mixtures of organelle fragments, membranes, amorphous debris, and large complex myeloid bodies. Occasionally, cytosomes appeared to have ruptured, their contents spilling into the surrounding cytoplasm.

After 7 or 10 days of aminoglycoside therapy, myeloid bodies were apparent in small numbers in cytosomes in other parts of the kidney such as distal and collecting tubules, but subsequent injury was never observed at these sites. Glomerular alterations were never identified. In many tubules, myeloid bodies appeared to be passing into the intervillous spaces from the apical cytoplasm as if they were being extruded. Consistent with this possibility was the observation of dense collections of myeloid bodies in the tubular lumina of kidneys in which there was no evidence of cell disruption.

As described, cytosomal changes occurred in most cells of the proximal

tubules before any other evidence of injury was observed. These changes were similar in appearance and extent with both drugs at all dosages. When further injury occurred, the pattern was similar for all groups, but the severity and the rates at which they occurred varied with drug and dosage. Unlike the cytosomal changes, these later alterations were always focal, predominantly involving segments of the pars convoluta. They usually began with mitochondrial swelling (Figure 2). The mitochondrial matrices became coarse and floccular; the cristae became disordered and swollen. Segments of the endoplasmic reticulum became dilated. Cytoplasm was edematous and the cells appeared swollen. Ribosomal particles became dispersed and ill defined. By light microscopy, cells were swollen with coarsely granular or clumped cytoplasm.

Later changes of injury were probably irreversible. The cytoplasm became exceedingly edematous and completely disarrayed, containing fragments of mitochondria, lysosomes, free myeloid bodies, and unidentifiable organelle material (Figure 3). The cell membrane became irregular, often forming a series of folds and indentations with few microvilli. Tight junctions with neighboring cells were lost. Nuclei became edematous with heterochromatin clumped densely at their margins. At the times when cells manifested this extent of injury, other cells in the same tubules were rupturing and desquamating. Cytoplasmic debris and myeloid bodies filled tubular lumina. After tubular cells were sloughed, adjacent intact cells appeared to spread out and flatten over broad areas of the tubular basement membranes. As this happened the microvilli separated and appeared less numerous. Focal cell injury could usually be found in segments of the partes rectae at this stage.

The patchy nature of cell injury was striking by both electron and light microscopy (Figure 4). Adjacent segments of proximal tubules were often only minimally injured or showed only the cytosomal changes. Within the same tubules, the extent of cell change was highly variable. Even in the animals treated for 10 days with gentamicin, when proximal tubular necrosis had become nearly universal, there were scattered solitary residual proximal tubule cells with minor cytosomal changes and a few myeloid bodies. These cells were usually flattened on an otherwise denuded basement membrane.

After 7 days of treatment with either drug, perivascular infiltrates of mononuclear inflammatory cells were usually conspicuous in the cortices and at the corticomedullary junctions. These infiltrates were consistently heaviest in those kidneys which were most severely injured.

By 10 days of amnioglycoside administration there was a perceptible increase in mitotic activity throughout the proximal tubules. This in-

Table 2—Criteria for Grading Proximal Tubule Injury by Light Microscopy

Grade	Criteria
0	Normal
1	Desquamation of tubular epithelial cells in small foci (less than 1% of total tubule population involved)
	Areas of focal granulovaculor epithelial cell degeneration and granular debris in tubular lumina with or without evidence of desquamation
2	Tubular epithelial necrosis and desquamation are prominent but involve less than half of cortical tubules.
3	More than half of proximal tubules are undergoing necrosis and desquamation, but intact tubules are easily identified.
4	Total or near total proximal tubular necrosis

crease, as described below, was followed by substantial regenerative activity in the high-dose tobramycin animals which were killed after 14 days.

The extent of proximal tubular injury severe enough to be apparent by light microscopy was graded according to the criteria listed in Table 2. The results of this evaluation are provided in Table 1.

Gentamicin, 40 mg/kg/day

After 3 days of therapy, the renal cortices appeared normal by light microscopy. On electron microscopic examination, proximal tubule cells manifested the cytosomal changes described above (Figure 1). There was no evidence of other significant alteration.

After 7 days, all stages of cellular injury were apparent by light and electron microscopy (Figures 3 and 4). By light microscopy the extent of necrosis varied from less than 10% to nearly 50% of the proximal tubule population. Cytoplasmic and nuclear debris filled many tubular lumina, and corticomedullary lymphocytic infiltrates were present. By electron microscopy, many tubules were still showing only cytosomal changes.

After 10 days of gentamicin therapy, at least 75% and, in many sections, nearly all tubules of the outer two thirds of the renal cortices were necrotic (Figure 5). Collecting tubules, distal tubules, and rare straight segments of the proximal tubules formed small islands in this zone of destruction. The normal corticomedullary and medullary architecture prevailed. By electron microscopy, most of the proximal tubules were devoid of epithelium. Most, but not all, cells which were not frankly necrotic displayed the changes of severe, irreversible injury already described.

Tobramycin, 40 mg/kg/day

After 3 days of drug administration, the light microscopic appearance of the renal cortices was essentially the same as that of controls. Ultrastructurally, proximal tubular epithelial cells displayed characteristic cytosomal changes (Figure 6).

After 7 days of treatment, rare small foci of convoluted proximal tubule necrosis could be found on careful light microscopic examination of the mid and outer cortices. By electron microscopy, even with generous tissue sampling, necrotic proximal tubular cells were seldom observed. Usually the most extreme changes consisted of mitochondrial swelling and segmental dilatation of the endoplasmic reticulum. Dense collections of cytoplasmic debris in myeloid bodies were sometimes found within tubular lumina, suggesting that cell disruption had occurred at other sites in the nephron. Cytosomal changes were similar to those noted at 3 days.

After 10 days of drug administration, proximal tubular necrosis was only slightly more extensive than that observed 4 days earlier (Figure 7). Again, direct ultrastructural evidence of frank cellular necrosis was difficult to find. Most proximal tubules appeared no different than those observed at earlier intervals (Figure 2). Cytosomal changes were more pronounced.

Tobramycin, 120 mg/kg/day

After 3 days of drug administration, the renal cortex was essentially normal by light microscopy. By electron microscopy, cytosomal changes of the proximal tubule cells were not detectably different from those of the 3-day animals in the other aminoglycoside treatment groups. There was no other evidence of cellular injury.

After 7 days of treatment, there were rare foci of proximal tubule necrosis by light microscopy. Cytosomal changes were similar to those seen in the 3-day animals except that these vacuoles were larger and more irregular. Mitochondria in many cells were swollen. No epithelial necrosis was identified by electron microscopy.

After 10 days, proximal tubular necrosis was more conspicuous. However, the proportion of necrotic tubules was judged not to exceed 5% of the total proximal tubular area in any section. Epithelial cells of some intact tubules were vacuolated. Inflammatory infiltrates were present. By electron microscopy, all stages of cell injury were evident.

After 14 days, proximal tubular necrosis appeared no more extensive than 4 days before. Many tubular segments were lined by low cuboidal epithelial cells having large vesicular nuclei and basophilic granular cytoplasm signifying regenerative activity. The mitotic rate was high, particu-

larly in the areas of the junction between convoluted and straight segments of the proximal tubules. Few tubules contained necrotic debris. Mononuclear inflammatory infiltrates were conspicuous. Ultrastructurally, most tubular changes were comparable to those observed at 10 days (Figure 8). Many cells contained massive lysosomes and displayed the sorts of organelle injury already described. As indicated by light microscopy, many proximal tubules were lined by regenerating epithelial cells (Figure 9). They were generally low cuboidal or squamoid cells with simplified or absent microvillous borders, few organelles, and polyribosome-rich cytoplasm. These cells usually contained myeloid bodies.

Discussion

The renal injury induced by tobramycin is morphologically similar to that observed with gentamicin but is significantly less severe. Like gentamicin, tobramycin primarily affects the proximal tubule cell. Initially, cytosomes and cytosegresomes increase in numbers; many contain heterogeneous collections of granules, membranes, and increasingly complex aggregates of myeloid bodies. With time these vacuoles appear to coalesce, becoming large and irregular. These changes probably result from accelerated autophagic activity in response to focal cellular injury and/or interference with normal lysosomal digestive function.[12,13]

With both drugs, cytosomal changes are followed by evidence of progressive cell injury, including swelling and alteration of the organelles, loss of ribosomes, and cell edema progressing to a state of cytoplasmic chaos, organelle disruption, and, finally, to cell rupture and desquamation. Unlike the lysosomal changes which affected nearly all proximal tubular epithelial cells, these later manifestations of cell injury were characteristically focal; their extent varied with the drug, dose, and duration of treatment. Tobramycin caused much less tubular damage than gentamicin, even at a three times higher dose.

After 14 days of high-dose tobramycin administration, there was substantial proximal tubular epithelial regeneration. We have observed the same phenomenon in Fischer rats which had received gentamicin, 40 mg/kg daily, for 2 weeks.[14] The appearance of regenerating tubule cells despite continued drug administration raises several questions about their origin and ultimate fate. It seems probable that the cells arise from the relatively unaffected residual cells that can be found in proximal tubules even at the height of severe aminoglycoside-induced injury.[14] Regenerating cells develop some of the cytosomal changes described, but in these short trials they rarely display evidence of subsequent injury. We do not

know whether this apparent resistance to the drugs is related to the metabolic immaturity of the regenerating cells, is a characteristic of a subpopulation of tubule cells, or results from other phenomena such as acquired immunity to the drugs. In the first instance, tubular epithelial cells would probably become increasingly sensitive to the drug as they matured. With prolonged drug administration, the renal cortex would become a patchwork of active necrosis and regeneration, resulting perhaps in progressive chronic interstitial fibrosis and inflammation. Recent observations by Cuppage et al [15] are interesting in this regard. They found that neither Fischer nor Sprague-Dawley rats were azotemic after 28 days of gentamicin administered at a daily dose of 40 mg/kg. By light and electron microscopy there were focal proximal tubule necrosis and regeneration. The animals were not studied at earlier intervals, but, based on the present study and others,[8,9,14] it is reasonable to assume that the Fischer rats developed subtotal proximal tubule necrosis and severe azotemia during the second week of treatment, followed by significant recovery of tubule structure and glomerular filtration.

If the sensitivity of proximal tubule cells to the nephrotoxic effects of aminoglycosides were related to cell maturity, the focal, irregular distribution of proximal tubule injury initially induced by the drugs might reflect the local patterns of normal epithelial cell turnover. The sensitivity would increase with increasing age of the cell. The overall susceptibility of an animal to the nephrotoxic effects of the drugs may vary with its age. This is of potential importance in the comparison of the two tobramycin dosage groups which, on the basis of weights, had a mean age difference of 2 to 3 weeks. The younger animals, having received the higher dosage, may have been better able to withstand the drug, thus minimizing the functional and morphologic differences between the two groups.

If, however, regenerating cells derive their resistance to the toxic effects of gentamicin from a subgroup of tubular epithelial cells from which they arise, maturation might not be accompanied by increasing drug sensitivity and the cortex might be reconstituted with little or no further injury, even while drug administration continues. It is not likely, however, that resistance to the drug is absolute. If it were due to a slower rate of intracellular drug accumulation, the effect would be to delay rather than prevent cell injury if administration of the drug were continued and would probably result in a histologic picture of mixed proximal tubule necrosis and repair.

The possibility that antibodies to aminoglycoside antibiotics or some other adaptive phenomenon develop and subsequently limit the toxic effects of the drug on the kidney has not been studied.

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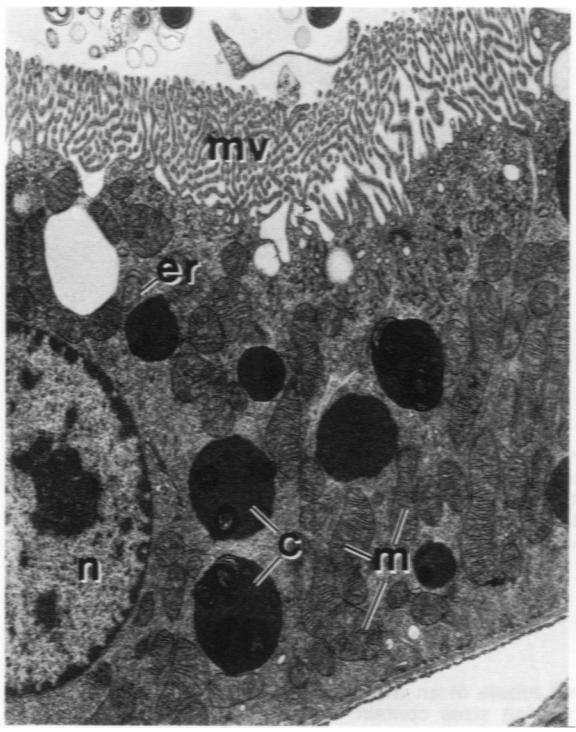
[*Illustrations follow*]

Figure 1—Electron micrograph of a proximal tubule of a rat receiving gentamicin, 40 mg/kg/day for 3 days. Myeloid bodies are present in several cytosomes (*c*) and in the tubular lumen. Other cellular components, including mitochondria (*m*), segments of endoplasmic reticulum (*er*), the microvillus border (*mv*) and nucleus (*n*), are shown without abnormalities. (× 14,600) (with printing reduction of 7%)

Figure 2—Electron micrograph of a proximal tubule cell from an animal that received tobramycin, 40 mg/kg/day for 10 days. Significant cell injury is indicated by the pronounced swelling of the mitochondria. In each the matrix is pale and unevenly dispersed. Large cytosomes contain numerous myeloid bodies. The cytosomal matrices are light and focally coarsened and granular. Their contours are irregular and the limiting membranes are ill defined. Cellular changes were, in most other areas, less severe than shown here. (× 14,600) (with printing reduction of 7%)

Figure 3— Electron micrograph of a proximal tubule after gentamicin, 40 mg/kg/day for 7 days. The epithelium is severely injured. A cell with apparently intact membrane but with marked cytoplasmic damage and disorganization (*a*) clings to the basal lamina. A much less severely injured cell (*b*) is flattened on the basal lamina. The lumen contains cytoplasmic debris and myeloid bodies. (× 14,600) (with printing reduction of 7%)

Figure 4—Photomicrograph of the outer renal cortex from an animal which received gentamicin, 40 mg/kg/day for 7 days, demonstrating the focality of early tubular necrosis. Some of the proximal tubules are devoid of viable epithelium; their lumina contain nothing but necrotic debris. Many others show little evidence of injury. The interstitium contains collections of lymphocytes. (H&E, × 300) (with printing reduction of 7%)



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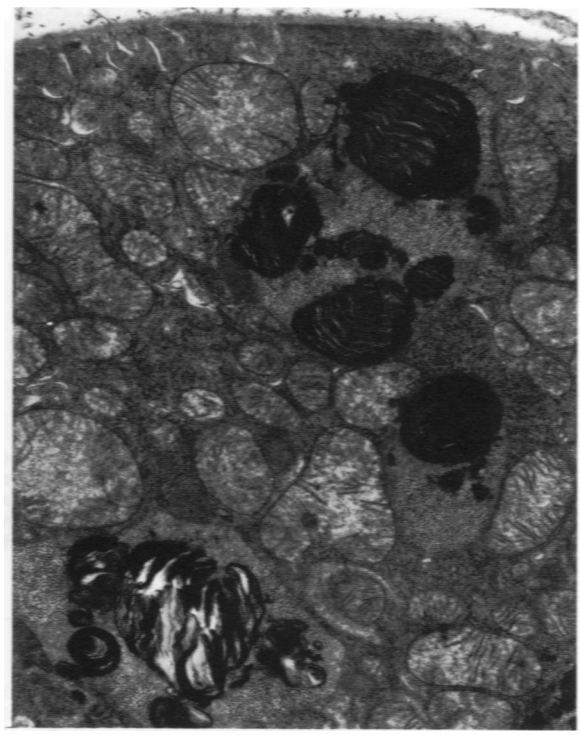

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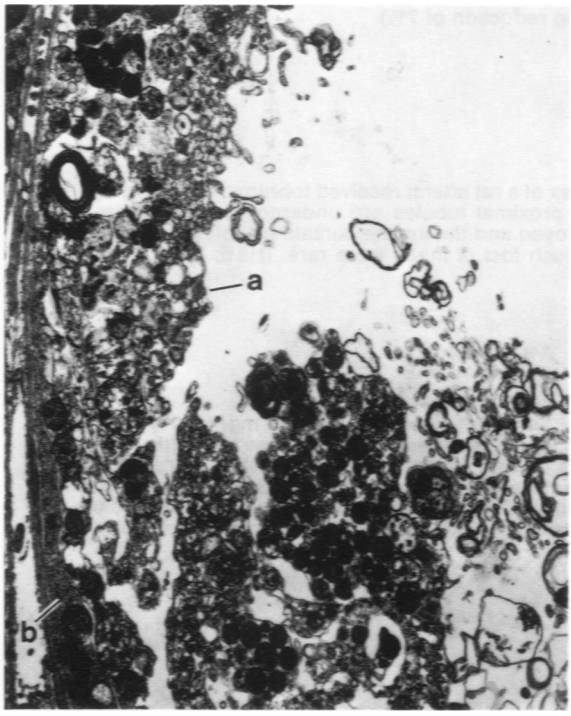

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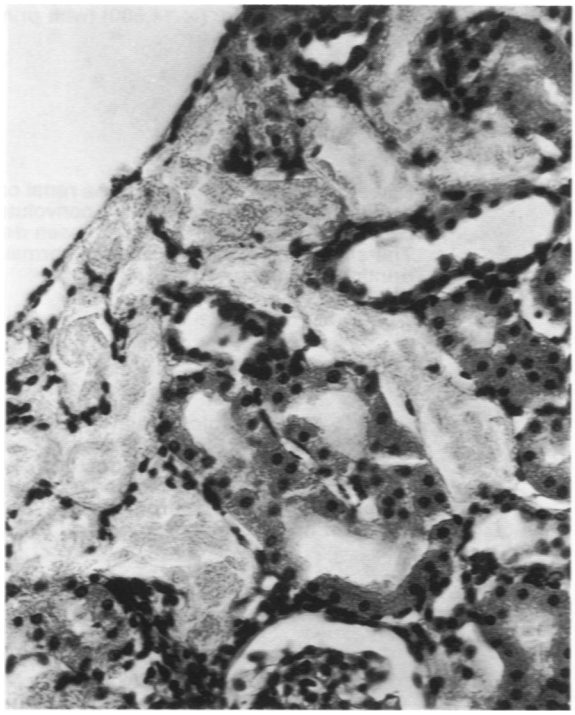

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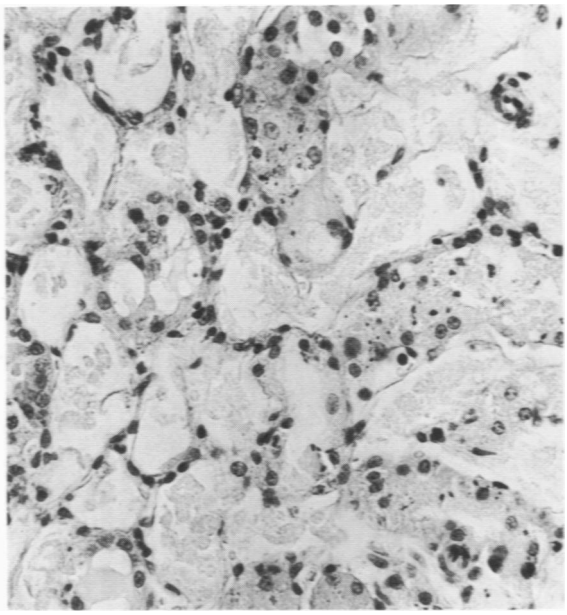
Figure 5—Photomicrograph of the renal cortex of an animal treated with gentamicin, 40 mg/kg/day for 10 days. Proximal tubular necrosis is nearly complete. Almost all tubules are devoid of epithelium. Lumina are filled with cytoplasmic and nuclear debris. Mononuclear inflammatory cells are scattered throughout the interstitium. Intact tubule cells are vacuolated and have coarsely granular cytoplasm. (H&E, × 300) (with printing reduction of 7%)

Figure 6—Electron micrograph of a proximal tubule of an animal treated with tobramycin, 40mg/kg/day for 3 days. Cytosomes of varying sizes contain myeloid bodies. One has apparently ruptured, leaving a cluster of myeloid bodies lying in the cytoplasm beside a remnant of cytosomal membrane (*arrows*). None of the other components of the cell is significantly altered. (× 14,600) (with printing reduction of 7%)

Figure 7—Photomicrograph of the renal cortex of a rat after it received tobramycin, 40 mg/kg/day for 10 days. Segments of convoluted proximal tubules are undergoing necrosis (*top center*). The epithelial cells have been destroyed and the lumina contain cytoplasmic debris. The surrounding tubules appear normal. Such foci of injury were rare. (H&E; × 300) (with printing reduction of 7%)

Figure 8—Electron micrograph of a proximal tubule after tobramycin, 120 mg/kg/day for 10 days. The lumen is filled with myeloid bodies. Note their presence at the base of the microvilli, and the absence of luminal necrotic debris. These findings suggest that this extracellular accumulation of the myeloid bodies resulted at least in part from their extrusion from proximal tubule cells rather than from cellular disruption. The cells contain numerous myeloid bodies but show no evidence of significant cell injury. (× 14,600) (with printing reduction of 7%)



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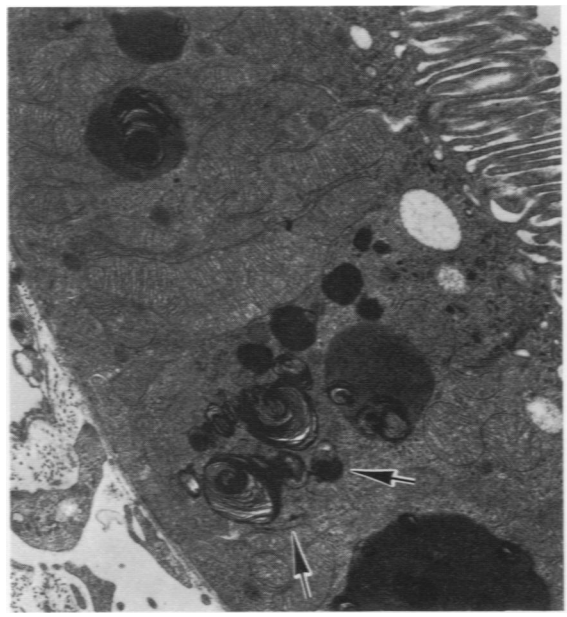

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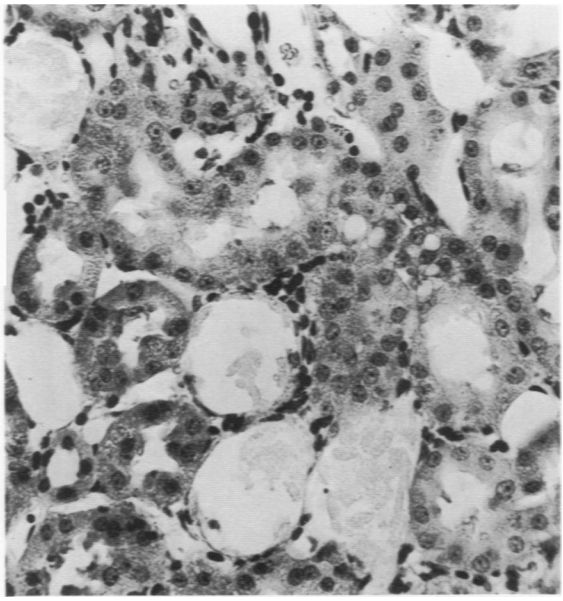

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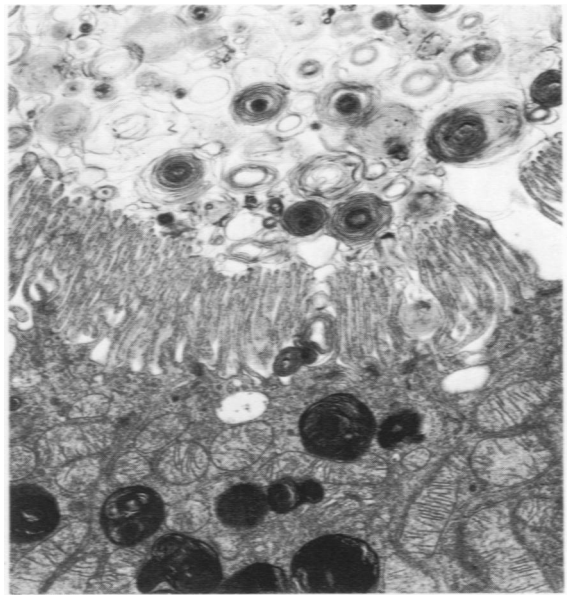

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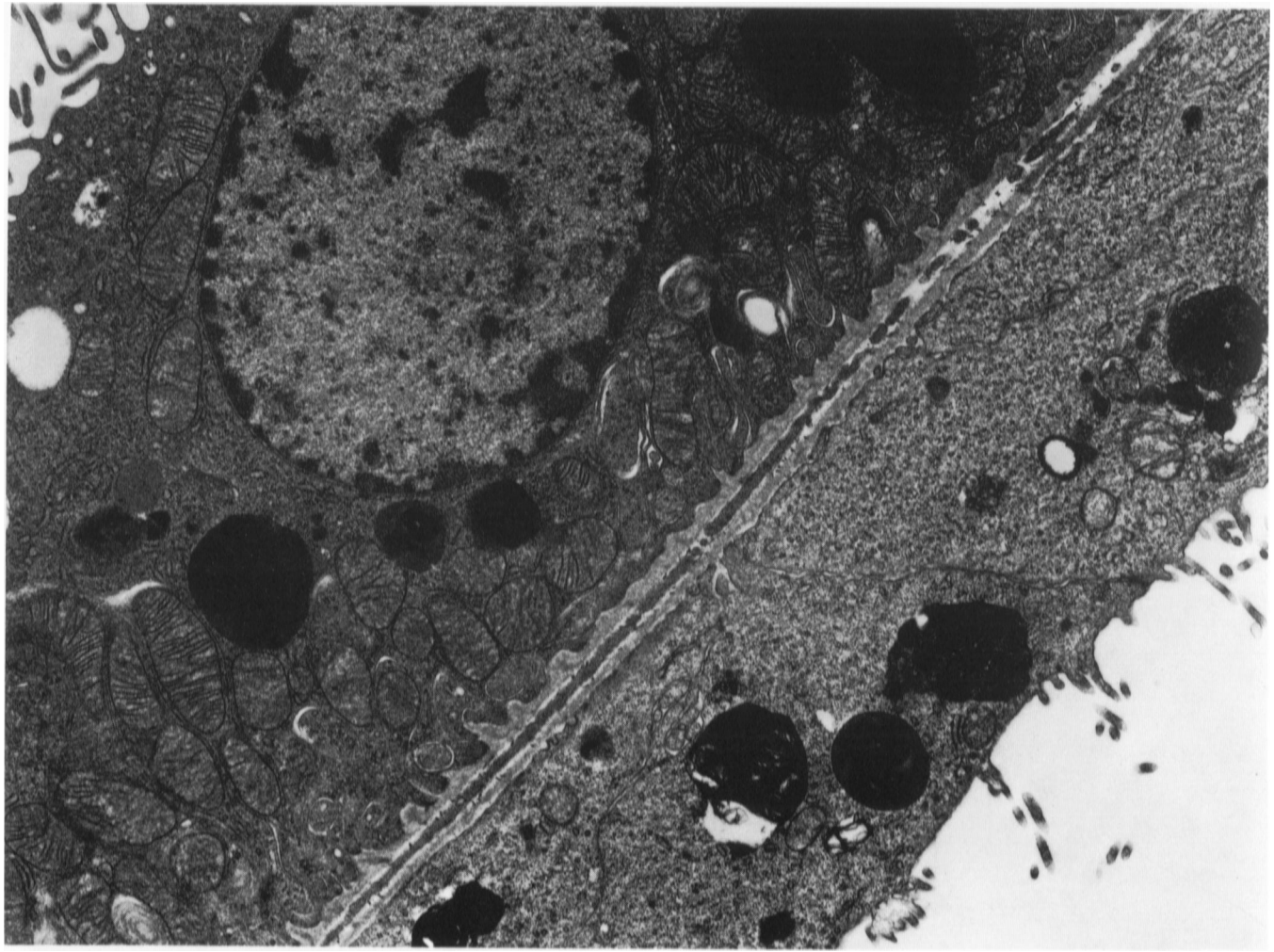

Figure 9—Electron micrograph of proximal tubules from an animal treated with tobramycin, 120 mg/kg/day for 14 days. The tubule at the *lower right* is lined by regenerating cells. These cells are cuboidal with only a few microvilli and simplified cytoplasm. Organelles consist of occasional mitochondria, rare segments of rough endoplasmic reticulum, numerous polyribosomes, and cytosomes packed with myeloid bodies. In contrast, the adjacent tubule is lined by minimally altered epithelial cells with characteristically complex cytoplasm and cytosomes containing dense membranous aggregates. (× 14,600)