ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1986, p. 96–100
0066-4804/86/070096-05$02.00/0

Vol. 30, No. 1

Comparative Uptake of Gentamicin, Netilmicin, and Amikacin in the Guinea Pig Cochlea and Vestibule

DIDIER DULON,[1]* JEAN-MARIE ARAN,[1] GARY ZAJIC,[2] AND JOCHEN SCHACHT[2]

Laboratoire d'Audiologie Expérimentale, Université de Bordeaux II, Institut National de la Santé et de la Recherche Médicale U 229, Hopital Pellegrin, 33076 Bordeaux, France,[1] and Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, Michigan 48109[2]

Received 20 December 1985/Accepted 14 April 1986

The kinetics of the entry of three aminoglycosides into inner-ear tissues of the guinea pig after acute and chronic administration were compared: gentamicin toxic to the cochlea and the vestibule, amikacin preferentially cochleotoxic, and netilmicin of low ototoxic liability. During constant intravenous infusion, levels of the three drugs in plasma tended to reach a plateau after 1 h, while levels in perilymph did not reach a plateau within 6 h. The drug concentrations in both vestibular and cochlear tissues quickly reached saturation. Amikacin and gentamicin concentrations were similar in vestibular and cochlear tissues, while netilmicin values were somewhat lower. After 1 week of chronic treatment (100 mg of drug per kg of body weight daily subcutaneously), levels of gentamicin and amikacin in tissue were similar to each other and were not significantly different between cochlear and vestibular tissues. Netilmicin concentrations again were somewhat lower in the tissues, but identical to those of the other drugs in the perilymph. After 3 weeks of treatment, all of the drugs were equally distributed in the inner-ear tissues. Release of the drug from the tissues after the 3-week treatment was faster for amikacin (83% decrease after 20 days) than for netilmicin and gentamicin (approximately 50% decrease). There was no correlation, under any of the experimental conditions, between the drug concentrations and their degrees of toxicity. These results demonstrate that selective aminoglycoside ototoxicity cannot be explained by a preferential uptake or accumulation of drugs in the afflicted tissues or in the perilymph.

Toxicity to both the vestibular and cochlear structures of the inner ear is a well-known adverse effect of aminoglycoside antibiotic therapy. The morphological changes and electrophysiological dysfunctions induced by different aminoglycosides have been extensively studied, and it is clearly established that the pattern of toxicity varies greatly within this family of antibiotics (1, 8, 12). For example, gentamicin affects the cochlear and vestibular systems to nearly the same extent, while amikacin preferentially damages the cochlea; netilmicin is significantly less toxic than either gentamicin or amikacin to both parts of the labyrinth (2, 5, 7). It has been postulated that this differential damage is related to the concentration of aminoglycoside achieved in the perilymph, i.e., the more toxic a drug, the higher its level in this inner-ear fluid (7, 20), although exceptions from this pattern had been noted (6, 15). Moreover, it has been argued that an accumulation of aminoglycosides in the inner-ear fluids is responsible for the organ-specific toxic action. However, recent detailed pharmacokinetic studies have not supported such an explanation of toxicity. Levels of aminoglycosides in inner-ear fluid did not exceed levels in plasma (21, 22). Furthermore, the concentrations of neomycin and gentamicin in cochlear tissues were not significantly higher than in other body tissues that do not show aminoglycoside-induced pathology (11; P. Tran Ba Huy, P. Bernard, and J. Schacht, J. Clin. Invest., in press).

Most of the pharmacokinetic studies of aminoglycosides in the inner ear have been concerned with the fluids and to some extent the cochlear tissues. Little is known about the distribution of these drugs in the vestibular system. The aim of this study was to determine whether cochlear or vestibular toxicity of the aminoglycoside antibiotics correlated with preferential uptake into these structures and also whether toxicity related to levels of the drug in inner-ear fluids or tissues. We selected three aminoglycosides with distinctly different patterns of ototoxicity, i.e., gentamicin, netilmicin, and amikacin, and studied their uptake into perilymph as well as into cochlear (organ of Corti and lateral wall) and vestibular (saccule, utricle, and crista ampullaris) tissues after acute and prolonged treatment.

MATERIALS AND METHODS

Drug administration. Experiments were performed on pigmented guinea pigs (190 to 250 g) with a normal Preyer reflex.

As acute administration, constant intravenous (i.v.) infusions were given at rates of 5, 15, or 50 μg of drug per min. For each rate, infusions were performed for 0.5, 1, 3, and 6 h, and three animals were tested for each condition. Animals were anesthetized with a mixture of Rompun (Xylazine, 20 mg/kg) and Ketaset (Ketamine, 100 mg/kg), and a tracheotomy was performed. The aminoglycosides were delivered in 0.15 M NaCl by a constant infusion pump (syringe pump model 351; Orion Research, Inc., Cambridge, Mass.) via a jugular catheter at 25 μl/min, and the different infusion rates were obtained by varying the concentration of the drug in the infusate. During the infusion, body temperature was maintained at 37.5°C by a heating pad.

For chronic administration, drugs were injected subcutaneously (100 mg of drug per kg of body weight) in saline daily for 1 week (five animals per drug) and 3 weeks (eight animals

* Corresponding author.

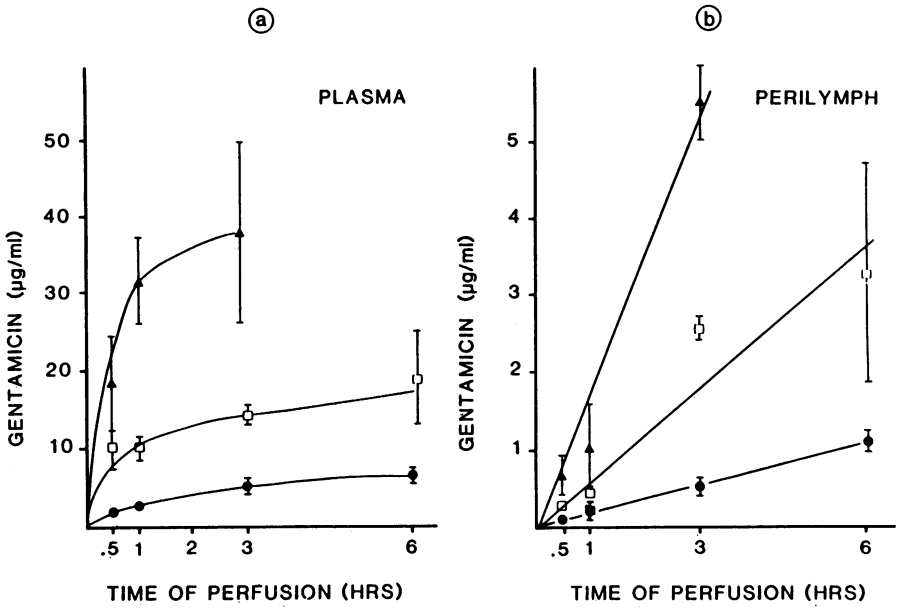

FIG. 1. Time course of gentamicin concentrations in plasma and perilymph. The drug was administered in different concentrations at 25 μl/min via the jugular vein, and the resulting concentrations in plasma and perilymph were determined at the time indicated as described in Materials and Methods. The rates of administration were 5 (●), 15 (□), and 50 (▲) μg/min. Numbers are means of at least three samples, and vertical bars indicate the standard deviation. Kinetics of gentamicin in plasma (a) and perilymph (b).

per drug). Animals were killed 24 h after the last injection or, for the release studies (eight animals per drug), 20 days after the last injection.

Sampling procedures. At the end of the infusion, the carotid artery was cannulated, and a blood sample was collected. The animal was then exsanguinated through the carotid catheter and perfused with isotonic saline to rinse the vascular bed. The blood samples were centrifuged at 1,000 × *g* for 2 min, and samples of plasma were stored at −20°C. The bullae were removed, and 5 μl of perilymph was collected from each ear with a microcapillary through the round window or from the second turn of the cochlea. The scalae were rinsed with isotonic saline, and the tissues of the lateral wall (i.e., stria vascularis and spiral ligament), organ of Corti, saccule, utricle, and crista ampullaris were dissected. The tissues were collected in 0.2 M sodium phosphate (pH 8) and homogenized as previously described (11). All samples were processed in polypropylene tubes (10).

Assays. Tissue homogenates were centrifuged for 2 min at 12,000 × *g*, and drug concentrations were measured in the supernatant fraction with a commercial radioimmunoassay kit (American Bioclinical Inc., Portland, Oreg.) modified according to Meulemans et al. (14). When known amounts of [^{3}H]gentamicin were homogenized with ear tissues, 97% ± 1% of the drug was found in the supernatant fraction (Tran Ba Huy et al., in press).

The sensitivities of the modified radioimmunoassays were 50, 100, and 150 pg, respectively, for gentamicin, netilmicin, and amikacin, with a coefficient of variation of less than 7%. Considering the amounts of fluids and tissues sampled, the detection limits for gentamicin, netilmicin, and amikacin were, respectively, 1, 2, and 3 ng/ml of plasma and 10, 20, and 30 ng/ml of perilymph. For the tissues, these limits ranged from 2, 4, and 6 ng/mg of protein for the lateral-wall tissues to 20, 40, and 60 ng/mg of protein for the smallest tissue, the saccule.

The total protein concentration was determined from the tissue homogenate (13). Statistical comparisons of aminoglycoside concentrations were made by the Student *t* test.

RESULTS

Kinetics of gentamicin. (i) Plasma and perilymph. At all three rates of infusion (5, 15, and 50 μg of gentamicin per min), the increase of drug concentrations in the plasma slowed after 1 h, suggesting that a plateau was being approached. The plateau levels correlated directly with the rate of infusion (Fig. 1a), as did the gentamicin concentrations in perilymph. Saturation, however, was not reached during the experiment (Fig. 1b). Drug concentrations in the perilymph were lower than those in the plasma at all times; the perilymph-to-plasma ratios of mean drug concentrations ranged from 0.05 to 0.15.

(ii) Tissues. At all rates of infusion, the drug quickly reached steady-state levels in all inner-ear tissues (Fig. 2a, for the organ of Corti). The drug concentrations leveled off at the same rate as in the plasma and correlated with the levels in plasma (Fig. 2b). Gentamicin concentrations overall were similar, with somewhat lower levels in the crista ampullaris and tissues of the lateral wall.

Comparison of gentamicin, netilmicin, and amikacin. (i) Acute administration. In plasma, gentamicin, netilmicin, and amikacin exhibited the same behavior of rapid saturation (after 1 h) and similar plateau values of 23 ± 8, 27 ± 4, and 30 ± 7 μg/ml, respectively, at an infusion rate of 15 μg/min (Fig. 3a).

The three drugs also showed similar kinetic behavior in perilymph. After 3 h of 15 μg of drug per min, drug levels were similar (Fig. 3a), and no saturation was observed for up to 6 h. The perilymph/plasma ratios were also nearly identical at 0.12 ± 0.04, 0.08 ± 0.03, and 0.07 ± 0.02, respectively, for gentamicin, netilmicin, and amikacin. Because of the rapid saturation in the tissues, the antibiotic concentrations were compared at these plateau values (Fig. 3b). Gentamicin

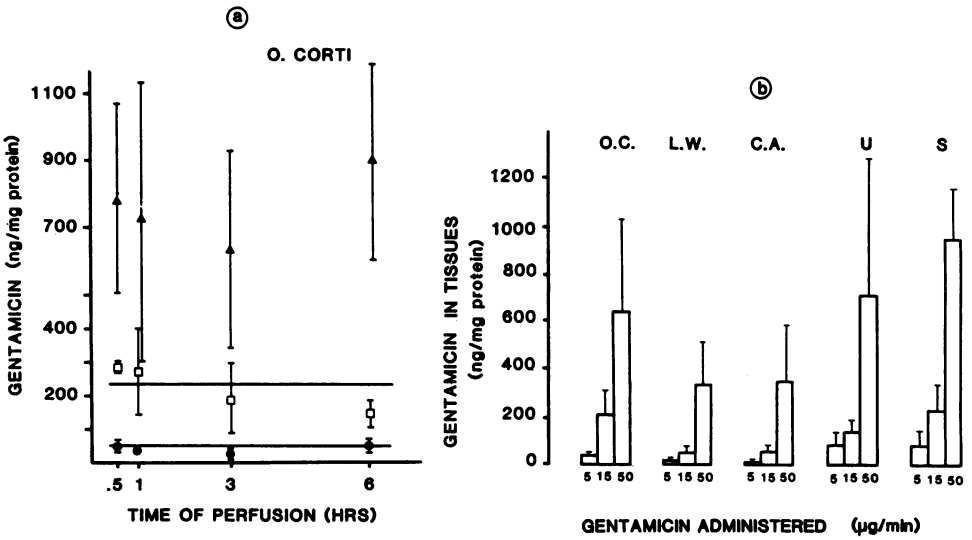

FIG. 2. Kinetics of gentamicin in inner-ear tissues. (a) Kinetics of gentamicin in the organ of Corti during constant infusion (i.v.) of 5 (●), 15 (□), and 50 (▲) µg of gentamicin per min. Similar kinetics were observed in all inner-ear tissues. (b) Gentamicin plateau concentrations in cochlear and vestibular tissues during constant infusion. The plateau values were calculated from the mean of the concentrations at 0.5, 1, 3, and 6 h, as seen in panel a for the organ of Corti. The bars indicate the standard deviation of 5 to 12 samples. Abbreviations: O.C., organ of Corti; L.W., lateral wall; C.A., crista ampullaris; U, utricle; S, saccule.

and amikacin reached similar levels in all tissues, while netilmicin was lower in the organ of Corti, lateral-wall tissues, utricle, and saccule.

(ii) Chronic administration for 1 week. After 1 week of treatment with 100 mg of drug per kg of body weight daily, there was no significant difference in the distribution of any drug between cochlear and vestibular structures. Amikacin and gentamicin reached similar concentrations in all tissues, while netilmicin was lower in the organ of Corti, lateral wall, and crista ampullaris ($P < 0.01$) (Fig. 4a). Concentrations of the three drugs also were similar in perilymph, ranging between 200 and 300 ng/ml.

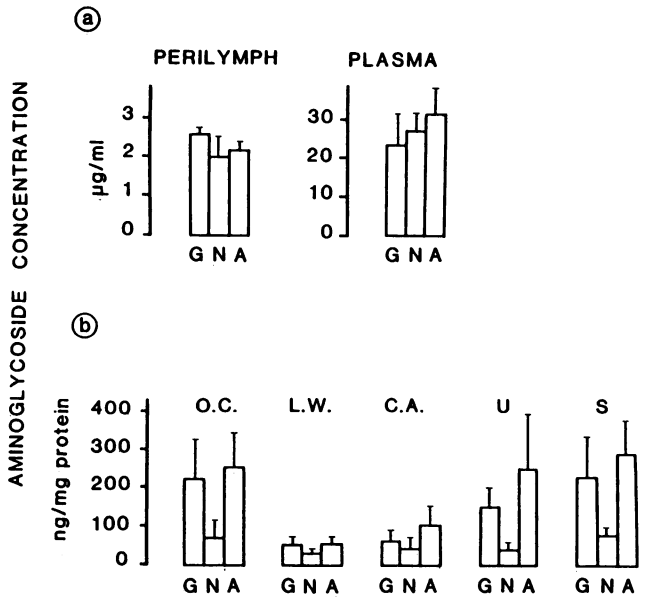

FIG. 3. Comparison of gentamicin (G), netilmicin (N), and amikacin (A) concentrations in inner-ear tissues and fluids after acute i.v. perfusion, as described in Materials and Methods. (a) The drug concentrations in plasma and perilymph were measured after 3 h of a constant i.v. infusion of 15 µg/min. The bars indicate the standard deviation of at least three samples. At all times tested, the three drugs reached essentially identical levels in the perilymph. (b) Values in tissues after constant i.v. infusion for up to 6 h (15 µg/min). Plateau values were determined as described in the legend to Fig. 2. The bars indicate the standard deviation of 5 to 12 samples. Netilmicin was lower in the organ of Corti, utricle, and saccule ($P < 0.01$). For abbreviations, see the legend to Fig. 2.

(iii) Chronic administration for 3 weeks. All of the antibiotics reached similar levels in tissue after prolonged treatment with the three drugs (Fig. 4b). These values were not higher than those measured at 7 days, except for netilmicin ($P < 0.01$). There was no preferential accumulation of a drug in any of the inner-ear structures.

(iv) Release of drugs from inner-ear tissues. The animals were treated for 20 days at 100 mg of drug per kg of body weight daily, and the antibiotic was assayed 20 days after the last injection (Fig. 4c). Drug levels were lower in all tissues compared with their levels at the end of the chronic administration. Gentamicin and netilmicin concentrations decreased to approximately 50%, while amikacin was generally reduced to about 17% of its original level. There was no differential loss of drug from any vestibular or cochlear structure.

DISCUSSION

The results clearly demonstrate that the selective ototoxicity of aminoglycosides cannot be explained on the basis of their accumulation in the tissues and fluids of the inner ear. When aminoglycoside levels in perilymph are specifically considered, it is important to note that concentrations were identical for a highly ototoxic drug such as gentamicin and a much less ototoxic drug such as netilmicin after both acute and chronic treatments. Furthermore, the perilymph/plasma ratio of all drugs remained well below 1, contradicting a possible accumulation in the inner-ear fluid for any of the drugs. These findings and their interpretation are in contrast to earlier pharmacokinetic studies of aminoglycosides which suggested a direct relationship between the severity of aminoglycoside ototoxicity and the levels of antibiotics

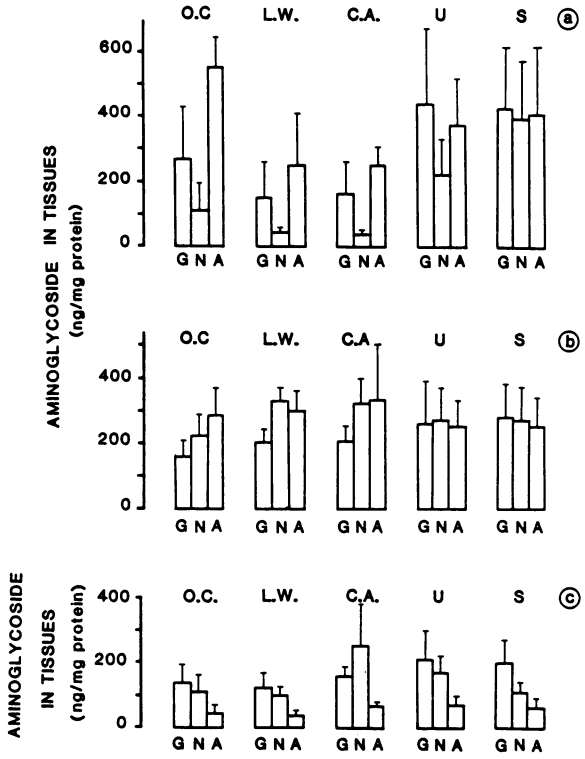

FIG. 4. Comparison of gentamicin (G), netilmicin (N), and amikacin (A) concentrations in inner-ear tissues after chronic administration (100 mg of drug per kg of body weight daily as described in Materials and Methods). (a) Treatment for 1 week. (b) Treatment for 20 days. The animals were killed 24 h after the last injection. Values are means of three to eight samples, and the bars indicate the standard deviation. (c) Release study. The animals were injected daily with 100 mg of aminoglycoside per kg of body weight for 20 days as for panel b and then were sacrificed 20 days after the last injection. Values are means of four to eight samples, and the bars indicate the standard deviation.

reached in the perilymph (7, 9, 12, 20, 23). However, they are in agreement with recent studies (15, 21, 22) challenging such a hypothesis.

Although analyses of drugs in inner-ear fluids have received considerable attention in the past, it may be more important to study drug uptake into the tissues, the site of action. Drug levels in cochlear tissues were first investigated by Desrochers and Schacht (11) and subsequently by Tran Ba Huy and Schacht (Abstr. Assoc. Res. Otolaryngol., abstr. no. 69, p. 52, 1983; Tran Ba Huy et al., in press). In these studies, aminoglycoside levels in cochlear tissues were found to be in the same range as levels in other body organs that remain unaffected by the antibiotics. Drug concentrations in the present study are in very good agreement with those reports, and we are now able to extend these observations to the vestibular structures. Levels of amikacin and gentamicin were similar in vestibular and cochlear tissues. Thus, the different toxic potentials of amikacin and gentamicin (1, 6, 8) cannot be explained by selective uptake. Moreover, amikacin, which has been shown to be highly cochleotoxic and much less vestibulotoxic (8, 17), reached similar levels in cochlear and vestibular tissues. Netilmicin appeared to enter most of the tissues more slowly, but reached levels similar to those of the other drugs after 3 weeks.

There was also no correlation between the documented toxicities of the drugs and their persistence in the tissues. Gentamicin and netilmicin, despite their different toxic potential (2, 5, 7), showed a similar rate of release from the tissues. Amikacin demonstrated a faster, albeit equal, release from cochlear and vestibular tissues, not accounting for the preferential cochleotoxicity of this drug.

These results strongly support the conclusion that the differences in ototoxicity between aminoglycosides can be explained neither by preferential uptake nor by persistence in the afflicted tissues. It is interesting in this context that the renal concentrations of aminoglycosides also do not correlate with their nephrotoxicity (4). Thus, the dissociation of toxicity from drug concentration in the tissues seems to be a general phenomenon of aminoglycoside oto- and nephrotoxicity.

Information on the pharmacokinetics of the aminoglycoside antibiotics is important for an understanding of the molecular mechanisms of ototoxicity. In light of our results, the differences in toxicity within the family of aminoglycosides would rely primarily on the intrinsic potency of the drugs to disrupt vital mechanisms of cellular metabolism. A model of toxicity based on the specific interactions between aminoglycosides and polyphosphoinositides (18, 19, 25), for example, attempts to explain the drug actions at this level.

Another point requires further consideration. While the pharmacokinetics of various aminoglycosides in inner-ear fluids and tissues have been established, information is still lacking at the cellular level. It remains possible that the distribution of the drugs in tissue is not uniform and that specific cells (such as hair cells of the organ of Corti and the cristae) are a preferred target. Determinations of the distribution of radioactive dihydrostreptomycin in the cochlear have so far yielded conflicting results (3, 16, 24). Thus, immunocytochemical or further radioautographic investigations of aminoglycoside distribution after acute and chronic administration at the light and electron microscopic level are required to solve this question. It is clear, however, that aminoglycoside concentrations in inner-ear fluids and whole tissues do not reflect the toxicities of the drugs.

ACKNOWLEDGMENTS

We acknowledge the capable assistance of M. Harrington and W. Frauenheim with the drug assays. We thank Y. Cazals and D. Peyret for discussions.

This research was supported by grant NS-13792 from the National Institutes of Health and grants from Roger Bellon (Neuilly sur Seine, France) and Meigi Seika Kaisha (Tokyo, Japan).

LITERATURE CITED

1. **Aran, J. M.** 1982. Aminoglycosides cochlear and vestibular action, p. 377–386. *In* J. P. Fillastre (ed.), Nephrotoxicity-ototoxicity of drugs. Editions I.N.S.E.R.M., Paris.
2. **Arpini, A., L. Cornacchia, L. Albiero, F. Bamonte, and L. Parravicini.** 1979. Auditory function in guinea pigs treated with netilmicin and other aminoglycosides. Arch. Otolaryngol. **244**:137–142.
3. **Balog, K., F. Hiraide, and D. Ishii.** 1970. Distribution of radioactive dihydrostreptomycin in the cochlea. Ann. Otol. Rhinol. Laryngol. **79**:641–652.
4. **Brier, M. E., P. R. Mayer, R. A. Brier, D. Visscher, F. C. Luft, and G. R. Aronoff.** 1985. Relationship between rat renal accumulation of gentamicin, tobramycin, and netilmicin and their nephrotoxicities. Antimicrob. Agents Chemother. **27**:812–816.
5. **Brummett, R. E., and K. E. Fox.** 1982. Studies of aminoglycoside ototoxicity in animal models, p. 419–451. *In* A. Whelton and H. C. Neu (ed.), The aminoglycosides: microbiology, clinical use and toxicology. Marcel Dekker, Inc., New York.

6. **Brummett, R. E., K. E. Fox, T. W. Bendrick, and D. L. Himes.** 1978. Ototoxicity of tobramycin, gentamicin, amikacin and sisomicin in the guinea pig. J. Antimicrob. Chemother. **4**(Suppl. A):73–83.
7. **Brummett, R. E., K. E. Fox, R. T. Brown, and D. L. Himes.** 1978. Comparative ototoxic liability of netilmicin and gentamicin. Arch. Otolaryngol. **104**:579–584.
8. **Christensen, E. F., J. C. Reiffenstein, and H. Madissoo.** 1977. Comparative ototoxicity of amikacin and gentamicin in cats. Antimicrob. Agents Chemother. **12**:178–184.
9. **Chung, M., L. Parravicini, B. M. Assael, G. Cavanna, E. Radwanski, and S. Symchowicz.** 1982. Comparative pharmacokinetics of aminoglycoside antibiotics in guinea pigs. Antimicrob. Agents Chemother. **22**:1017–1021.
10. **Desrochers, C. S., and J. Schacht.** 1981. Assay of aminoglycosides is influenced by tissue homogenization technique. Experimentia **37**:1357–1358.
11. **Desrochers, C. S., and J. Schacht.** 1982. Neomycin concentrations in inner ear tissues and other organs of the guinea pig after chronic drug administration. Acta Otolaryngol. **93**:233–236.
12. **Federspil, P., W. Schätzle, and E. Tiesler.** 1976. Pharmacokinetics and ototoxicity of gentamicin, tobramicin and amikacin. J. Infect. Dis. **134**(Suppl.):200–205.
13. **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.
14. **Meulemans, A., C. Manuel, and P. Tran Ba Huy.** 1981. Radioimmunoassay of gentamicin in microliter and nanoliter samples of biological fluids. Chemotherapy **27**:29–33.
15. **Ohtani, I., K. Ohtsuki, T. Aikawa, Y. Sato, T. Anzai, J. Ouchi, and J. Saito.** 1985. Low ototoxicity and its mechanism of netilmicin. ORL J. Otorhinolaryngol. Relat. Spec. **47**:84–89.
16. **Portmann, M., J. Darrouzet, and C. Coste.** 1974. Distribution within the cochlea of dihydrostreptomycin injected into the circulation. Arch. Otolaryngol. **100**:473–475.
17. **Reiffenstein, J. C., S. W. Holmes, G. H. Hottendorf, and M. E. Bierwagen.** 1973. Ototoxicity studies with BBK8, a new semisynthetic aminoglycoside. J. Antibiot. **26**:94–100.
18. **Schacht, J.** 1979. Isolation of an aminoglycoside receptor from guinea pig inner ear tissues and kidney. Arch. Otorhinolaryngol. **224**:129–134.
19. **Schacht, J., B. M. Wang, and N. D. Weiner.** 1983. Aminoglycoside-cell receptor interactions: implications for toxicity and in vitro-models. Proc. 13th Int. Congr. Chemother. **105**:39–44.
20. **Stupp, H., K. Kupper, F. Lagler, H. Sous, and M. Quante.** 1973. Inner ear concentrations and ototoxicity of different antibiotics in local and systemic application. Audiologie **12**:350–363.
21. **Tran Ba Huy, P., C. Manuel, A. Meulemans, O. Sterkers, and C. Amiel.** 1981. Pharmacokinetics of gentamicin in perilymph and endolymph of the rat as determined by radioimmunoassay. J. Infect. Dis. **143**:476–486.
22. **Tran Ba Huy, P., A. Meulemans, M. Wassef, C. Manuel, O. Sterkers, and C. Amiel.** 1983. Gentamicin persistence in rat endolymph and perilymph after a two-day constant infusion. Antimicrob. Agents Chemother. **23**:344–346.
23. **Voldrich, L.** 1965. The kinetics of streptomycin, kanamycin and neomycin in the inner ear. Acta Otolaryngol. **60**:243–248.
24. **Von Ilberg, C., H. Spoendlin, and W. Arnold.** 1971. Autoradiographical distribution of locally applied dihydrostreptomycin in the inner ear. Acta Otolaryngol. **71**:159–165.
25. **Wang, B. M., N. D. Weiner, A. Takada, and J. Schacht.** 1984. Characterization of aminoglycoside-lipid interactions and development of a refined model for ototoxicity testing. Biochem. Pharmacol. **33**:3257–3262.