Kidney International, Vol. 18 (1980), pp. 583–590

Gentamicin-induced nephrotoxicity: A cell biology approach

J. P. MORIN, G. VIOTTE, A. VANDEWALLE, F. VAN HOOF, P. TULKENS, and J. P. FILLASTRE

Groupe de Physiopathologie Tissulaire, Université de Rouen, Service de Néphrologie, Hôpital de Bois Guillaume, Bois Guillaume, France; International Institute of Cellular and Molecular Pathology and Laboratoire de Chimie Physiologique, Université Catholique de Louvain, Brussels, Belgium; and Unité de Recherches en Pathologie Cardiovasculaire, Hôpital Léon Bernard, Limeil Brevannes, France

Gentamicin is an antibiotic that exhibits a broad spectrum of activity and is particularly valuable in severe sepsis. Its use is, however, restricted because of the development of ototoxicity and nephrotoxicity (for the latter, see review in Ref. 1). At physiologic pH, the drug is highly charged and water soluble, and therefore it is practically unable to diffuse through biologic membranes.

Nephrotoxicity has been related to a selective accumulation of gentamicin in the renal cortex [2, 3]. Morphologic lesions of proximal tubules have been documented in optic microscopy [4–6]. At the ultrastructural level, the earliest lesions observed concern the lysosomes, which show an accumulation of myeloid bodies [7–9].

The mechanism of the gentamicin toxicity is, however, unknown. In this paper, we present studies on the localization of the drug at the cellular and subcellular levels, and on the enzymatic alterations that develop during gentamicin treatment.

Autoradiography of microdissected nephrons. Autoradiographic studies were performed on isolated microdissected tubules by the technique of Vandervalle et al [10]. The proximal tubular segments were divided in three groups: the *initial portion*, corresponding to segments taken from the first 3 mm following the glomerulus; the *pars recta*, segments belonging to the straight proximal tubules, corresponding to the 3 mm preceeding the beginning of the thin descending limb of Henle; and the *mid portion*, segments not belonging to the above mentioned groups, that is, situated approximately between the 4th and the 7th mm from the glomerulus. Segments of the distal part of the tubule were defined according to the following classification [10]: thin descending limb (TDL), thin ascending limb (TAL), thick medullary limb (MAL), cortical ascending limb (CAL), distal convoluted tubule (DCT) (without distinction between bright and granular portion of this segment), cortical collecting tubule (CCT), and medullary collecting tubule (MCT). Autoradiography was performed by dry film technique [10].

Figure 1 shows the distribution of tritiated gentamicin in the various segments of rabbit kidney nephron 4 hours after a single injection (100 μCi/kg of body wt; that is, 100 μg of drug/kg). Thin-layer chromatography showed that the intrarenal radioactivity consisted only in true gentamicin, with a relative contribution of the three major subcomponents (C_1, C_{1a}, and C_2) similar to that of the injected drug. No silver grain could be detected in the glomerulus, and only very low amounts were detected in all parts of the nephron beyond the proximal tubule. The distribution of the drug among this latter segment is, however, not homogenous: the largest part of the drug is recovered in the pars recta and a lesser amount in the early part of the tubule. Autoradiography of kidney cortex slices processed according to standard histologic techniques showed only intracellular label. No silver grains were seen in the tubular lumen and in blood vessels.

Cell fractionation studies. Fractionation by density equilibration was performed on postnuclear supernatants prepared in 0.25 M sucrose and 1 mM EDTA. The sample was placed at the top of a linear sucrose gradient with densities extending from 1.10 to 1.30 g/cm^3. The gradient was centrifuged at

Received for publication May 29, 1980
0085–2538/80/0018-0583 $01.60

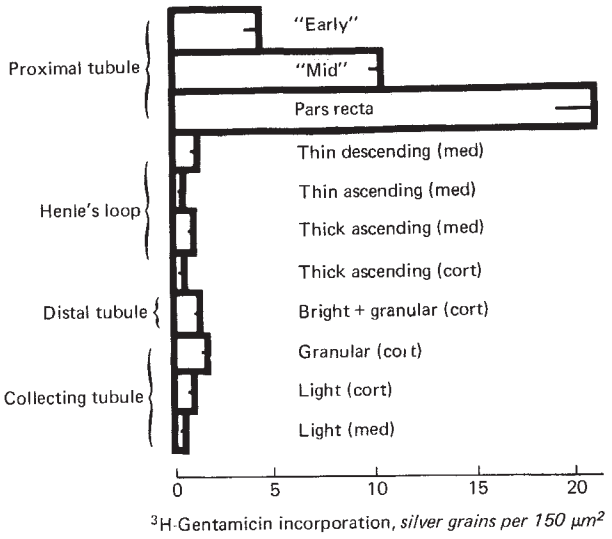

Fig. 1. *Incorporation of tritiated gentamicin in tubular cells.* Silver grains were counted in ten areas at random, each 150 μm^2 wide for each segment. The administration of tritiated drug was performed on two animals, as described in the text. Individual variations from nephron to nephron are expressed as the standard variation.

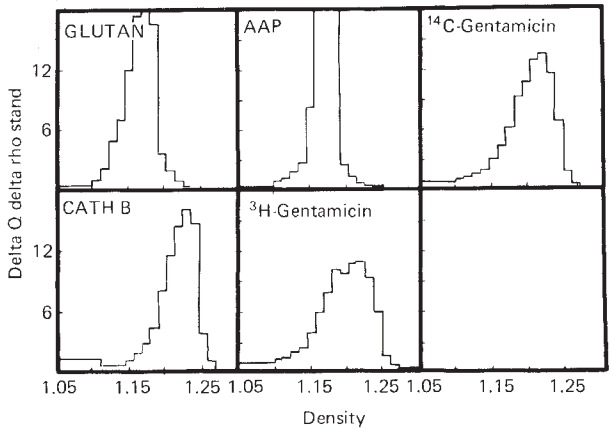

Fig. 2. *Cell fractionation studies on distribution of gentamicin.* Isopycnic centrifugation of a postnuclear supernatant was obtained from isolated proximal tubules of a rabbit injected with ^{14}C-gentamicin and tritiated gentamicin, as indicated in the text. The technique for isolating the tubules is derived from that of Carlson et al [44], and will be described in detail in a forthcoming publication (Hjelle et al). Results are given as density distribution histograms, constructed following the method of Leighton et al [45]. GLUTAN is γ-glutamyl-transpeptidase; AAP, alanyl-amino-peptidase; and CATH B, cathepsin B.

49,000 rpm for 2 hours in a vertical rotor (VTi50; Beckman Instruments, Palo Alto, California) with the slow acceleration accessory and collected in 13 to 15 fractions, whose densities and weights were recorded. Enzymes and constituents were assayed after exposure of the fractions to 0.1% Triton X-100 in order to achieve complete disruption of the cell organelles.

Figure 2 shows the results of an experiment in which an homogenate of isolated proximal tubules from rabbit has been fractionated by isopycnic centrifugation. This technique allows one to resolve, in a clearcut fashion, lysosomes (detected by cathepsin B and *N*-acetyl-β-glucosaminidase) from mitochondria (cytochrome oxidase) and the pericellular membrane, especially the brush border (alanine aminopeptidase and γ-glutamyl-transpeptidase) (Hjelle and Morin, unpublished). The animal was injected with ^{14}C-gentamicin (13 μCi; that is, 2 mg/kg of body wt) and with tritiated gentamicin (26 μCi; that is, 26 μg/kg of body wt), 6 hours and 45 min prior to the sacrifice, respectively.

The ^{14}C-gentamicin injected 6 hours before sacrifice consistently and exclusively distributes in the same manner as the two lysosomal enzymes. In a control experiment where the homogenate was frozen and thawed before centrifugation to disrupt lysosomes, the antibiotic dissociated from the lysosomal enzymes and displayed a broad distribution pattern similar to that of protein.

On the other hand, the tritiated gentamicin injected only 45 min prior to sacrifice showed a slightly bimodal distribution, which is suggestive of an association partly to lysosomes and to a lesser extent to other subcellular structures, such as the brush border (alanine aminopeptidase).

Lysosomal structural latency. This was estimated by measuring the free/total activity ratio of the *N*-acetyl-β-D-glucosaminidase, in a lysosomal preparation freshly isolated from cortex homogenate, as previously described [11, 12]. This ratio is assumed to reflect the integrity of the lysosomal membrane at the time of the assay [13]. A low ratio reflects membrane integrity, and a high ratio reflects membrane disruption and an increase of membrane permeability.

The variation of the latency of the lysosomal enzyme *N*-acetyl-β-glucosaminidase is shown in Fig. 3. On a freshly isolated lysosomal fraction, we observed that the treatment of the animals with gentamicin results in a significant increase of the percentage of free activity, that is, the part of activity of the enzyme that is demonstrable in the absence of detergent. The addition of detergent induces the total disruption of the lysosomal membrane. Similar results are obtained with lysosomal fractions incubated at 37° C for 60 min, except that under those conditions a higher level of free activity is observed in the controls, due to the spontaneous rupture of

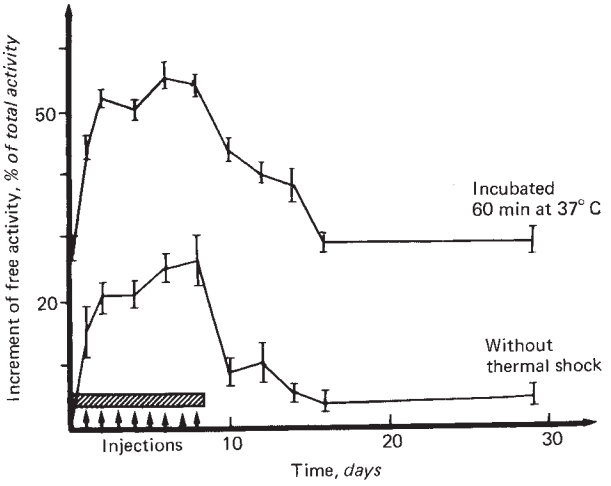

Fig. 3. *Effect of gentamicin treatment on the structural latency of lysosomal N-acetyl-β-glucosaminidase (mean of four independent experiments* ± SEM*).* Animals were injected with 50 mg/kg gentamicin during the first 8 days (*arrows*).

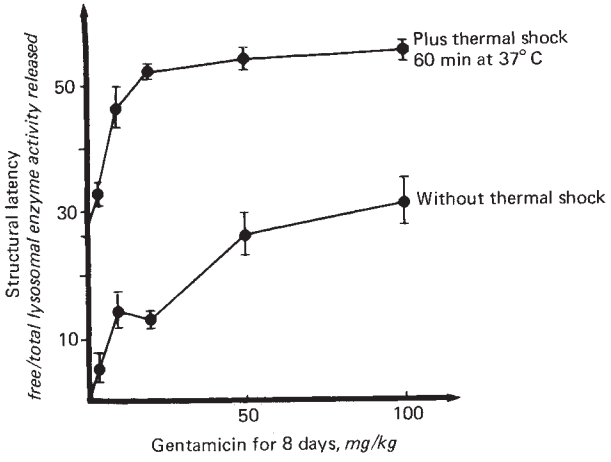

Fig. 4. *Structural latency of lysosomal N-acetyl-β-glucosaminidase following gentamicin treatment (mean of four independent experiments* ± SEM*).*

lysosomes during the incubation [14, 15]. The cessation of the treatment allows a recovery to control values within approximately 8 days. The increase of free activity of the *N*-acetyl-β-glucosaminidase is already significant after 8 days of treatment with 4 mg/kg gentamicin and is almost maximal at 50 mg/kg (Fig. 4).

Correlation between biochemical and morphological changes. For biochemical analyses, kidney cortices were dissected, dried on filter paper, and frozen at −20° C until analysis. They were homogenized (1/200 wt:vol) in distilled water by a Kontess conical, sintered glass tissue grinder. The following enzymes were assayed with chromogenic or fluorogenic substrates according to standard techniques [16]. With 4-methyllumbelliferyl derivatives, acid α-L-fucosidase, acid α-D-galactosidase, acid α-D-mannosidase were assayed. With para-nitrophenol derivative, *N*-acetyl-β-D-glucosaminidase was assayed. With para-nitranilide derivative, alanine aminopeptidase and γ-glutamyl transpeptidase were assayed. Cathepsin B was measured with α-*N*-benzoyl-D-L-arginine-2-naphtylamide [17]; sphingomyelinase was assayed with 2-hexadecanoyl amino-4-nitrophenyl-phosphorylcholine [18]. True glucose 6-phosphatase was assayed with glucose 6-phosphate as substrate [16]. Proteins were measured by the method of Lowry et al [19] with bovine serum albumin as standard. Gentamicin was assayed microbiologically with *Bacillus subtilis* as test organism.

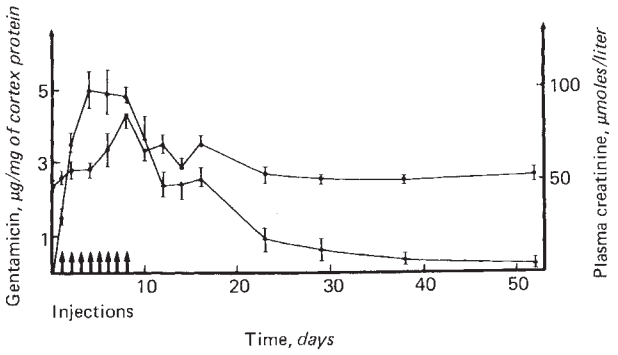

Fig. 5. *Level of the blood creatinine and the gentamicin content of kidney cortex during gentamicin treatment and after withdrawal of the drug (mean of four experiments* ± SEM *on Wistar rats given 50 mg/kg daily for 8 days).*

In a first series of experiments, we have examined the development of biochemical lesions during a treatment of Wistar rats with gentamicin, 50 mg/kg for 8 days, and the recovery of these lesions following cessation of treatment. Figure 5 shows that blood creatinine rises after day 4, but returns to control values within 15 days after treatment. The elevation is highly significant and suggests prominent renal failure. Histologic examination revealed large zones of necrosis throughout the cortex. The lesions had completely disappeared at day 30; that is, 22 days after gentamicin withdrawal. Also as indicated in Fig 5, the intracortical content of gentamicin increases almost linearly up to day 4 and then reaches a plateau value at a level of about 6 μg of antibiotic per milligram of protein; that is, about 0.9 mg/g of wet tissue. On discontinuation of the treatment, the cortical content decreases rapidly to about one third of the plateau value, and then much more slowly during the next 40 days.

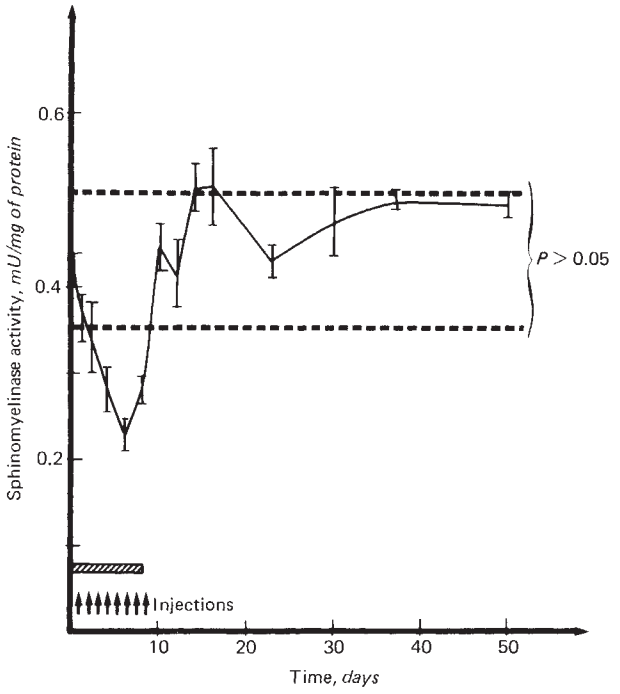

Fig. 6. *Activity of sphingomyelinase of kidney cortex during and after gentamicin treatment (50 mg/kg; 8 days; four experiments* ± SEM*).*

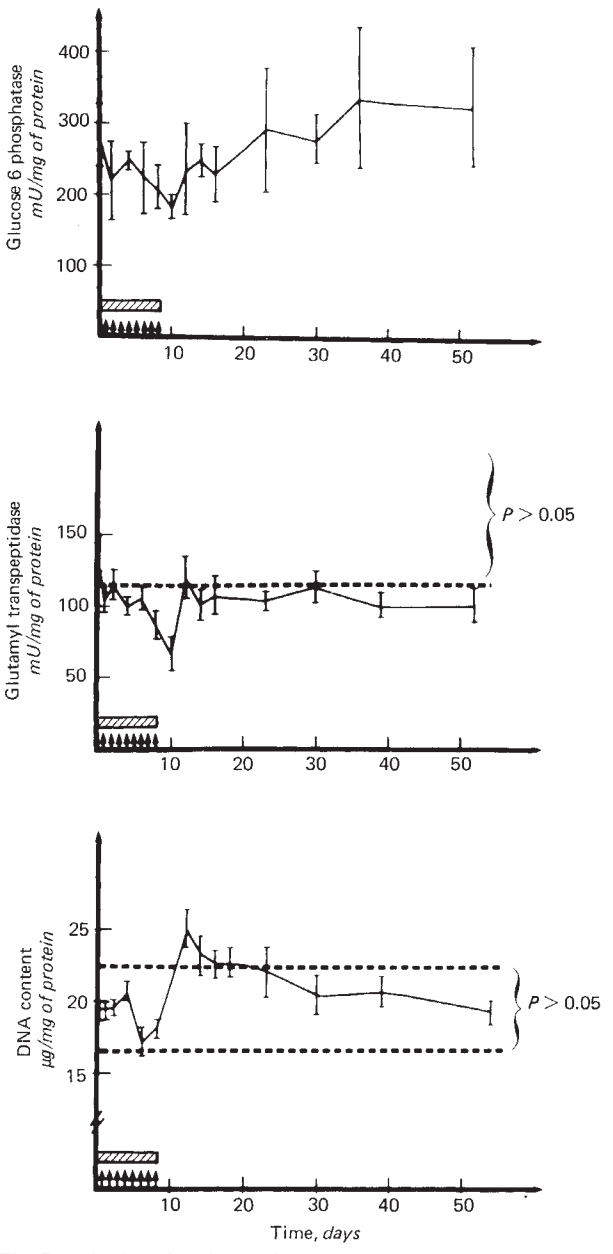

Fig. 7 *Activities of γ-glutamyl-transpeptidase (brush border) and of glucose 6-phosphatase (endoplasmic reticulum) of kidney cortex during and after gentamicin treatment.* The animals were injected with 50 mg/kg gentamicin during the first 8 days (*arrows*). The bottom part of the figure shows the DNA content of tissue. (All values are the means ± SEM, $N = 4$ experiments).

The activity of several lysosomal enzymes was systematically measured in two sets of experiments: *(1)* an 8-day treatment at 50 mg/kg followed by gentamicin withdrawal, and *(2)* an 8-day treatment at dosages varying from 0 to 100 mg/kg. Renal function was evaluated by measuring the blood creatinine. The most striking feature is the decrease of the activity of the sphingomyelinase (Fig. 6), an enzyme responsible for the intralysosomal breakdown of sphingomyelin [18, 21]. The effect is already significant after 2 to 4 days of treatment with 50 mg/kg or after 8 days with 4 mg/kg. Recovery of the enzyme activity is rapid after cessation of treatment. Another lysosomal activity affected is that of cathepsin B, an acid thiol-dependent endopeptidase of broad specificity [21]. Like sphingomyelinase, this shows a decrease in activity. This effect is, however, seen only after 6 days and at doses of 50 mg/kg or more. Other lysosomal enzymes assayed in this study and several others, mainly glycosidases and esterases, measured in similar experiments were not significantly affected, with the exception of acid α-galactosidase, the activity of which is slightly decreased.

The activity of γ-glutamyl transpeptidase, an enzyme of the brush border [22], is significantly decreased. Surprisingly, this effect is already maximum after 4 mg/kg for 8 days; larger doses have no further effect. Figure 7 shows, however, that the decrease of activity of γ-glutamyl-transpeptidase is related to the duration of the treatment at 50 mg/kg.

Figure 7 indicates also that glucose 6-phosphatase (endoplasmic reticulum) is not affected. The DNA-protein ratio shows little change, except for a

transient but distinct rise immediately on gentamicin withdrawal.

Discussion

Cellular and subcellular localization of intrarenal gentamicin. The preferential association of gentamicin with the kidney cortex has been known for several years [2], but its cellular and intracellular distribution has been largely overlooked. Recently, several studies have pointed out that the drug distributes in a very discrete fashion within the cortex. Just, Erdmann, and Haberman [23], Silverblatt and Kuehn [24], and Kuhar, Mak, and Lietman [25] showed an almost exclusive association of the drug with the proximal tubules, with, however, a large degree of heterogeneity among these structures. The microdissection technique has allowed us to locate unambiguously gentamicin all along the nephron. We demonstrate that the heterogeneity of concentration does not reflect an internephron variation of uptake, but rather a gradient of accumulation along the proximal tubule of each individual nephron. In accordance with other investigators, only very low amounts of gentamicin are found in other parts of the nephron, including the glomerulus.

The studies of Just, Erdmann, and Haberman [23] and Silverblatt and Kuehn [24] have strongly suggested that the intratubular gentamicin is not distributed throughout the cells, but rather is found accumulated within the lysosomes. Our biochemical studies confirm clearly the localization of the intrarenal gentamicin within the lysosomes, from which it can be released only by disruption of the lysosomal membrane. This pattern is observed, however, only a few hours after injection of drug, and shorter periods of time yield less clearcut results. We interpret the more complex distribution of gentamicin 45 min after injection, as reflecting its mode of entry within the tubular cells, which most likely is by adsorptive pinocytosis from the tubular lumen. Adsorptive pinocytosis is the process by which substances bind to the pericellular membrane and are thereafter included into intracytoplasmic vacuoles that arise from this membrane and that eventually fuse with lysosomes. This process allows a very efficient uptake of a large number of exogenous compounds into lysosomes [26, 27].

In accordance with this interpretation are the following. The autoradiographic studies cited above show an association of the drug with the brush border and then with apical vesicles, prior to its intralysosomal deposition. Our fractionation studies agree with these findings, in that the amount of drug that is clearly not associated with lysosomes 45 min after injection can be assigned to the brush border (alanyl-aminopeptidase). The occurrence of an adsorptive process at the level of the brush border is all the more evident because binding of gentamicin to this structure has been demonstrated in vitro [28, 29]. The association constant and the number of binding sites revealed by these studies are consistent with a rate of uptake of the drug about 20-fold faster than that of substances that enter cells by fluid pinocytosis (that is, without prior binding to the pericellular membrane, but through inclusion in vesicles) such as inulin or PVP [30] (see also the models of pinocytosis in Refs. 31–33). Interestingly enough, a survey of the available data shows that the renal uptake of gentamicin is 10- to 25-fold more efficient than that of inulin or PVP [11, 34–36]. It must also be stressed that pinocytosis is a vesicular transport process; thus, no transmembrane transport of the molecules is required for their final sequestration in lysosomes. This is all the more important because gentamicin is a highly hydrophilic substance.

The intracellular uptake and intralysosomal accumulation of gentamicin is not a phenomenon unique to the kidney. Tulkens and Trouet [37] showed that cultured rat fibroblasts readily take up gentamicin and several other aminoglycosides as well; drugs accumulate in lysosomes where they reach concentrations 100- to 200-fold larger than in the culture fluid. A salient observation is that rat fibroblasts take up gentamicin more slowly and less efficiently than does the kidney, but they eventually accumulate very large amounts of drugs (up to 30 μg or more per milligram of cell protein; that is, 6 mg/g of wet wt). The slower rate of uptake can almost completely be accounted for by the lack of binding sites at the surface of fibroblasts, but the mechanism of uptake (pinocytosis) seems similar in both cell types (Morin and Tulkens, unpublished data).

Metabolic alterations induced by gentamicin. Gentamicin has been reported to provoke characteristic and dose-related anatomic renal changes both in animals and in man [7–9]. The first lesions observed concern the lysosomes that show a conspicuous accumulation of osmiophilic material, organized in a multilamellar and concentric disposition (myeloid bodies). Hruban, Slesers, and Hopkins [43] have suggested that myeloid bodies within lysosomes indicate an impairment of the degradation of complex polar lipids. Such situations occur in several inborn enzyme defects [39]. For instance, Tay-Sachs disease (deficiency of lysosomal

β-hexosaminidase A), Niemann-Pick disease (a defect of sphingomyelinase), or Fabry disease (deficiency of thermolabile α-galactosidase) result in the intralysosomal accumulation of complex lipids (mainly GM_2 gangliosides, sphingomyelin and ceramide dihexosides and trihexosides, respectively). Morphologically, the accumulation of these lipids bears some similarity to the images found by Kosek et al [7] and his followers.

As mentioned above, gentamicin has been shown to enter fibroblasts in culture. Using this model, Aubert-Tulkens, Van Hoof, and Tulkens [38] have studied the cellular toxicity of the drug. When the intracellular concentration reaches about 4 μg/mg of cell protein (800 μg/g of wet wt), lysosomes show a conspicuous overloading with myeloid bodies, similar in appearance to those observed in the kidney. Other subcellular oganelles showed no lesion. Assaying systematically for a large number of acid hydrolases, Aubert-Tulkens et al [38] showed a constant and highly significant decrease of the activity of the lysosomal sphingomyelinase. Recent studies from the same laboratory (Van Hoof, unpublished) have also shown a decrease of the level of acid phospholipase A ($A_1 + A_2$). Concomitantly, the total lipid phosphorus rises up to 170% of the control value. Thin-layer chromatography indicates that the concentration of all major phospholipids is increased without appreciable change in their relative amounts. Thus, these observations are consistent with the hypothesis that gentamicin impairs the lysosomal catabolism of phospholipids, by decreasing the activities of the corresponding hydrolases. The molecular mechanism of this toxicity is under investigation.

Our biochemical data with rat kidney cortices suggest that the mechanism of gentamicin toxic action may be similar to that reported for fibroblasts. The activity of sphingomyelinase is significantly decreased, under all conditions investigated, even at low doses. It has, however, been difficult to demonstrate unambiguously an accumulation of phospholipids, due to the heterogeneity of the kidney cortex and the much larger amount of nonlysosomal phospholipids (mainly intracellular and pericellular membranes) as compared with fibroblasts. Morphologic studies in progress leave, however, no doubt regarding the huge accumulation of myeloid bodies in lysosomes, in an apparent correlation with the decrease of sphingomyelinase activity. Also, we have been unable to detect lesions in other subcellular structures, as long as the cells do not exhibit signs of necrosis.

To the concept of gentamicin-induced phospholipidosis, as developed by Aubert-Tulkens et al [38] for the fibroblasts, we should, however, add the other features revealed by this work in kidney. Cathepsin B and α-D-galactosidase, two other lysosomal enzymes, are also impaired. But this occurs only at large doses and may be a secondary phenomenon. Intralysosomal deposition of undigested proteins and of galactocerebrosides [37] has not yet been investigated.

We also have noted a sharp decrease of the activity of γ-glutamyltranspeptidase and of alanyl-amino peptidase (for the latter, data not shown), two enzymes of the brush border. This is consistent with the increased urinary excretion of these enzymes observed during aminoglycoside treatment (for review, see Ref. 40). The significance of this finding in relation with gentamicin-induced toxicity is, however, unclear because no dose-dependence of the effect is observed. Finally, we could not demonstrate any gross modification of the tissue protein content.

Relation of lysosomal dysfunction with acute renal failure. Elevation of the blood creatinine concentration is clearly observed for gentamicin dosages above 20 mg/kg, at which an extensive cell necrosis is observed in proximal tubules, but no lesions are recorded in glomeruli or other parts of the nephron. Thus, the decrease of the glomerular filtration is most likely related to proximal tubule injury. This is consistent with the concept that the nephron constitutes a unit, all parts of which must be functional for efficient activity.

Accepting this hypothesis, we may consider that the site of the toxic action of gentamicin is exclusively the proximal tubule. Lysosomal overloading could lead to cell death, either because of mechanical of chemical alteration of the lysosomal membrane, or because of an impairment of some important lysosomal function. In this connection, recent studies from our laboratory using cultured *human* fibroblasts have indicated that gentamicin readily kills the cells when the intracellular concentration reaches about 3 μg/mg of cell protein (600 μg/g of wet wt).

We cannot, however, exclude another site of toxicity of gentamicin besides lysosomes. For instance, in vitro studies have shown that gentamicin alters the respiratory control of isolated mitochondria. In vivo, however, no such effect could be evidenced, nor was the localization of gentamicin within the mitochondria demonstrated (either in kidney or in cultured fibroblasts). Similarly, no oth-

er site of storage, besides lysosomes, has been unambiguously documented. In this respect, we should point out the difficulty in interpretating the cell fractionation data on animals injected with large doses of gentamicin, or for prolonged periods, or both.

As cells become necrotic, intralysosomal gentamicin will be released and will reabsorb unspecifically to various structures. In addition, studies reported so far [46, 47] are deficient in the strict monitoring of the homogenization procedure, evaluation of the integrity of the subcellular structures, and determination of the actual composition of the isolated fractions, three major prerequisites in tissue fractionation (see Ref. 41 and 42). Our observation that the latency of the lysosomal enzyme *N*-acetyl-β-glucosaminidase decreases during gentamicin treatment reinforces this *caveat*, because it suggests an alteration of the lysosomal membrane.

We have observed an apparent saturation of the uptake of gentamicin, after 4 days of treatment with 50 mg/kg, concomitantly with the increase of blood creatinine. This plateau could reflect a complex equilibrium between (*1*) a decrease of the uptake due to the decrease of glomerular filtration and (*2*) an excretion of gentamicin from the kidney through the elimination of necrotic cells. This should be further investigated.

Summary. By autoradiography of microdissected isolated nephrons, we show that gentamicin distributes almost exclusively in the proximal tubule, where an increasing concentration gradient takes place from the initial to the distal part. On isopycnic centrifugation of homogenates from isolated tubules, the drug is found exclusively associated with the lysosomes 6 hours after injection. At a shorter time, the distribution is slightly bimodal and consistent with an association of part of the drug with brush border. This agrees with the suggestion that gentamicin enters cells and accumulates in lysosomes by absorptive pinocytosis.

In gentamicin-treated animals, we showed (*1*) a decrease of the latency of lysosomes; (*2*) a decrease of the activity of lysosomal sphingomyelinase and, at large doses, of cathepsin B and α-D-galactosidase; (*3*) a decrease of the activity of alanylaminopeptidase and γ-glutamyl-transpeptidase. Unlike the others, the latter effect is not dose-related. All these alterations showed complete reversibility within 15 to 21 days after gentamicin withdrawal.

These findings are consistent with the proposal that a central feature of the mechanism of gentamicin nephrotoxicity involves the accumulation of the drug in the lysosomes of the cells of the proximal tubule, leading to an extensive dysfunction of these cells through (*1*) the subsequent inhibition of the activities of the enzymes that are involved in the degradation of polar lipids, (*2*) the alteration of the properties of the lysosomal membrane permeability.

From in vitro studies on cultured cells (fibroblasts), these alterations of the cell metabolism seem to be relevant for cell necrosis and cell death.

Acknowledgments

This work was supported by the Belgian Fonds de la Recherche Scientifique Médicale (grant 3.4516.79), the US Public Health Service (grant 9235) and the French I.N.S.E.R.M. (grant 76.1176.5). J. P. Morin is Fellow of the International Institute of Cellular and Molecular Pathology (ICP). P. Tulkens is *Chercheur Qualifié* of the Belgian F.N.R.S. Mrs. F. Andries-Renoird and Mr. O. M. Vu (ICP) gave technical help, and Mr. Y. Marchand (ICP) and Mrs. D. Falize typed the manuscript.

Reprint requests to Dr. J. P. Morin, Groupe de Physiopathologie Tissulaire, Université de Rouen, Service de Néphrologie, Hôpital de Bois Guillaume, F 76230 Bois Guillaume, France

References

1. Appel GB, Neu HC: The nephrotoxicity of antimicrobial agents [Three parts]. *N Engl J Med* 269:663-670, 722-728, 784-787, 1977
2. Alfthan O, Renkonen OV, Sivonen A: Concentration of gentamicin in serum, urine and urogenital tissue in man. *Acta Pathol Microbiol Scand (B)* 81 (suppl. 241):92, 1973
3. Luft FC, Kleit SA: Renal parenchymal accumulation of aminoglycoside antibiotics in rats. *J Infect Dis* 130:656, 1974
4. Abramowicz M, Edelman CM: Nephrotoxicity of anti-infective drugs. *Clin Pediatr* 7:389, 1968
5. Falco FG, Smith HM, Acieri GM: Nephrotoxicity of aminoglycosides and gentamicin. *J Infect Dis* 119:406-409, 1969
6. Kleinknecht D, Fillastre JP: *La Tolérance Rénale des Antibiotiques*. Paris, Masson Publ. 1973, p. 89.
7. Kosek JD, Mazze RI, Cousins MJ: Nephrotoxicity of gentamicin. *Lab Invest* 30:48-57, 1974
8. Houghton DC, Harnett M, Campbell-Boswell M, Porter G, Bennett WM: A light and electron microscopic analysis of gentamicin nephrotoxicity in rats. *Am J Pathol* 82:589-612, 1976
9. Houghton DC, Campbell-Boswell MV, Bennett WM, Porter AJ, Brooks RE: Myeloid bodies in the renal tubules of humans: Relationship to gentamicin therapy. *Clin Nephrol* 10:140-145, 1978
10. Vandervalle A, Farman N, Morin JP, Fillastre JP, Hatt PY, Bonvalet JP: Gentamicin incorporation along

the nephron: Autoradiographic study on isolated tubules. *Kidney Int*, in press
11. MORIN JP, FILLASTRE JP, VAILLANT R: Prediction of aminoglycoside-cephalosporin nephrotoxicity. *Current Chemotherapy, Proc 10th Int Congr Chemother* 2:960–962, 1978
12. MORIN JP, FRESEL J, FILLASTRE JP, VAILLANT R: Aminoglycoside actions on rat kidney lysosomes "in vivo" and "in vitro," in *Nephrotoxicity: Interaction of Drugs with Membrane Systems: Mitochondria-Lysosomes*, edited by FILLASTRE JP, New York and Paris, Masson Pub. Inc. U.S.A., 1978, pp. 253–263
13. DE DUVE C, WATTIAUX R, WIBO M: Effects of fat soluble compounds on lysosomes in vitro. *Biochem Pharmacol* 9:97, 1962
14. BERTHET J, BERTHET L, APPELMANS F, DE DUVE C: Tissue fractionation studies: II. The nature of the linkage between acid phosphatase and mitochondria in rat liver tissue. *Biochem J* 50:182–189, 1951
15. DE DUVE C: The lysosome concept. in *Ciba Foundation Symposium on Lysosomes*, edited by DE REUCK AVS, CAMERON MP, London, J. & A. Churchill Ltd., 1963, pp. 1–31
16. VAN HOOF F, HERS HG: The abnormalities of lysosomal enzymes in mucopolysaccharidose. *Eur J Biochem* 7:34, 1968
17. BARRETT AJ: A new assay for cathepsin B_1 and other thiol proteinases. *Anal Biochem* 47:280, 1972
18. GAL AE, BRADY RD, HIBBERT SR, PENTCHEV PG: A practical chromogenic procedure for the detection of homozygotes and heterozygous carriers of Niemann-Pick disease. *N Engl J Med* 293:632–636, 1975
19. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265, 1951
20. BRADY RO, KANFER JN, MOCK MB, FREDERICKSON DS: The metabolism of sphingomyelin. II. Evidence of an enzymatic deficiency in Niemann-Pick disease. *Proc Natl Acad Sci USA* 55:366–369, 1966
21. BARRETT AJ, HEATH MF: Lysosomal enzymes, in *Lysosomes, A Laboratory Handbook* (2nd ed.),, edited by DINGLE JT, Amsterdam, Elsevier/North Holland Publ. Co., 1977, pp. 19–145
22. GEORGE SG, KENNY AJ: Studies on the enzymology of purified preparations of brush-border from rabbit kidney. *Biochem J* 134:43–57, 1973
23. JUST M, ERDMANN G, HABERMAN E: The renal handling of polybasic drugs: 1. Gentamicin and aprotinin in intact animals. *Naunyn-Schmiedebergs Arch Pharmacol* 300:57–66, 1977
24. SILVERBLATT F, KUEHN C: Autoradiography of gentamicin uptake by the rat proximal tubule cell. *Kidney Int* 15:335–345, 1979
25. KUHAR MJ, MAK LL, LIETMAN PS: Autoradiographic localization of [^{3}H]gentamicin in the proximal renal tubules of mice. *Antimicrob Agents Chemother* 15:131–133, 1979
26. JACQUES P: Endocytosis, in *Lysosomes in Biology and Pathology*, edited by DINGLE JT, FELL HB, Amsterdam, North Holland Publ. Co., 1979, vol. 2, pp. 395–420
27. SILVERSTEIN SC, STEINMAN RM, COHN ZA: Endocytosis. *Ann Rev Biochem* 46:669–722, 1977
28. JUST M, HABERMAN E: The renal handling of polybasic drugs: 2. In vitro studies with brush-border and lysosomal preparations. *Naunyn-Schmiedebergs Arch Pharmacol* 300:67–76, 1977
29. JERAULD RS, SILVERBLATT FJ: Gentamicin binding to renal proximal tubule brush-border membranes. *Proc 11th Int Congr Chemother and 19th Intersci Conf Antimicrob Agents*, Boston, 1979, abstr. no. 935
30. ROBERTS AVS, WILLIAMS KE, LLOYD JB: The pinocytosis of ^{125}I-labelled poly vinyl pirrolidone, ^{14}C-sucrose and colloidal ^{198}Au gold by rat yolk sac cultured in vitro. *Biochem J* 168:239–244, 1977
31. SCHNEIDER YJ, TULKENS P, DE DUVE C, TROUET A: Fate of plasma membrane during endocytosis: I. Uptake and processing of anti-plasma membrane and control immunoglobulins by cultured fibroblasts. *J Cell Biol* 82:449–465, 1979
32. SCHNEIDER YJ, TULKENS P, DE DUVE C, TROUET A: Fate of plasma membrane during endocytosis: II. Evidence for recycling (shuttle) of plasma membrane constituents. *J Cell Biol* 82:466–474, 1979
33. DE DUVE C, DE BARSY T, POOLE B, TROUET A, TULKENS P, VAN HOOF F: Lysosomotropic agents. *Biochem Pharmacol* 23:2495–2531, 1974
34. SCHILLER A, RER G, TAUGNNER R: Excretion and intrarenal distribution of low molecular poly vinyl pyrrolidone and inulin in rats. *Arzneim Forsch* 28:2064–2070, 1978
35. FABRE J, RUDHART M, BLANCHARD P, REGAMEY C: Persistence of sisomicin and gentamicin in renal cortex and medulla compared with other organs and serum of rats. *Kidney Int* 10:444–449, 1976
36. LUFT FC, KLEIT SA: Renal parenchymal accumulation of aminoglycoside antibiotics in rats. *J Infect Dis* 130:656–659, 1974
37. TULKENS P, TROUET A: The uptake and intracellular accumulation of aminoglycoside antibiotics in lysosomes of cultured rat fibroblasts. *Biochem Pharmacol* 27:415, 1978
38. AUBERT-TULKENS G, VAN HOOF F, TULKENS P: Gentamicin-induced lysosomal phospholipidosis in cultured rat fibroblasts: Quantitative ultrastructural and biochemical study. *Lab Invest* 40:481–493, 1979
39. BRADY RO: Elucidation of clinical lysosome deficiencies, in *Molecular Basis of Biological Degradative Processes*. New York, Academic Press, 1978, pp. 39–64
40. SOEP HH, DE BROE ME: Enzymurie: toxicité rénale et aminosides. *Schweiz Rundschau Med (Praxis)* 67:1721–1725, 1978
41. DE DUVE C: Principles of tissue fractionation. *J Theor Biol* 6:33–59, 1964
42. DE PIERRE JW, KARNOVSKY ML: Plasma membrane of mammalian cells: A review of methods for their characterization and isolation. *J Cell Biol* 56:275–303, 1973
43. HRUBAN Z, SLESERS A, HOPKINS E: Drug induced and naturally occurring myeloid bodies. *Lab Invest* 27:62–70, 1972
44. CARLSON EC, BRENDEL K, HJELLE JT, MECZAN E: Ultrastructural and biochemical analyses of isolated basement membranes from kidney glomeruli and tubules and brain and retinal microvessels. *J Ultrastruct Res* 62:26–53, 1978
45. LEIGHTON F, POOLE B, BEAUFAY H, BAUDHUIN P, COFFEY J, FOWLER S, DE DUVE C: The large-scale separation of peroxisomes, mitochondria, and lysosomes from the livers of rats injected with Triton WR-1339. *J Cell Biol* 37:482–513, 1968
46. VERA-ROMAN J, KRISHNAKANTA TP, CUPPAGE FE: Gentamicin nephrotoxicity in rats: I. Acute biochemical and ultrastructural effects. *Lab Invest* 33:412–417, 1975
47. KOHLHEPP SJ, THOMPSON S, HOUGHTON DC, CONNOR R, GILBERT DN: Renal subcellular distribution of gentamicin during experimental nephrotoxicity. *Proc 11th Int Cong Chemother and 19th Intersci Conf Antimicrob Agents*, Boston, 1979, abstr. no. 934
48. FILLASTRE JP: *Nephrotoxicity: Interaction of drugs with membrane systems: Mitochondria-Lysosomes*. edited by FILLASTRE JP, New York and Paris, Masson Pub. Inc. U.S.A., 1978