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Comparative Low-Dose Nephrotoxicities of Gentamicin, Tobramycin, and Amikacin

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Most investigations of the comparative nephrotoxicities of aminoglycosides in animals have utilized large multiples of the human dose. Furthermore, many of these assessments have used only one or two dose levels and have not described a dose-response comparison among antibiotics. Because of this lack of comparative dose-response data over a range of low multiples of the human dose, the nephrotoxicities of gentamicin, tobramycin, and amikacin were investigated in 180 rats, utilizing doses ranging from one to seven times the equivalent human clinical doses. Histopathological evaluations of both kidneys from each rat were scored without knowledge of the treatment, and statistical analyses of the results indicated that a linear and parallel dose-response relationship existed for each drug, the relative nephrotoxicity over the range of doses analyzed was gentamicin > tobramycin > amikacin ($P = 0.0001$), and, unlike amikacin, the human dose equivalents (milligrams per kilogram) of gentamicin and tobramycin were significantly nephrotoxic in rats ($P < 0.05$).

The prevalent utilization of doses many times greater than those used in human antibacterial therapy when comparing the nephrotoxicities of aminoglycosides in animals has been questioned (1, 31). Additionally, many of these studies have employed only one or two dose levels and have not defined relative dose-response relationships. Relative toxicity is classically defined by comparing dose-response relationships which are linear and parallel over a range of responses of a similar magnitude (11, 15). Based on results of a previous limited comparison (13), the relative dose-response nephrotoxicities of gentamicin, tobramycin, and amikacin were explored in rats by utilizing a range of low doses anticipated to produce similar magnitudes of nephrotoxic responses.

MATERIALS AND METHODS

Animals. A total of 180 adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc.), weighing between 105 and 140 g upon arrival, were conditioned for 14 days before initiation of the study. The rats were housed individually in cages of appropriate type and size in an environmentally controlled room, given Purina Laboratory Chow and fresh drinking water ad libitum, ranked by body weight, and then randomly divided into groups of 10 after being individually identified by a tag number attached to an ear.

Aminoglycoside administration. The 18 groups of 10 rats each were dosed for 28 days as shown in Table 1. Doses were selected with regard to antibiotic activity, split, and administered twice a day at approximately 9 a.m. and 3 p.m. by subcutaneous injection through a 27-gauge needle. The five gentamicin doses (4 to 20 mg/kg per day), six tobramycin doses (4 to 40.5 mg/kg per day), and six amikacin doses (15 to 98 mg/kg per day) were each geometrically spaced, except for the lowest doses of tobramycin and amikacin. The human therapeutic doses recommended in the package inserts are as follows: gentamicin and tobramycin, 3 to 5 mg/kg per day (we used 4 mg/kg per day); amikacin, 15 mg/kg per day. The equivalents of these therapeutic doses for all three aminoglycosides, expressed in milligrams per kilogram, were also included. Because of the differences in therapeutic doses, the daily doses of each aminoglycoside were normalized and expressed as multiples of the respective therapeutic dose to facilitate comparisons.

The concentrations utilized were as follows: 2 mg/ml for gentamicin, 4 mg/ml for tobramycin, and 10 mg/ml for amikacin. The daily half-dose injection volumes varied from 0.075 to 0.5 ml/100 g, depending on the dose. Ten control rats received 0.5 ml of antibiotic-free saline per 100 g.

Antemortem observations. Signs of toxicity, changes in general health and behavior, and body weights were recorded just before the administration of the first dose each day. Serum creatinine and blood urea nitrogen were determined on individual blood samples of all rats before the start of dosing and on days 14 or 15 and 27 or 28 of dosing. Urinalyses were performed on pooled 18-h urine samples collected from each treated group and the control group at the same times. Urine volume, specific gravity, and pH were determined for each sample, and protein, sugar, ketones, bilirubin, hemoglobin, erythrocytes, leukocytes, epithelial cells, bacteria, and casts were measured semiquantitatively.

Postmortem evaluation. All rats were sacrificed by an overdose of sodium pentobarbital on the day after the last day of dosing. Cardiac blood samples were taken immediately, after which both kidneys were removed and examined grossly. A longitudinal section of the left kidney and a cross section of the right kidney from each rat were preserved in 10%

neutral buffered Formalin for histopathological evaluation. Tissue sections (6 μm each) were stained with hematoxylin and eosin, and the resulting tissue slides were randomized, masked, and examined by a single pathologist without knowledge of the animal's treatment.

Lesions encountered in the renal cortex included tubular vacuolar or granular degeneration, peritubular inflammation, tubular necrosis, tubular dilatation, tubular basophilia, and interstitial fibrosis. The extent and distribution of each of these lesions in both kidneys of every rat were scored as follows: 0, absence of lesion; 1, lesion represented in fewer than 10% of the nephrons; 2, lesion represented in 10 to 50% of the nephrons; 3, lesion represented in 50 to 90% of the nephrons; and 4, lesion represented in more than 90% of the nephrons. Since these lesions are all interrelated and represent various stages of proximal tubular damage (13), the lesion scores were summed to produce a single nephrotoxicity response for each animal, with a possible severity range of 0 to 24.

Statistical analysis. Regression analyses (20) were performed for each aminoglycoside, relating the nephrotoxicity response to the multiple of the therapeutic dose. Each analysis tested the significance of the linear dose response and the departure from linearity, and the slopes of the dose-response curves were also tested for parallelism. An analysis of covariance was then performed to determine whether the mean nephrotoxicity responses defined by the dose-response curves differed significantly from each other after being adjusted for the effect of dose (20). In addition, the nephrotoxicity responses obtained at the human equivalent therapeutic doses of the aminoglycosides were compared with those of the control by an analysis of variance, followed by the Dunnett procedure for comparison of all treatment means against a control (32).

RESULTS

Antemortem observations. All of the animals survived the 28-day experimental dosing period. No treatment-related effects on body weights were encountered in any group. Serum creatinine values were within normal limits (Table 1). Blood urea nitrogen values (21 to 25 mg/dl) were slightly increased in several high-dose gentamicin and tobramycin rats at various times, but the terminal group means of all groups were within the normal limits of this laboratory (Table 1). Some casts (three to four per high-power field) were observed in the pooled urine

TABLE 1. *Aminoglycoside nephrotoxicity response in rats*

Group (n = 10)	Aminoglycoside[a]	Daily dose (mg/kg)[b]	Dose multiple	Mean terminal BUN (mg/dl)[c]	Mean terminal creatinine (mg/dl)	Mean nephrotoxicity score for following lesion[d]: a	b	c	d	e	f	Total mean nephrotoxicity score ± SE[e]
1	Gentamicin	4	1	16	0.7	0.4	1.2	0.1	1.0	1.1	0.2	4.0 ± 0.4
2		6	1.5	15	0.6	0.3	1.0	0	1.1	1.4	0.2	4.0 ± 0.3
3		9	2.3	15	0.6	0.8	1.6	0.3	1.5	1.8	0.2	6.2 ± 0.5
4		13.5	3.4	15	0.6	1.1	1.9	0.5	2.4	2.4	0.7	9.0 ± 0.6
5		20	5	16	0.6	1.5	2.5	0.6	3.1	3.0	1.8	12.5 ± 1.0
6	Tobramycin	4	1	15	0.7	0.2	0.5	0	0.6	0.9	0.2	2.4 ± 0.4
7		8	2	13	0.8	0.6	0.8	0	0.8	0.9	0.1	3.2 ± 0.4
8		12	3	14	0.7	0.9	1.5	0.2	1.6	1.6	0.2	6.0 ± 0.6
9		18	4.5	17	0.7	1.3	2.1	0.6	2.9	2.5	1.2	10.6 ± 0.9
10		27	6.8	19	0.7	1.1	2.7	0.8	3.4	3.4	2.3	13.7 ± 0.6
11		40.5	10.1	19	0.7	1.5	2.8	1.4	3.2	3.3	2.2	14.4 ± 0.8
12	Amikacin	15	1	15	0.7	0.1	0.4	0	0.4	0.7	0	1.6 ± 0.3[f]
13		40	2.7	15	0.6	0.5	0.9	0	0.9	0.9	0.2	3.4 ± 0.6
14		50	3.3	15	0.7	0.8	1.2	0.1	1.4	1.3	0.4	5.2 ± 0.4
15		62.5	4.2	15	0.6	1.3	1.3	0.2	2.0	2.1	0.5	7.4 ± 0.8
16		78	5.2	15	0.6	1.2	1.4	0.8	2.3	2.6	0.6	8.9 ± 0.7
17		98	6.5	17	0.8	2.0	2.2	1.1	3.3	3.2	1.7	13.5 ± 0.7
18	Saline control	0	—	13	0.7	0	0.4	0	0.2	0.5	0	1.1 ± 0.4

[a] Administered twice a day for 28 days.

[b] Geometric except for the lowest doses of tobramycin and amikacin.

[c] BUN, Blood urea nitrogen.

[d] a, Tubular degeneration; b, peritubular inflammation; c, tubular necrosis; d, tubular dilatation; e, tubular basophilia; f, interstitial fibrosis.

[e] Group means of individual rat total scores. SE, Standard error.

[f] Only value not significantly different from the control value ($P > 0.05$).

obtained from the higher-dose groups of all three aminoglycosides. These minor clinicopathological changes could not be correlated with the moderate degree of histopathological renal lesions produced in these animals. This anticipated observation supports the reported insensitivity of renal function tests in rats in the presence of mild to moderate histological nephrotoxicity (7, 13, 26, 27).

Postmortem evaluation. The gross examination at necropsy revealed some slight mottling of the kidneys of some rats in all of the higher-dose groups. Histopathological examination of both kidneys of all of the rats revealed only a moderate degree of bilateral proximal tubular damage at the highest doses of all three aminoglycosides. The production of such an intermediate grade of nephrotoxicity facilitates the development of a dose response and permits a more accurate microscopic comparison than do larger, more destructive doses (13). The light microscopic changes were confined to the proximal tubules and were consistent with those ascribed to aminoglycosides in rats and humans (10, 13, 24, 29). Some acute focal tubular epithelial degeneration and necrosis were evident, particularly at the higher doses of all aminoglycosides (Fig. 1). However, the focal proximal tubular damage in this 28-day model was represented predominantly by less acute changes, such as dilatation of the lumens of proximal tubules (Fig. 2 and 3), slight peritubular areas of round cell infiltrates (Fig. 4), tubular basophilia (Fig. 5), and small focal areas of peritubular interstitial fibrosis (Fig. 5). More than one lesion was usually represented in any individual kidney (Fig. 5), and the mean severity of each lesion increased with the dose of each aminoglycoside.

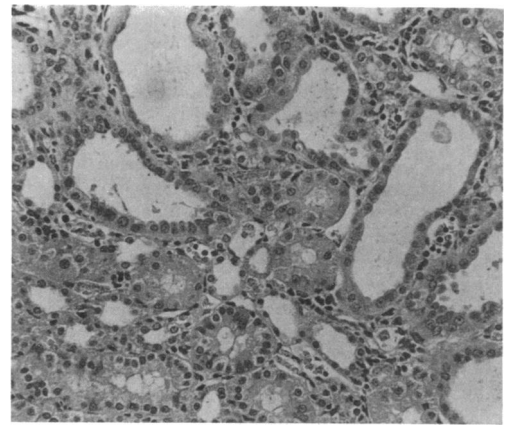

FIG. 2. *Dilated proximal tubules in a rat given 20 mg of gentamicin per kg per day (5 times the human therapeutic dose equivalent) for 28 days. ×150.*

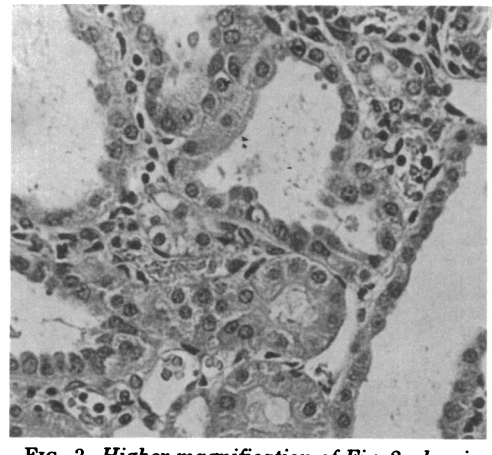

FIG. 3. *Higher magnification of Fig. 2, showing a brush border on some of the cells lining this dilated proximal tubule. ×300.*

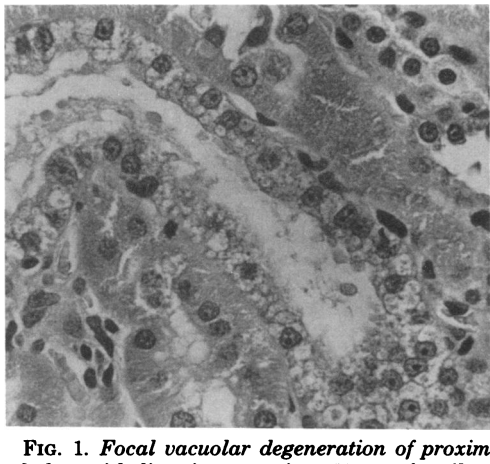

FIG. 1. *Focal vacuolar degeneration of proximal tubular epithelium in a rat given 78 mg of amikacin per kg per day (5.2 times the human therapeutic dose equivalent) for 28 days. ×900.*

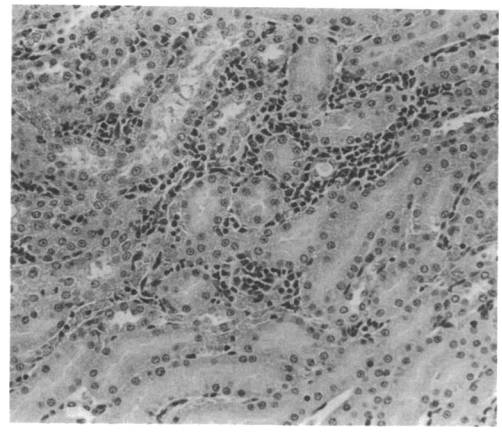

FIG. 4. *Focal peritubular round cell infiltrates in a rat given 8 mg of tobramycin per kg per day (2 times the human therapeutic dose equivalent) for 28 days. ×150.*

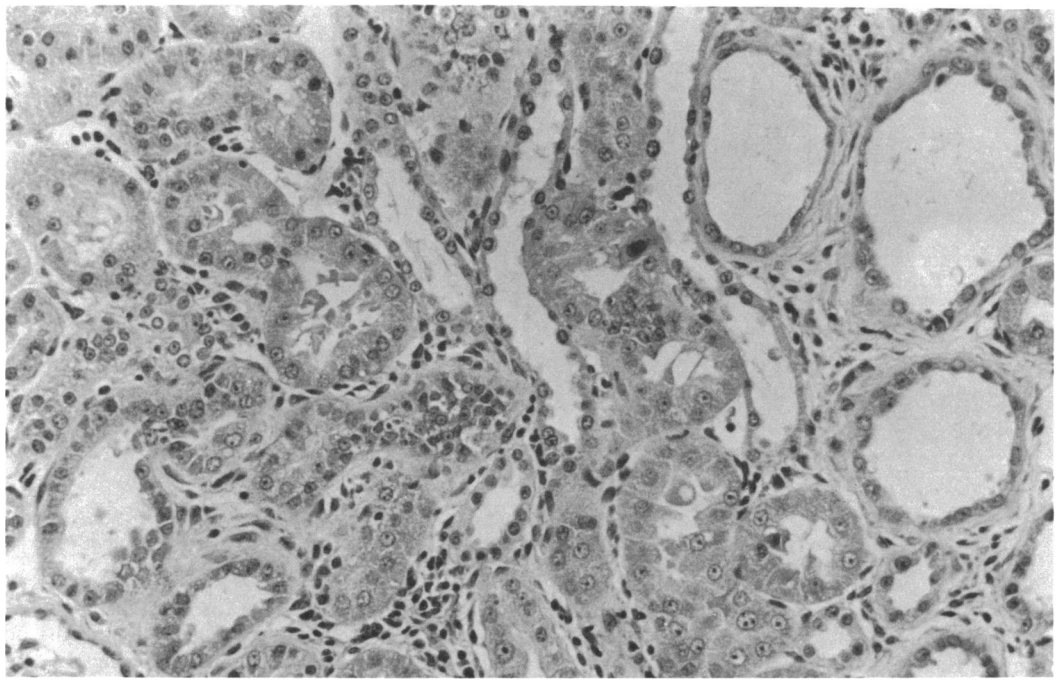

FIG. 5. *Spectrum of changes in a rat given 18 mg of tobramycin per kg per day (4.5 times the human therapeutic dose equivalent) for 28 days. Dilated tubules and interstitial fibrosis are shown on the right, with tubular regeneration through the center, and a small focus of round cells has infiltrated into the lower-left center.* ×300.

A mean nephrotoxicity response for the 10 rats in each treatment group and the control group was obtained from the individual rat scores. These mean nephrotoxicity responses and the means for each lesion by treatment group are given in Table 1.

Recovery from nephrotoxicity has been reported in rats receiving large doses of aminoglycosides, with active necrosis and regeneration occurring simultaneously (7, 9, 10, 17). This recovery is manifested histologically by the regeneration of proximal tubular epithelium, which is distinguished by its basophilia (7, 10). Tubular basophilia was one of the six nephrotoxic lesions evaluated in this study, and the tubular basophilia scores were highly correlated with the total histological nephrotoxicity scores for each aminoglycoside (partial correlation analysis, $P < 0.0001$). Therefore, the degree of recovery was proportional to the total renal toxicity observed with each aminoglycoside, and recovery within the duration of dosing should be equally represented for each aminoglycoside.

Statistical analysis. The nephrotoxicity response at the highest dose (10.1 times the human therapeutic dose equivalent) of tobramycin was excluded because it did not appear to lie on the linear portion of the dose-response curve and was outside the range of the dose multiples of the other two aminoglycosides. The nephrotoxicity responses are plotted on an arithmetic scale directly against the multiple of the therapeutic dose in Fig. 6. Other models in which log transformations were used did not offer any advantages over the arithmetic plot.

The three dose-response curves were significantly linear and parallel. The order of decreasing toxicity was gentamicin > tobramycin > amikacin, with each aminoglycoside differing significantly from the others ($P = 0.0001$). At the human therapeutic dose equivalents (milligrams per kilogram), gentamicin and tobramycin exhibited significant nephrotoxicity in rats ($P < 0.05$, treated versus control). The renal reactions to the equivalent dose of amikacin could not be distinguished from those of the control group ($P > 0.2$).

DISCUSSION

Nephrotoxicity is the most serious adverse effect of aminoglycosides in clinics (19, 23). Gentamicin has produced degenerative epithelial changes detectable by light microscopy in the proximal tubules of rats given daily doses of as

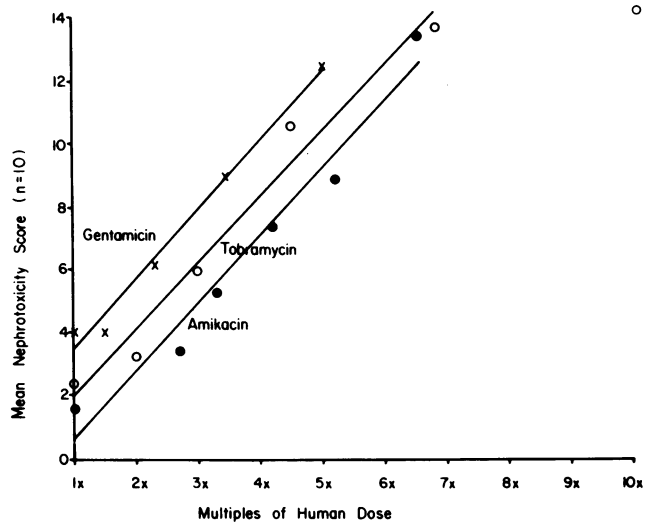

FIG. 6. *Nephrotoxicity dose-response curves for gentamicin* (×), *tobramycin* (○), *and amikacin* (●).

low as 3 mg/kg per day for 28 days (7) and 5 mg/kg per day for 21 days (30). Ultrastructural changes have been reported in rats 48 h after receiving doses of 1 mg/kg per day (14).

Gentamicin has been shown to be more nephrotoxic than tobramycin in animals (2, 10, 17, 19, 23, 27, 29). Four reports indicate that amikacin is less nephrotoxic than both gentamicin and tobramycin (2, 4, 24; J. S. Wold, D. O. Robbins, C. L. Gries, B. L. Miller, and S. A. Turnipseed, Toxicol. Appl. Pharmacol. 48:A20, 1979).

The relevance to humans of such findings observed in animals is controversial. However, several clinicians have declared that it is very difficult to determine the incidence of antibiotic-induced nephrotoxicity in patients (6, 10, 12). Compounding the problems of distinguishing between the patient's illness and drug-induced nephrotoxicity are the inaccuracies and lack of specificity of renal function tests, including blood urea nitrogen and serum creatinine (3, 8), endogenous creatinine and inulin clearance (5, 18, and quantitation of urinary enzymes (22, 25, 28). Unfortunately, there is no satisfactory marker to predict impending nephrotoxicity in clinics (6). Microscopic examination of kidney sections is an accurate and sensitive method of assessing subclinical nephrotoxicity in animals, particularly if there is only slight to moderate focal renal damage (13, 14, 26, 29, 33). Comprehensive prospective, double-blind clinical studies comparing various aminoglycosides would be highly desirable, especially if more sensitive renal function tests and estimates of glomerular filtration more reliable than creatinine were included, but "until such data in man are available, reliance must be placed on animal studies" (16).

The utilization of rats for comparing the nephrotoxicities of aminoglycosides has some advantages over clinical trials, including the possibilities of studying large numbers of disease-free subjects, of having a negative control, and of making histological evaluations of renal tissues on test subjects not previously or concomitantly exposed to other nephrotoxins. Furthermore, there are well-validated similarities in the pharmacokinetics (13, 21) and toxicities (1, 13) of aminoglycosides in rats and humans.

Based on accurate and sensitive microscopic examinations of renal tissues, gentamicin and tobramycin produced statistically significant subclinical nephrotoxicity in rats given the equivalent human therapeutic dose expressed in milligrams per kilogram ($P > 0.05$). The group given therapeutic doses of amikacin could not be distinguished from the control group ($P > 0.2$). The relative nephrotoxicity over the entire range of doses tested was gentamicin > tobramycin > amikacin ($P = 0.0001$). Since the ability to compare the nephrotoxicities of aminoglycosides in clinical trials is suspect (6, 9, 12, 16), the greater safety of amikacin versus gentamicin and tobramycin predicted in rats may be undetectable in clinics.

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LITERATURE CITED

1. **Appel, G. B., and H. C. Neu.** 1978. Gentamicin in 1978. Ann. Intern. Med. **89**:528–538.
2. **Barza, M., V. Pinn, P. Tanguay, and T. Murray.** 1978.

Nephrotoxicity of newer cephalosporins and aminoglycosides alone and in combination in a rat model. J. Antimicrob. Chemother. 4(Suppl. A):59-68.
3. **Baum, N., C. C. Dichoso, and C. E. Carlton.** 1975. Blood urea nitrogen and serum creatinine. Physiology and interpretations. Urology 5:583-588.
4. **Beck, Von H., B. Züllich, R. Marre, and K. Sack.** 1979. Tierexperimentelle Untersuchungen zur Nephrotoxizität von 7 Aminoglykosiden bei Langzeitbehandlung. Arzneim. Forsch. 29:935-939.
5. **Berlyne, G. M., S. Nilwarangkur, H. Varley, and M. Hoerni.** 1964. Endogenous creatinine clearance and glomerular filtration rate. Lancet ii:874-876.
6. **Cronin, R. E.** 1979. Aminoglycoside nephrotoxicity: pathogenesis and prevention. Clin. Nephrol. 11:251-256.
7. **Cuppage, F. E., K. Setter, L. P. Sullivan, E. J. Reitzes, and A. O. Melnykovych.** 1977. Gentamicin nephrotoxicity. II. Physiological, biochemical and morphological effects of prolonged administration to rats. Virchows Arch. B 24:121-138.
8. **Dossetor, J. B.** 1966. Creatininemia versus uremia. The relative significance of blood urea nitrogen and serum creatinine concentrations in azotemia. Ann. Intern. Med. 65:1287-1299.
9. **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.
10. **Gilbert, D. N., C. Plamp, P. Starr, W. M. Bennett, D. C. Houghton, and G. Porter.** 1978. Comparative nephrotoxicity of gentamicin and tobramycin in rats. Antimicrob. Agents Chemother. 13:34-40.
11. **Goldstein, A., L. Aronow, and S. M. Kalman.** 1974. Principles of drug action: the basis of pharmacology, 2nd ed. John Wiley & Sons, Inc., New York.
12. **Hewitt, W. L.** 1973. The cephalosporins—1973. J. Infect. Dis. 128(Suppl.):S312-S319.
13. **Hottendorf, G. H.** 1977. Comparative ototoxicity (cats) and nephrotoxicity (rats) of amikacin and gentamicin. Am. J. Med. 62(Amikacin Suppl.):97-104.
14. **Kosek, J. C., R. I. Mazze, and M. J. Cousins.** 1974. Nephrotoxicity of gentamicin. Lab. Invest. 30:48-57.
15. **Loomis, T. A.** 1974. Essentials of toxicology, 2nd ed. Lea & Febiger, Philadelphia.
16. **Luft, F. C.** 1978. Netilmicin: a review of toxicity in laboratory animals. J. Int. Med. Res. 6:286-299.
17. **Luft, F. C., L. I. Rankin, R. S. Sloan, and M. N. Yum.** 1978. Recovery from aminoglycoside nephrotoxicity with continued drug administration. Antimicrob. Agents Chemother. 14:284-287.
18. **Miller, B. F., A. Leaf, A. R. Mamby, and Z. Miller.** 1952. Validity of the endogenous creatinine clearance as a measure of glomerular filtration rate in the diseased human kidney. J. Clin. Invest. 31:309-313.
19. **Ormsby, A. M., R. A. Parker, C. E. Plamp, P. Stevens, D. C. Houghton, D. N. Gilbert, and W. M. Bennett.** 1979. Comparison of the nephrotoxic potential of gentamicin, tobramycin and netilmicin in the rat. Curr. Ther. Res. Clin. Lab. Study 25:335-343.
20. **Ostle, B. O., and R. W. Mensing.** 1975. Statistics in research. Iowa State University Press, Ames.
21. **Pechere, J.-C., and R. Dugal.** 1979. Clinical pharmacokinetics of aminoglycoside antibiotics. Clin. Pharmacokinet. 4:170-199.
22. **Reed, M. D., M. W. Vermeulen, T. F. Boat, R. C. Stern, and P. W. Cheng.** 1979. Enzymuria does not predict aminoglycoside-associated nephrotoxicity. Pediatr. Res. 13:518.
23. **Reiner, N. E., D. D. Bloxham, and W. L. Thompson.** 1978. Nephrotoxicity of gentamicin and tobramycin given once daily or continuously in dogs. J. Antimicrob. Chemother. 4(Suppl. A):85-101.
24. **Sairio, E., A. Kasanen, K. Kangas, A. L. Nieminen, and L. Nieminen.** 1978. The nephrotoxicity and renal accumulation of amikacin, tobramycin and gentamicin in rats, rabbits and guinea pigs. Exp. Pathol. 15(Suppl.): 370-375.
25. **Schentag, J. J., F. M. Gengo, M. E. Plaut, D. Danner, A. Mangione, and W. J. Jusko.** 1979. Urinary casts as an indicator of renal tubular damage in patients receiving aminoglycosides. Antimicrob. Agents Chemother. 16:468-474.
26. **Sharratt, M., and A. C. Frazer.** 1963. The sensitivity of function tests in detecting renal damage in the rat. Toxicol. Appl. Pharmacol. 5:36-48.
27. **Soberon, L., R. L. Bowman, E. Pastoriza-Munoz, and G. J. Kaloyanides.** 1979. Comparative nephrotoxicities of gentamicin, netilmicin and tobramycin in the rat. J. Pharmacol. Exp. Ther. 210:334-343.
28. **Stroo, W. E., and J. B. Hook.** 1977. Enzymes of renal origin in urine as indicators of nephrotoxicity. Toxicol. Appl. Pharmacol. 39:423-434.
29. **Welles, J. S., J. L. Emmerson, W. R. Gibson, R. Nickander, N. V. Owen, and R. C. Anderson.** 1973. Preclinical toxicology studies with tobramycin. Toxicol. Appl. Pharmacol. 25:398-409.
30. **Wellwoood, J. M., D. Lovell, A. E. Thompson, and J. R. Tighe.** 1976. Renal damage caused by gentamicin: a study of the effects on renal morphology and urinary enzyme excretion. J. Pathol. 118:171-182.
31. **Whelton, A., G. G. Carter, T. J. Craig, H. H. Bryant, D. V. Herbst, and W. G. Walker.** 1978. Comparison of the intrarenal disposition of tobramycin and gentamicin: therapeutic and toxicologic answers. J. Antimicrob. Chemother. 4(Suppl. A):13-22.
32. **Winer, B. J.** 1971. Statistical principles in experimental design, 2nd ed. McGraw-Hill Book Co., New York.
33. **Wright, P. J., and D. T. Plummer.** 1974. The use of urinary enzyme measurements to detect renal damage caused by nephrotoxic compounds. Biochem. Pharmacol. 23:65-73.