ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1987, p. 570–574 Vol. 31, No. 4
0066-4804/87/040570-05$02.00/0
Copyright © 1987, American Society for Microbiology

Correlation between Renal Membrane Binding and Nephrotoxicity of Aminoglycosides

P. D. WILLIAMS,* D. B. BENNETT, C. R. GLEASON, AND G. H. HOTTENDORF

Departments of Experimental Toxicology and Biostatistics, Pharmaceutical Research and Development Division, Bristol-Myers Co., Syracuse, New York 13221-4755

Received 28 October 1986/Accepted 18 January 1987

The kinetics of aminoglycoside binding to renal brush border and basolateral membrane vesicles from rat renal cortex were studied by using [^{3}H]amikacin. [^{3}H]amikacin binding to renal membranes was found to be a rapid, saturable process with a fourfold greater affinity for basolateral membranes than for brush border membranes (K_d basolateral = 607 μM; K_d brush border = 2,535 μM). Renal membranes prepared from immature rats (2 to 3 weeks old) exhibited a significantly lower affinity compared with membranes from adults (K_d basolateral = 2,262 μM; K_d brush border = 6,216 μM). Additionally, the inhibitory behavior of several aminoglycosides versus [^{3}H]amikacin binding to brush border membranes revealed the following rank order of potency: neomycin > tobramycin ~ gentamicin ~ netilmicin > amikacin ~ neamine > streptomycin. The relative insensitivity of immature rats to aminoglycoside-induced nephrotoxicity in vivo and the comparative nephrotoxicity of the various aminoglycosides suggest that renal membrane-binding affinity is closely correlated to the nephrotoxic potential of these antibiotics.

Aminoglycoside antibiotics are nephrotoxic in humans and experimental animals, in which they induce necrosis of the proximal tubule (4, 13, 26). The nephrotoxicity of these drugs is associated with selective accumulation of aminoglycosides in the kidney, with cortical levels reaching as high as 20 times the circulating levels in serum (10, 40). Aminoglycosides are known to accumulate in renal tissue via tubular reabsorption as well as by extraction from the circulation at the basolateral surface of the kidney, although brush border uptake is thought to contribute more on a quantitative basis (6, 7, 17, 34). However, it remains to be established whether the nephrotoxicity is a consequence of accumulated drug or relates to an interaction at the initial point of contact between the renal cell and the aminoglycoside, the plasma membrane. Williams et al. (43) recently reported the inhibition of aminoglycoside nephrotoxicity by polyamino acids associated with decreased renal brush border and basolateral membrane binding without inhibition of total cortical accumulation. Effects of aminoglycosides on plasma membrane structure and function have been reported (11, 20, 29, 36, 44).

To examine the possible correlation between renal membrane binding and the nephrotoxicity of aminoglycosides, it is appropriate to compare the relative affinity of the various aminoglycosides for the membrane-binding site. Renal membrane-binding kinetics have been described for gentamicin (18, 28, 42) and netilmicin (19). It was the purpose of this investigation to broaden the basis for comparison by studying the renal membrane kinetics of another aminoglycoside, amikacin. Furthermore, by using [^{3}H]amikacin as a prototypical aminoglycoside, we posed several question which would clarify the possible role of membrane-binding affinity in the nephrotoxic expression of aminoglycosides. The first question involved a comparison of [^{3}H]amikacin-binding kinetics between immature and adult rats, since immature rats are known to be less sensitive to aminoglycoside-induced nephrotoxicity (33). The second question involved the possible relationship between the potency for inhibiting [^{3}H]amikacin binding to the renal membrane receptor and the nephrotoxic potential of several aminoglycosides.

MATERIALS AND METHODS

Materials. [^{3}H]amikacin (10 mCi/mmol) was synthesized by Amersham Radiochemicals (Arlington Heights, Ill.) and was purified by high-pressure liquid chromatography methodology developed at Bristol Myers Co., Pharmaceutical Research and Development Division, Syracuse, N.Y. Neomycin sulfate and gentamicin constituents C_1, C_2, and C_{1a} were obtained from Schering Corp., Bloomfield, N.J. Amikacin sulfate and neamine were obtained from Bristol-Myers. Netilmicin and gentamicin sulfates, horseradish peroxidase (HRP) type VI, hydrogen peroxide, and pyrogallol were purchased from Sigma Chemical Co., St. Louis, Mo. Tobramycin sulfate was purchased from Eli Lilly & Co., Indianapolis, Ind.

Membrane isolation and binding studies. Adult male Sprague-Dawley rats (250 to 300 g) were sacrificed by decapitation, and the kidneys were immediately removed and placed in ice-cold saline. Kidneys from immature male rats, between 2 to 3 weeks of age and weighing 60 to 100 g, were also used. Cortical tissue was separated from the medulla, weighed, and homogenized in 10 volumes (wt/vol) of 0.25 M sucrose by using a Teflon (Du Pont Co., Wilmington, Del.)-glass homogenizer.

Brush border and basolateral membrane vesicles were prepared according to the method of Kinsella et al. (23) as modified by Williams et al. (41). Basolateral membranes were collected on a discontinuous sucrose gradient composed of 8 and 38.7% sucrose. The purity of membranes isolated by this procedure was established by marker enzyme analyses which demonstrate enhancement of the brush border membrane marker enzyme, alkaline phosphatase, and the basolateral membrane marker, Na^+,K^+-ATPase, in the respective fractions and the absence of mitochondrial (succinic dehydrogenase) contamination in either membrane fraction. To assay the extent of contamination of membrane fractions with "light" microsomes which may contain pinocytotic vesicles, experiments were done in which rats

* Corresponding author.

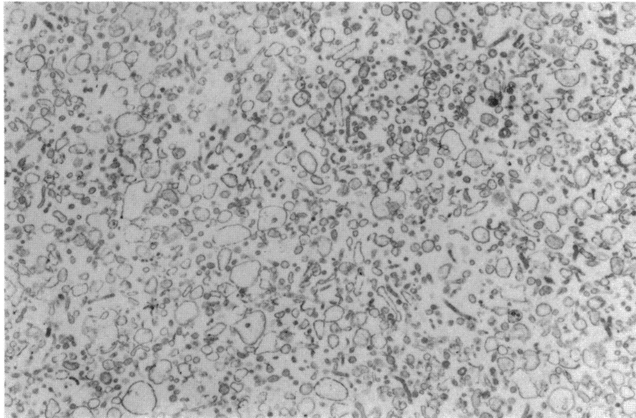

FIG. 1. Electron micrograph of brush border membranes, showing a section fixed for 10 min in formaldehyde (0.5%) and glutaraldehyde (1.0%). Magnification, ×17,500.

were injected (intravenously via tail vein) with HRP, a protein known to be reabsorbed in the proximal tubule by pinocytosis (38). In vivo labeling with HRP is used as a marker for purifying pinocytotic vesicles (22). After HRP injection, rats were sacrificed (10 min postlabeling), and their kidneys were prepared as described above. Brush border and basolateral membrane fractions were then assayed spectrophotometrically for HRP activity. Membranes were suspended in a buffer consisting of 100 mM mannitol and 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.0, with KOH.

Uptake assays were done by incubating membrane vesicles with radiolabeled amikacin at various time intervals and concentrations. The reaction was initiated by the addition of membrane protein (100 μg) and was stopped by the addition of excess cold buffer. The membranes were collected on 0.45-μm-pore-size membrane filters (Millipore Corp., Bedford, Mass.) under vacuum, and uptake was quantitated by counting radioactivity by liquid scintillation spectroscopy. The uptake was also examined in the presence of altered osmolarity (50 to 800 mM sucrose) and at various incubation temperatures (4, 25, and 37°C) to determine whether the observed uptake represented binding or transport across the membrane vesicles. All uptake values represent specific aminoglycoside uptake as determined in the presence of 100× excess cold amikacin.

Protein and enzyme assays. Protein was determined by the method of Lowry et al. by using bovine serum albumin as the standard (30). Na^+,K^+-ATPase was measured by a linked-enzyme assay in which oxidation of NADH was continuously monitored at 340 nm in the presence or absence of 1 mM ouabain (35). Alkaline phosphatase was measured at 420 nm by the *p*-nitrophenyl phosphate method (27), and succinic dehydrogenase was monitored at 600 nm by a method described by Earl and Korner (9). HRP was assayed by a colorimetric method with hydrogen peroxide and pyrogallol as substrates (39).

Electron microscopy. The purity of brush border membrane fractions was also examined by transmission electron microscopy. Membrane pellets were prepared and fixed according to published procedures (2). Subsequent analyses were done by an impartial source (Michigan Cancer Foundation, Detroit, Mich.).

Statistical analyses. Comparisons of [^{3}H]amikacin uptake in both membranes as a function of temperature and osmolarity were made on the basis of the Dunn procedure at the 5% significance level (two tailed).

Kinetic evaluations and comparative ($\log_{10}$) dose-response analyses were done by using linear regression techniques (12). Rankings of the potency of the various aminoglycosides were made by comparing adjacent dose-response curves at the values for concentrations producing 50% inhibition of [^{3}H]amikacin binding. The dose-response curves were compared under the constraints of parallelism when differences in slope estimates were not evident at the 5% significance level.

RESULTS

Brush border and basolateral membrane purity were confirmed by marker enzyme analyses, which revealed approximately 10-fold enrichment of Na^+,K^+-ATPase in basolateral membranes and alkaline phosphatase in brush border membranes. Both membrane fractions showed little cross-contamination and no evidence of contamination with mitochondria as analyzed by succinic dehydrogenase. HRP was not enriched in either membrane fraction, indicating a lack of contamination by pinocytotic vesicles. Electron microscopic analyses of brush border membranes revealed vesicular structures ranging in size from 3.8×10^{-3} to 1.4×10^{-1} μm^2, with the majority of the vesicles in the range of 2×10^{-2} μm^2. Additionally, no evidence of ribosomes, glycogen, rough endoplasmic reticulum, or other cytoplasmic inclusions was found. A photomicrograph of the brush border vesicles is shown in Fig. 1.

[^{3}H]amikacin uptake in renal brush border and basolateral membranes was found to be a rapid, saturable process as shown in Fig. 2 and 3. However, brush border uptake reached equilibrium by 30 s, whereas basolateral uptake required 5 to 10 min to exhibit saturation. The uptake of [^{3}H]amikacin in both membranes was unaffected by changes in temperature or osmolarity, suggesting that the observed uptake represents a binding rather than a transport process (24). Binding kinetics summarized in Table 1 reveal a higher affinity for basolateral membranes compared with brush border membranes. The specificity of amikacin binding to the renal membranes in these experiments was very high (≧95%).

Renal membranes prepared from immature rats exhibited binding affinities significantly lower than those for adult membranes as depicted in Fig. 4. The K_ds for [^{3}H]amikacin binding to brush border and basolateral renal membranes in the immature rat were 6,216 and 2,262 μM, respectively,

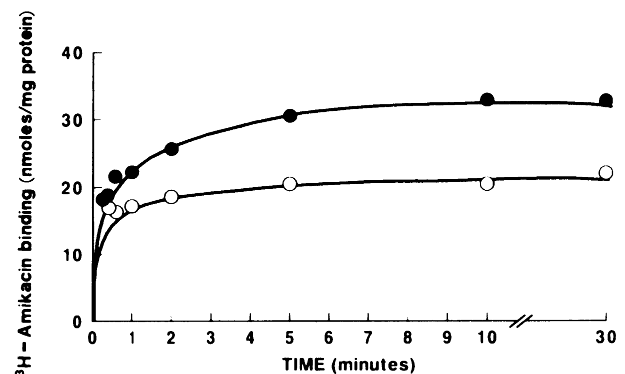

FIG. 2. Time course of 1 mM [^{3}H]amikacin uptake in renal brush border (○) and basolateral (●) membranes. Each datum point represents the average of four to six experiments.

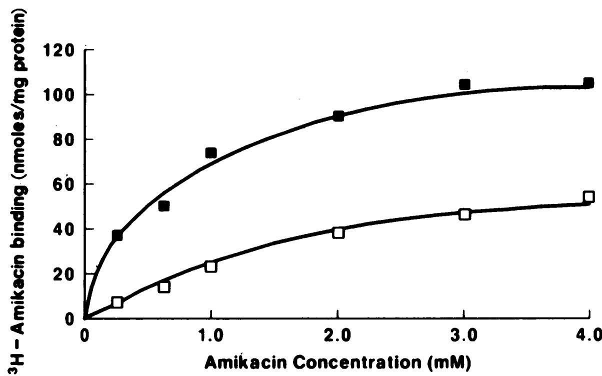

FIG. 3. [^{3}H]amikacin uptake in renal brush border (□) and basolateral (■) membranes as a function of amikacin concentration. Uptakes (30 min) were done as described in the text. Each datum point represents the average of four to six experiments.

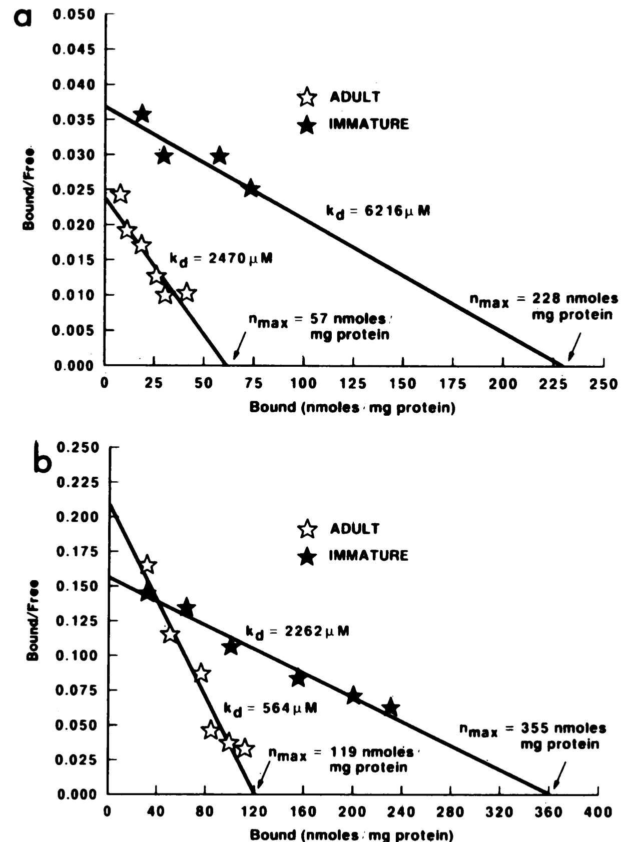

FIG. 4. Scatchard analysis of [^{3}H]amikacin binding to adult and immature rat brush border (a) and basolateral (b) renal membranes. Shown are the data from a single adult and immature rat experiment. Uptakes (30 min) were done as described in the text. Each datum point represents the average of four to six experiments.

approximately 2.5- to 4-fold higher than the values from adult rat membranes.

When several aminoglycosides were tested for inhibition of [^{3}H]amikacin membrane binding, the following rank order of potencies was obtained: neomycin > tobramycin ~ gentamicin ~ netilmicin > amikacin ~ neamine > streptomycin. The dose-response curves for inhibition of [^{3}H]amikacin are displayed in Fig. 5. The 50% inhibitory values are listed in Table 2.

DISCUSSION

The uptake of [^{3}H]amikacin in rat renal membrane vesicles parallels the characteristics of a rapid, saturable binding process previously described for gentamicin (42, 44). As described for both gentamicin (18, 42,44) and netilmicin (19), binding of the aminoglycoside was higher in basolateral membranes compared with brush border membranes. For example, amikacin had a fourfold greater affinity for basolateral (K_d = 607 μM) versus brush border membranes (K_d = 2,535 μM). Of interest is the comparison among the affinities of amikacin, gentamicin, and netilmicin for renal membranes. Amikacin affinity, as compared by K_d values, is approximately 100-fold lower in brush border membranes and 15-fold lower in basolateral membranes compared with gentamicin and netilmicin (18, 19, 42). The comparative renal membrane-binding affinities of amikacin, gentamicin and netilmicin correlate well with their nephrotoxic potentials (15).

The observation that immature rats are relatively resistant to aminoglycoside-induced nephrotoxicity compared with adult rats (33) provided an opportunity to further examine the possible correlation between renal membrane-binding affinity and nephrotoxicity. Renal membranes from young rats exhibited a lower affinity for [^{3}H]amikacin binding in both brush border and basolateral membranes, suggesting a direct correlation between membrane-binding affinity and nephrotoxic potential. Aladjem et al. (1) reported an age-related increment in gentamicin uptake in rat renal cortical slices, which may be a result of the altered membrane-binding affinities revealed in this study. Reduced suscepti-

TABLE 1. Amikacin-binding parameters in rat kidney brush border and basolateral membranes from adult rats[a]

Membrane	K_d	N_{max}
Brush border	2,535 ± 71	87 ± 20
Basolateral	607 ± 82[b]	126 ± 15

[a] Data are the means of values calculated from at least three separate experiments ± standard error of the mean. K_d = μM; N_{max} = nanomoles per milligram of protein.

[b] Significantly different from brush border value. $P < 0.05$ by Student's t test.

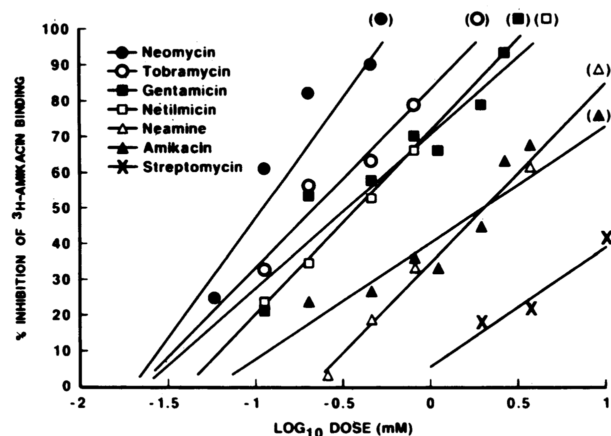

FIG. 5. Dose-response of the inhibition of 1 mM [^{3}H]amikacin binding to renal brush border membranes by various aminoglycosides. Uptakes (30 s) were done as described in the text. Each datum point represents the average of four to six experiments.

TABLE 2. Effects of various aminoglycosides on the binding of [^{3}H]amikacin to rat kidney brush border membranes

Aminoglycoside	Estimated IC_{50}[a]
Neomycin	0.108
Tobramycin	0.254
Gentamicin C_2	0.341
Gentamicin complex	0.345
Gentamicin C_{1a}	0.416
Netilmicin	0.425
Gentamicin C_1	0.874
Amikacin	2.07
Neamine	2.11
Streptomycin	10.80

[a] Values represent the estimated 50% inhibitory concentration from the various dose-response curves generated by at least three datum points. IC_{50} = concentration of the aminoglycoside (mM) inhibiting [^{3}H]amikacin binding (1 mM) by 50%. Uptake (30 s) assays were as described in the text.

bility to aminoglycoside-induced nephrotoxicity as well as lower cortical accumulation of aminoglycosides has also been reported in female rats compared with male rats (5). Williams and Hottendorf (42) recently demonstrated a lower affinity for [^{3}H]gentamicin binding to renal brush border membranes from female rats compared with male rats, which reinforces the relationship between membrane-binding affinity and nephrotoxicity. These observations may shed further light upon the phenomenon of the development of resistance of nephrotoxicity with continued aminoglycoside treatment (14, 32). The regenerated tubules resulting from repair of damaged tissue with continuous aminoglycoside treatment may be reflective of an immature stage of development, particularly since the regenerated tubules become sensitive with time (14). Assuming a relationship between regenerated tubules in adult rats and tubules in immature rats, our data would propose that resistance to aminoglycoside nephrotoxicity in these two states is related to a decreased affinity for aminoglycoside binding which is lost with cellular maturity.

The ability of aminoglycosides to compete for binding sites on renal membranes provides a means of comparing relative membrane-binding affinities with in vivo nephrotoxic potentials. As shown in Fig. 5 and Table 2, the inhibitory activity of several aminoglycosides versus [^{3}H]amikacin binding revealed a rank order of potency of neomycin > tobramycin ~ gentamicin ~ netilmicin > amikacin ~ neamine > streptomycin. These inhibitory potencies reflect the relative affinity of these aminoglycosides for the renal membrane-binding site. The relative affinities are in good agreement with the in vivo nephrotoxic potentials of these aminoglycosides (14–16, 30). Neomycin is consistently the most nephrotoxic aminoglycoside, whereas streptomycin is essentially nonnephrotoxic. Amikacin possesses a low nephrotoxic potential, followed by tobramycin and gentamicin. There is less agreement in the literature with respect to the relative nephrotoxicity of netilmicin. High-dose comparisons in rats predicted a low nephrotoxic potential of netilmicin relative to other aminoglycosides (31). However, netilmicin has a shallow dose-response curve relative to other aminoglycosides, and when tested at low multiples of the human clinical dose, netilmicin nephrotoxicity was comparable to that of gentamicin (15). The clinical use of netilmicin has also been associated with appreciable nephrotoxicity (8, 37). The gentamicin constituents C_1, C_{1a}, and C_2 have been reported to possess different nephrotoxic potentials in vivo (25). The inhibitory potencies of these three constituents (Table 2) are consistent with their reported nephrotoxic potentials. Gentamicin C_2 is the most nephrotoxic, followed by gentamicin C_{1a}. Gentamicin C_1 was found to be the least nephrotoxic of the three consitituents. Neamine, a component of the neomycin molecule containing 2,6-diaminoglucose and 2-deoxystreptamine, was similar to amikacin in its inhibitory potency versus [^{3}H]amikacin binding. This structure has been found to possess a nephrotoxic potential in vivo approximately fivefold less than that of gentamicin and comparable to that of amikacin (unpublished data). Unlike the positive relationship between membrane-binding affinities and nephrotoxic potential, the nephrotoxic potentials of aminoglycosides do not correlate with the degree of renal cortical accumulation (21). Of additional interest is the recent report of Au et al. (3) which reported a correlation between aminoglycoside ototoxicity and interactions with liposomal membranes. Thus, the ototoxic as well as nephrotoxic potential of these drugs may correlate with membrane-associated events.

Williams et al. (43) recently provided evidence that renal membrane binding of aminoglycosides assumes a direct role in the renal pathogenesis. Coadministration of the polyamino acid polyaspartic acid reduced the levels of amikacin in brush border and basolateral membranes and was associated with reduced nephrotoxicity. The lack of pinocytotic vesicle contamination in our membrane preparation as reported in this investigation further indicates that the brush border binding observed and its inhibition by polyaspartic acid represents an association of the aminoglycoside with brush border microvilli, not pinocytotic vesicles. Thus, the correlation between brush border membrane-binding inhibition and nephrotoxicity of aminoglycosides discussed above reflects the relative affinity for the brush border microvilli.

The results of this investigation demonstrate that the renal membrane binding of a prototypical aminoglycoside antibiotic, amikacin, is decreased under conditions of resistance of aminoglycoside nephrotoxicity in vivo and is inhibited to various degrees by other aminoglycosides. The relative affinities of the aminoglycosides for their renal membrane-binding sites appear to correlate well with their known nephrotoxic potentials, indicating the utility of this membrane model for assessing the nephrotoxicity of new aminoglycosides.

LITERATURE CITED

1. **Aladjem, M., Y. Aladjem, G. Koren, and H. Boichis.** 1984. Maturation of renal tubular transport of gentamicin. Dev. Pharmacol. Ther. **7**:82–86.
2. **Arnold, J., H. Soule, and J. Russo.** 1975. Fine structure of a human mammary carcinoma cell line. In Vitro (Rockville) **10**:356–359.
3. **Au, S., N. Weiner, and J. Schacht.** 1986. Membrane perturbation by aminoglycosides as a simple screen of their toxicity. Antimicrob. Agents Chemother. **30**:395–397.
4. **Bennett, W. M.** 1983. Aminoglycoside nephrotoxicity. Nephron **35**:73–77.
5. **Bennett, W. M., R. A. Parker, W. C. Elliott, D. N. Gilbert, and D. C. Houghton.** 1982. Sex-related differences in the susceptibility of rats to gentamicin nephrotoxicity. J. Infect. Dis. **145**: 370–373.
6. **Chiu, P. J. S., A. Brown, G. Miller, and J. F. Long.** 1976. Renal extraction of gentamicin in anesthetized dogs. Antimicrob. Agents Chemother. **10**:277–282.
7. **Collier, V. U., P. S. Lietman, and W. E. Mitch.** 1979. Evidence for luminal uptake of gentamicin in the perfused rat kidney. J. Pharmacol. Exp. Ther. **210**:247–251.
8. **Cronin, R. D.** 1979. Aminoglycoside nephrotoxicity: pathogenesis and prevention. Clin. Nephrol. **11**:251–256.

9. **Earl, D. C. M., and A. Korner.** 1965. The isolation and properties of cardiac ribosomes and polysomes. Biochem. J. **94**:721–734.
10. **Fabre, J., J. P. Fillastre, J.-P. Morin, and M. Rudhardt.** 1978. Nephrotoxicity of gentamicin: action on subcellular organelles and pharmacokinetics in the kidney. Contrib. Nephrol. **10**:53–62.
11. **Feldman, S., M. Wang, and G. J. Kaloyanides.** 1982. Aminoglycosides induce a phospholipidosis in the renal cortex of the rat: an early manifestation of nephrotoxicity. J. Pharmacol. Exp. Ther. **220**:514–520.
12. **Finney, D. J.** 1978. Statistical method in biological assay, 3rd ed., p. 1–508. MacMillan Publishing Co., New York.
13. **Francke, E., and H. C. Neu.** 1983. Nephrotoxicity of aminoglycosides. Infect. Surg. **1983(3)**:205–214.
14. **Gilbert, D. N., D. C. Houghton, W. M. Bennett, C. E. Plamp, K. Reger, and G. A. Porter.** 1979. Reversibility of gentamicin nephrotoxicity in rats: recovery during continuous drug administration. Proc. Soc. Exp. Biol. Med. **160**:99–103.
15. **Hottendorf, G. H., D. Barnett, L. L. Gordon, E. F. Christensen, and H. Madissoo.** 1981. Nonparallel nephrotoxicity dose-response curves of aminoglycosides. Antimicrob. Agents Chemother. **19**:1024–1028.
16. **Hottendorf, G. H., and L. L. Gordon.** 1980. Comparative low-dose nephrotoxicities of gentamicin, tobramycin, and amikacin. Antimicrob. Agents Chemother. **18**:176–181.
17. **Hsu, C. H., T. W. Kurtz, and J. M. Weller.** 1977. In vitro uptake of gentamicin by rat renal cortical tissue. Antimicrob. Agents Chemother. **12**:192–194.
18. **Ishikawa, Y., K. Inui, and R. Hori.** 1985. Gentamicin binding to brush border and basolateral membranes isolated from rat kidney cortex. J. Pharmacobio-Dyn. **8**:931–941.
19. **Josepovitz, C., R. Levine, T. Farruggella, B. Lane, and G. J. Kaloyanides.** 1985. ^{3}H-netilmicin binding constants and phospholipid composition of renal plasma membranes of normal and diabetic rats. J. Pharmacol. Exp. Ther. **233**:298–303.
20. **Kaloyanides, G. J.** 1984. Aminoglycoside-induced functional and biochemical defects in the renal cortex. Fundam. Appl. Toxicol. **4**:930–943.
21. **Kaloyanides, G. J., and E. Pastoriza-Munoz.** 1980. Aminoglycoside nephrotoxicity. Kidney Int. **18**:571–582.
22. **Kinne, R., and K. Baumann.** 1974. Analysis of the pinocytic process in rat kidney. J. Cell Biol. **63**:998–1008.
23. **Kinsella, J. L., P. D. Holohan, N. I. Pessah, and C. R. Ross.** 1979. Isolation of luminal and antiluminal membranes from dog kidney cortex. Biochim. Biophys. Acta **552**:468–477.
24. **Kinsella, J. L., P. D. Holohan, and C. R. Ross.** 1979. Transport of organic ions in renal cortical luminal and antiluminal membrane vesicles. J. Pharmacol. Exp. Ther. **209**:443–450.
25. **Kohlhepp, S. J., M. O. Loveless, P. W. Kohnen, D. C. Houghton, W. M. Bennett, and D. N. Gilbert.** 1984. Nephrotoxicity of the constituents of the gentamicin complex. J. Infect. Dis. **149**:605–614.
26. **Kosek, J. C., R. I. Mazze, and M. J. Cousins.** 1974. Nephrotoxicity of gentamicin. Lab. Invest. **30**:48–57.
27. **Lansing, A. I., M. L. Belkhode, W. E. Lynch, and I. Lieberman.** 1967. Enzymes of plasma membranes of liver. J. Biol. Chem. **242**:1772–1775.
28. **Lipsky, J. J., L. Cheng, B. Sacktor, and P. S. Lietman.** 1980. Gentamicin uptake by renal tubule brush border membrane vesicles. J. Pharmacol. Exp. Ther. **215**:390–393.
29. **Lodhi, S., N. Weiner, and J. Schacht.** 1976. Interactions of neomycin and calcium in synaptosomal membranes and polyphosphoinositide monolayers. Biochim. Biophys. Acta **426**:781–785.
30. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
31. **Luft, F. C., R. Bloch, R. S. Sloan, M. N. Yum, R. Costello, and D. R. Maxwell.** 1978. Comparative nephrotoxicity of aminoglycoside antibiotics in rats. J. Infect. Dis. **138**:541–545.
32. **Luft, F. C., L. E. Rankin, R. S. Sloan, and M. N. Yum.** 1978. Recovery from aminoglycoside nephrotoxicity with continued drug administration. Antimicrob. Agents Chemother. **14**:284–287.
33. **Marre, R., N. Tarara, T. Louton, and K. Sack.** 1980. Age-dependent nephrotoxicity and the pharmacokinetics of gentamicin in rats. Eur. J. Pediatr. **133**:25–29.
34. **Pastoriza-Munoz, E., R. L. Bowman, and G. J. Kaloyanides.** 1979. Renal tubular transport of gentamicin in the rat. Kidney Int. **16**:440–450.
35. **Schwartz, A., J. C. Allen, and S. Harigaya.** 1969. Possible involvement of cardiac Na^+, K^+-adenosine triphosphatase in the mechanism of action of cardiac glycosides. J. Pharmacol. Exp. Ther. **168**:31–41.
36. **Schwertz, D. W., J. I. Kreisberg, and C. Venkatachalam.** 1984. Effects of aminoglycosides on proximal tubule brush border membrane phosphatidylinositol-specific phospholipase. J. Pharmacol. Exp. Ther. **231**:48–55.
37. **Snydman, D. R., F. P. Tally, S. H. Landesman, M. Barza, and S. L. Gorbach.** 1979. Netilmicin in gram-negative bacterial infections. Antimicrob. Agents Chemother. **15**:50–54.
38. **Straus, W.** 1960. Localization of intravenously injected horseradish peroxidase in the cells of the convoluted tubules of rat kidney. Exp. Cell Res. **20**:600–603.
39. **Straus, W.** 1962. Colorimetric investigation of the uptake of an intravenously injected protein (horseradish peroxidase) by rat kidney and effects of competition by egg white. J. Cell Biol. **12**:231–246.
40. **Whelton, A., and W. B. Walker.** 1974. Intrarenal antibiotic distribution in health and disease. Kidney Int. **6**:131–137.
41. **Williams, P. D., P. D. Holohan, and C. R. Ross.** 1981. Gentamicin nephrotoxicity. II. Plasma membrane changes. Toxicol. Appl. Pharmacol. **61**:243–251.
42. **Williams, P. D., and G. H. Hottendorf.** 1986. ^{3}H-gentamicin uptake in brush border and basolateral membrane vesicles from rat kidney cortex. Biochem. Pharmacol. **35**:2253–2256.
43. **Williams, P. D., G. H. Hottendorf, and D. B. Bennett.** 1986. Inhibition of renal membrane binding and nephrotoxicity of aminoglycosides. J. Pharmacol. Exp. Ther. **237**:919–925.
44. **Williams, P. D., M. E. Trimble, L. Crespo, P. D. Holohan, J. D. Freedman, and C. R. Ross.** 1984. Inhibition of renal Na^+, K^+-adenosine triphosphatase by gentamicin. J. Pharmacol. Exp. Ther. **231**:248–253.