Increased Renal DNA Synthesis In Vivo After Administration of Low Doses of Gentamicin to Rats

GUY LAURENT,1,2* PAUL MALDAGUE,3 MARIE-BEATRICE CARLIER,1,2 AND PAUL M. TULKENS1,2

Laboratoires de Chimie Physiologique1 and Pathologie et Cytologie Experimentales.3 Université Catholique de Louvain, and International Institute of Cellular and Molecular Pathology,2 B-1200 Brussels, Belgium

Received 15 April 1983/Accepted 20 July 1983

Kidney cortex DNA synthesis was studied in female rats treated with a low dose of gentamicin (10 mg/kg) up to 14 days. Synthesis was measured by incorporation of [3H]thymidine into DNA 1 h after intraperitoneal injection of the labeled precursor (200 μCi per animal). Gentamicin given in one injection per day resulted in a greater incorporation of [3H]thymidine into DNA after both 7 and 14 days of treatment as compared with control animals. When the daily dose was divided into three equal injections given at 8-h intervals, a statistically significant increase in thymidine incorporation was observed as early as 4 days after starting gentamicin administration. Excellent agreement was found between DNA specific radioactivity and kidney cortex nuclear labeling, as measured by histotautoradiography. The greatest amount of [3H]thymidine incorporation occurred within proximal tubular cells and interstitial cells. We conclude that a finite duration of gentamicin treatment at low dosage induces an increased DNA synthesis in vivo in rat kidney cortex. We suggest that this reaction results from cellular proliferation and could reflect a regenerative process after focal necrosis induced by gentamicin at low doses. The demonstrated early increase in DNA synthesis could be a useful tool to measure kidney cortex alterations caused by various aminoglycosides at low, therapeutic doses.

The histological manifestation of aminoglycoside-induced nephrotoxicity is proximal tubule necrosis (for review, see references 1, 13). Mechanistically, this is supported by the observation that aminoglycosides accumulate in this part of the nephron (15, 28; M. E. De Broe, G. J. Paulus, G. A. Verpooten, F. Roels, N. Buysens, R. Wedeen, F. van Hoof, and P. H. Tulkens, Kidney Int., in press). In rats treated with low doses of gentamicin (<20 mg/kg), histologically confirmed necrosis is infrequently encountered and animals show no alteration of renal function. A conspicuous lysosomal phospholipidosis is seen in proximal tubular cells under these experimental conditions (12, 18, 19), but its relationship to tubular necrosis and renal dysfunction remains to be elucidated (19, 22, 27).

High doses of gentamicin induce considerable postnecrotic regeneration, even under continuous drug administration (9, 20). Tubular regeneration is also observed after treatment at low doses, but becomes unambiguously detectable by conventional histology only after prolonged treatment (28 days or more) (4, 11).

In this study, we have directly examined the influence of a short course of low-dose gentamicin treatment on renal cortical DNA synthesis in vivo. For this purpose, we have performed biochemical and autotitoradiographic measurements of [3H]thymidine incorporation into kidney cortex DNA. Our observation is that gentamicin treatment considerably increases this incorporation. This result may have important toxicological and clinical implications.

MATERIALS AND METHODS

Animals and treatments. Young female Sprague-Dawley rats (200 to 220 g; 8 to 10 weeks old) were used throughout the experiments. For each experiment, treated and control animals were obtained at the same time from the supplier and were exposed to the same environmental conditions. Food and water was given ad libitum. The total daily dose of gentamicin was consistently and strictly 10 mg/kg of body weight (each animal was weighed before injection and the dose was adjusted accordingly; weights did not, however, vary by >15%). Animals receiving the daily dose in one administration were injected at 3 p.m., in two fractions, at 9 a.m. and 9 p.m.; and in three fractions (TID), at 8 a.m., 4 p.m., and 12 a.m. (midnight). Each injection was given intraperitoneally with 0.5 to 0.6 ml of 0.9% NaCl containing the suitable amount of drug. Controls received 0.5 ml of 0.9% NaCl on the same schedule.
Measurements of $[^3]$Hthymidine incorporation into DNA. The study of $[^3]$Hthymidine incorporation started 9 to 15 h after the last drug injection. One hour before sacrifice, each rat was injected intraperitoneally with 200 $\mu$Ci of [methyl-$^3$]thymidine (47 Ci/mmol) diluted in 0.5 ml of 0.9% NaCl. After the animals were killed by decapitation and bleeding, the kidneys were quickly removed and usually cut sagittally into two parts. The cortex, including the inner cortex, was dissected from one part and placed in a small plastic tube quickly dropped in dry ice. Samples were stored at $-20^\circ$C for a maximum of 4 days before further processing. Each cortex sample was thawed and homogenized in 49 volumes of ice-cold distilled water, using a Potter-Elvehjem tissue grinder fitted with a Teflon pestle. DNA was purified from 2 ml of homogenate (40 mg of tissue), following the procedure described by Munro and Fleck (21), except that the DNA was extracted, in the last step, with 2 ml of 1 M perchloric acid for 15 min at 65°C. Extracted DNA was assayed by the method of Burton (3), using herring sperm DNA as a reference standard. Radioactivity of the DNA extract was measured by scintillation spectrometry (800 $\mu$l of sample in 10 ml of Aqualuma [Lumac Systems Inc., Titusville, Fla.]). Counting efficiency was estimated by the internal-standard method.

Autoradiography and histology of kidney cortex. The other half of the kidneys, or fragments of the cortex, were fixed in Bouin solution (in some experiments 10% neutral buffered Formalin was also used) and embedded in paraffin. Tissue sections (ca. 6 $\mu$m) were coated with NTB Kodak emulsion. After drying, the specimens were stored in the dark and at 4°C for 14 or 28 days and processed in Ilford developer. The sections were counterstained with hematoxylin-eosin. Duplicate tissue sections were also stained with hematoxylin-eosin combined with the periodic acid-Schiff stain procedure. For quantitative evaluation, sagittal or parasagittal sections were examined at 400-fold magnification on a Zeiss photomicroscope, with a photographic setup giving a rectangular field divided in quadrants by reticules (to avoid overlapping of fields within a section). Enumeration was made along parallel straight lines, extending from the subcapsular to the juxtamedullary region. Every nucleus in each field was recorded, and the corresponding cell type was identified. A total of 10,000 to 15,000 nuclei were analyzed for each specimen.

Materials. Animals were supplied by Ilfa-Credo (L’Arbresle, France). Gentamicin (sulfate salt, in the form used in clinical practice in Belgium) was obtained from Schering Corp. (Kenilworth, N.J.). [Methyl-$^3$]thymidine (47 Ci/mmol) was purchased from the Radiochemical Center (Amersham, England), and herring sperm DNA (type VII) was from Sigma Chemical Co. (St. Louis, Mo.). All other reagents were of analytical grade.

RESULTS

Biochemical studies. Control measurements were done to ensure that differences in DNA specific radioactivity were really due to different rates of $[^3]$Hthymidine incorporation and not to changes in availability of labeled precursors. Treatment with gentamicin did not significantly modify the serum levels of $[^3]$Hthymidine (typically, ca. 1,550 dpm/$\mu$l) or the amount of acid-soluble radioactivity in kidney homogenates (typically, ca. 1,950 dpm/mg of tissue). The amount of DNA extracted from the cortex under our conditions was also not significantly modified by treatment (e.g., 0.162 ± 0.03 mg of DNA per 40 mg [wet weight] of cortex sample [$n = 8$] versus 0.165 ± 0.05 [$n = 8$] in animals receiving gentamicin or 0.9% NaCl for 10 days, TID, respectively).

In the first set of experiments, rats were treated for 7 and 14 days with gentamicin given as a single injection per day. Figure 1 shows that a significantly higher incorporation of $[^3]$Hthymidine occurred in kidney cortex DNA from treated rats. Whereas the specific radioactivity of DNA was variable between animals, especially after 14 days of treatment, comparison between right and left kidneys for individual animals provided good consistency.

It has been reported that the schedule of administration of gentamicin is an important modifier of nephrotoxicity. Thus, splitting the daily dose into multiple injections produces more renal alterations than single daily administration of the same dose (2, 8, 23; P. Tulkens, M. E. De Broe, P. Malagde, G. Verpoorten, and S. Scharpe, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, A30, p. 6). A comparison of $[^3]$Hthymidine incorporation was therefore made on rats receiving the same total daily dose of gentamicin in either two or three (TID) injections per day. These are the common schedules of injection of aminoglycosides in clinical practice, as indicated in the package inserts provided by the manufacturer. We selected 4 and 10 days for the duration of the treatment, since no significant increase of $[^3]$Hthymidine incorporation was seen between days 7 and 14. On the other hand, we wished to explore a shorter duration of treatment. Table 1 shows that, by day 4 with the TID regimen, mean DNA specific radioactivity was double that of the control animals. At day 10, its value was ca. fourfold higher than that of controls. The twice-daily regimen produced a similar effect, albeit more slowly; at day 4, only a marginal effect was noticeable.

Histological studies. Histoaautoradiographic studies were undertaken to determine the cellular localization of the label and to establish whether the increase in $[^3]$Hthymidine incorporation is due to a proliferative process in kidney cortex. All label was confined to nuclei, and the medulla showed much less labeling than the cortex. The abundance of labeled nuclei was related to the DNA specific radioactivity measured in corresponding kidneys. In the cortex, labeled nuclei appeared as small foci, distributed throughout the section, with an apparent abun-
Quantitative evaluation of the cortical labeling was performed on sections obtained from four animals used in the experiments shown in Table 1. These animals were selected on the basis of large differences in \(^3\text{H}\)thymidine incorporation levels (one control and three treated animals). Figure 3 (upper part) shows that the labeling index (total number of labeled nuclei per 10,000 nuclei) was in good agreement with the specific radioactivity of total cortex DNA. We also measured the labeling index of each of the major cell types encountered in kidney cortex (proximal tubules, interstitial cells [endothelial plus fibroblast-like cells], distal tubules, and glomerular cells [mesangial plus endothelial plus epithelial cells]). Figure 3 (lower part) shows that gentamicin treatment, for up to 10 days at 10 mg/kg, does not distort the respective abundance of each of these cell populations. The distributions of nuclei, for all four animals studied, were 52.3 ± 1.3, 28.5 ± 2.0, 7.8 ± 0.1, and 11.2 ± 2.2\% in proximal tubules, interstitium, distal tubules, and glomeruli, respectively. Figure 3 also shows, in a combined fashion, this distribution expressed as the relative labeling index of each of these cell populations and their respective contribution to the global labeling index. The bulk of the label in treated animals was associated with proximal tubules and interstitial cells, with the latter showing the highest relative labeling index. By contrast, distal tubules and glomeruli showed no important change in their relative labeling index, except in the animal that incorporated the largest amount of \(^3\text{H}\)thymidine. Yet, this increase was less than for proximal tubules or interstitial cells, and the contribution of glomeruli and distal tubules to global labeling remained low. Values for distal tubules may be somehow overestimated, because of possible confusion between genuine distal tubules and proximal tubules in an early stage of regeneration (see below).

By conventional histology, sections from animals treated 4 days could not be successfully sorted out in a blind evaluation. At day 10, mitoses were observed, especially with the TID schedule. Examination of two treated animals showed a mitotic index of ca. 0.12\% compared to <0.01\% in control animals. The mitotic index, however, proved to be unsatisfactory for accurate determination of cellular proliferation because of the low numbers recorded. At days 10 and 14, we also observed isolated necrotic cells (eosinophilic cells with pycnotic nuclei and apoptosis, i.e., cells demonstrating shrinkage necrosis [34]). They were, however, infrequent and often surrounded by seemingly normal tubules (Fig. 4a). On occasions, zones of peritubular infiltration were encountered (Fig. 4b). We also observed, especially on a TID schedule, typical
TABLE 1. Effect of administration schedule on [3H]thymidine incorporation into kidney cortex DNA in rats treated with gentamicin (10 mg/kg daily)\(^a\)

| Schedule | Duration of treatment (days) | DNA specific radioactivity (dpm/mg) | No. of animals\(^b\) | Student’s t test (P) as compared with: |
|----------|-----------------------------|------------------------------------|---------------------|---------------------------------------|
|          |                             | Control                            | BID schedule        |
| BID\(^c\) | 4                           | 22,902 ± 8,334                     | 4                   | NS\(^d\)                              |
|          | 10                          | 79,847 ± 51,556                    | 4                   | <0.025                                |
| TID\(^e\) | 4                           | 38,628 ± 11,016                    | 8                   | <0.001                                |
|          | 10                          | 89,121 ± 24,278                    | 7                   | <0.001                                |
| Controls\(^f\) |                             | 16,959 ± 4,915                    | 23                  | NS                                    |

\(^a\) [3H]thymidine (200 μCi per animal) was given intraperitoneally 1 h before sacrifice. DNA was purified as detailed in the text.

\(^b\) For each animal, both kidneys were processed separately and data were pooled. The mean of the absolute differences between kidneys from the same animal was 5,241 ± 7,092 dpm/mg of DNA.

\(^c\) BID, Daily dose in two equal injections per day at a 12-h interval.

\(^d\) NS, Nonsignificant.

\(^e\) TID, daily dose in three equal injections per day at 8-h intervals.

\(^f\) Pooled data of control animals treated for 4 and 10 days on BID and TID schedules with 0.9% NaCl only; statistical analysis (parametric and nonparametric) did not reveal differences among the various groups of controls.

regenerative tubules (basophilic cytoplasm, ill-developed brush border, flatter shape of cells, enlarged lumen). These tubules often contained mitotic figures (Fig. 4c). From comparable autoradiographs, many labeled nuclei were seen in sections of such tubules (Fig. 4d). Under the experimental conditions used (<15 days; 10 mg/kg), these lesions were, however, too focal to allow reliable estimation of toxicity, especially upon routine examination at 100- to 400-fold magnification.

DISCUSSION

Histoautoradiographic detection of [3H]thymidine incorporation in tubular cells of kidney cortex has been used by Cuppage and Tate (5) to study regeneration after partial necrosis induced by mercuric chloride. We have used a similar approach to study the alterations induced by gentamicin at low doses, with quantitative analysis of this process by both biochemical and morphological methods. As indicated by the determination of serum levels and tissue content in acid-soluble radioactivity, gentamicin treatment did not alter the distribution of [3H]thymidine in the body or its incorporation into kidney nucleotide pools. An excellent correlation was observed between the frequency of labeled nuclei seen in histoaautoradiography and the specific radioactivity of total cortex DNA. These data indicate an increased synthesis of nuclear DNA in kidney cortex dur-
FIG. 3. Quantitative evaluation of the histoautoradiographic labeling in a control and three gentamicin-treated animals. The four animals used in this study were selected among those used for the experiment described in Table 1. Animal A is a control; the other three animals were treated TID for 4 (B) or 10 (C and D) days. Animal B showed a specific radioactivity of 40,418 dpm/mg of DNA (i.e., close to the mean value for this experimental group); animal C showed 71,500 and animal D showed 129,775 dpm/mg of DNA (i.e., a low and a high value, respectively, compared with the mean of this experimental group). A total of 10,000 to 15,000 nuclei were scored for each animal, but all data are expressed per 10,000 nuclei. The upper part of the diagram shows the labeling index of the whole cortex (ordinate) as a function of the specific radioactivity of the cortex DNA (abscissa) in the corresponding animal. The lower part of the diagram shows the distribution of the nuclear label among the main cell types of the cortex (PTC, proximal tubular cells; INC, interstitial cells; DTC, distal tubular cells; GOC, glomerular cells) for each animal. Each cell type is represented by a block which spans on the abcissa a length equal to its proportions of total cortex nuclei (ΔNj = Nj/ΣNj, where Nj = number of nuclei of a given cell type and ΣNj = the sum of the nuclei of all cell types). The ordinate (heights of the blocks) shows the relative labeling index of each cell type (number of labeled nuclei per ΔNj). The area of each block is thus equal to the absolute number of labeled nuclei seen for each class, and the total area of each diagram (A, B, C, D) is equal to the global labeling index of each sample (number of labeled nuclei per 10,000 nuclei).

ing gentamicin treatment at low doses. All together, they also show that more cells synthesize DNA in kidney cortex after treatment with gentamicin at a low dose than is observed in control animals.

It might be thought that gentamicin produces this effect by direct binding to cell DNA and by inducing a repair DNA synthesis (for review, see references 16, 17, 24). Although this mechanism seems possible in view of the polycationic character of aminoglycosides, it is unlikely to operate in vivo at the low dose used. First, the magnitude of the effect is difficult to reconcile with a repair process, unless gentamicin behaved like a very reactive compound, inducing multiple lesions in the DNA molecule and, moreover, accumulating in the nuclei in vivo. The first two properties have not been uncovered so far for aminoglycosides (25). Studies on the localization of gentamicin in kidneys of
FIG. 4. Selected fields in sections obtained from rats treated for 10 days with gentamicin (10 mg/kg TID). (a) Typical image of shrinkage necrosis (apoptosis) in a seemingly normal proximal tubule (open arrows); (b) peritubular infiltration by endothelial and fibroblast-like cells; (c) mitotic figure (filled arrow) in a tubule showing signs of regeneration (basophilic cytoplasm; less developed brush border); (d) autoradiographic demonstration of [3H]thymidine incorporation in several nuclei (arrow heads) of a regenerating tubule. Bar, 10 μm.

animals treated with low doses of drug show no evidence of its accumulation in nuclei, but rather a sequestration into lysosomes and related vacuoles (15, 28, 33). A similar conclusion was reached from fractionation studies of fibroblasts cultivated in the presence of aminoglycosides (31).

Although a direct effect on DNA cannot entirely be ruled out, the available evidence suggests that the present observations are best explained as a regenerative process. We have observed an increase of the mitotic index. Less differentiated, regenerating tubules are also seen in our animals, very much resembling those seen during postnecrotic regeneration after mercuric chloride intoxication (5). Moreover, isolated necrotic cells were actually seen, albeit infrequently. We believe that the focal necrotic process occurring at low doses of gentamicin is actually underestimated because dead cells are quickly swept away in the tubular fluid. When animals are treated with higher doses, large zones of necrosis and adjacent zones of regeneration are easily seen during treatment (9, 20) even upon continuous drug administration. High doses are also associated with plugging of tubules by cellular impaction, causing a rise in intratubular pressure (6).
Cellular proliferation without concomitant necrosis, such as that seen in compensatory hyperplasia (32), can probably be ruled out, since the doses of gentamicin used in the present experiments do not affect kidney function significantly (12, 18) and are therefore unlikely to act as a trigger for such compensatory hyperplasia. In the present experiments, cellular proliferation was not restricted to proximal tubular cells, but also involved the interstitium. A similar pattern of reaction is seen after mercuric chloride intoxication (5). Moreover, long-term exposure to gentamicin induces marked interstitial infiltration, leading to fibrosis and focal inflammatory peritubular reaction (11).

The present observations may have both toxicological and clinical implications. Regeneration may confer protection against development of kidney failure upon gentamicin treatment. Healthy volunteers (<40 years of age) have shown no significant alteration of kidney function upon a 10-day exposure to gentamicin (29). Conversely, elevation of serum creatinine was seen in 10 to 37% of ill patients (average age, >60 years) treated for the same time period or less (7, 26, 30). Other situations in which kidney regeneration rates might differ from normal would be worthy of investigation. The measure of kidney cortex DNA synthesis may also allow comparison of various aminoglycosides at low doses and after short-term administration, i.e., under conditions closer to those prevailing in the clinical use of these antibiotics. This is especially important since uptake of aminoglycosides by kidney cortex is dose saturable (14) and aminoglycosides do not always show parallel nephrotoxicity–dose–response curves (10).

ACKNOWLEDGMENTS

We thank G. Porter for critical reading of this manuscript. M. C. Cambier and J. Gilson provided expert technical assistance.

This work was supported in part by the Belgian Ministère de la Santé Publique. P.M.T. is Maitre de Recherche of the Belgian Fonds National de la Recherche Scientifique.

LITERATURE CITED

1. Appel, G. B., and H. C. Neu. 1978. Gentamicin in 1978. Ann. Intern. Med. 89:528–538.
2. Bennett, W. M., C. E. Plamp, D. N. Gilbert, R. A. Parker, and G. A. Porter. 1979. The influence of dosage regimen on experimental gentamicin nephrotoxicity: dissociation of peak serum levels from renal failure. J. Infect. Dis. 140:576–580.
3. Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315–323.
4. Cuppage, F. E., K. Setter, L. P. Sullivan, E. J. Reitzer, and A. O. Melnykovev. 1977. Gentamicin nephrotoxicity. II. Physiological, biochemical and morphological effects of prolonged administration to rats. Vitchows Arch. B 24:121–138.
5. Cuppage, F. E., and A. Tate. 1967. Repair of the nephron following injury with mercuric chloride. Am. J. Pathol. 51:405–429.

6. De Rougemont, D., A. Oeschger, L. Konrad, G. Thiel, J. Thorhors, M. Werck, P. Wunderlich, and F. P. Brunner. 1981. Gentamicin-induced acute renal failure in the rat. Nephron 29:176–184.
7. Fong, I. W., R. S. Fenton, and R. Bird. 1981. Comparative toxicity of gentamicin versus tobramycin: a randomized prospective study. J. Antimicrob. Chemother. 7:81–88.
8. Frame, P. T., J. P. Phair, C. Watanakunakorn, and T. W. P. Bannister. 1977. Pharmacologic factors associated with gentamicin nephrotoxicity in rabbits. J. Infect. Dis. 135:952–956.
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 continued drug administration. Proc. Soc. Exp. Biol. Med. 160:99–103.
10. Hottendorf, G. H., D. Bartnett, L. L. Gordon, E. F. Christensen, and H. Madisson. 1981. Nonparallel nephrotoxicity dose–response curves of aminoglycosides. Antimicrob. Agents Chemother. 19:1024–1028.
11. Hottendorf, G. H., and L. L. Gordon. 1980. Comparative low-dose nephrotoxicities of gentamicin, tobramycin, and amikacin. Antimicrob. Agents Chemother. 18:176–181.
12. Houghton, C. M., M. Hartnett, M. Campbell-Boswell, G. Porter, and W. Bennett. 1976. A light and electron microscopic analysis of gentamicin nephrotoxicity in rats. Am. J. Pathol. 82:589–612.
13. Humes, H. D., J. M. Weinberg, and T. C. Knauss. 1982. Clinical and pathophysiological aspects of aminoglycoside nephrotoxicity. Am. J. Kidney Dis. 25:29–29.
14. Josepovitz, C., E. Pastoria-Munoz, D. Timmerman, M. Scott, S. Feldman, and G. Kaloyanides. 1982. Inhibition of gentamicin uptake in rat renal cortex in vivo by aminoglycosides and other organic polycations. J. Pharmacol. Exp. Ther. 223:314–321.
15. Just, M., G. Erdmann, and E. Haberman. 1977. The renal handling of polybasic drugs. 1. Gentamicin and aprotinin in intact animals. Naunyn-Schmiedeberg’s Arch. Pharmacol. 300:57–66.
16. Kohn, K. W. 1979. Drug-induced macromolecular damage of nuclear DNA. p. 207–239. In H. Busch, T. S. Crooke, and Y. Daskal (ed.), Effects of drugs on the cell nucleus. Academic Press, Inc., New York.
17. Kohn, K. W., L. C. Erickson, and G. Laurent. 1981. DNA alklylation, crosslinking and repair, p. 33–48. In B. Serro, P. S. Schein, and J. L. Imbach (ed.), Nitrosoarene in cancer treatment. INSERM symposium no. 19. Elsevier/North-Holland Biomedical Press, Amsterdam.
18. Kosk, J. D., R. I. Mazze, and M. J. Cousins. 1974. Nephrotoxicity of gentamicin. Lab. Invest. 30:48–57.
19. Laurent, G., M. B. Carlier, B. Rollman, F. Van Hoof, and P. Tulken. 1982. Mechanism of aminoglycoside-induced lysesosomal phospholipidosis: in vitro and in vivo studies with Gentamicin and Amikacin. Biochem. Pharmacol. 31:3861–3870.
20. 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.
21. Munro, H. N., and A. Fleck. 1966. The determination of nucleic acids. Methods Biochem. Anal. 14:113–176.
22. Porter, G. A., and W. M. Bennett. 1981. Nephrotic acute renal failure due to common drugs. Am. J. Physiol. 241:F1–F8.
23. Reiner, N. E., D. C. Bloxham, and W. L. Thompson. 1978. Nephrotoxicity of gentamicin and tobramycin given once daily or continuously in dogs. J. Antimicrob. Chemother. 4(Suppl.) A:83–101.
24. Roberts, J. J. 1980. Cellular responses to carbenicin-induced DNA damage and the role of DNA repair. Br. Med. Bull. 36:25–31.
25. Sande, M. E., and G. L. Mandell. 1980. Antimicrobial agents. The aminoglycosides, p. 1162–1180. In A. Goodman Gilman, L. S. Goodman, and A. Gilman (ed.), The
pharmacological basis of therapeutics. Macmillan Publishing Co., New York.

26. Schentag, J. J., M. E. Plaut, and F. B. Cerra. 1981. Comparative nephrotoxicity of gentamicin and tobramycin: pharmacokinetic and clinical studies in 201 patients. Antimicrob. Agents Chemother. 19:859–866.

27. Schor, N., I. Ichikawa, H. G. Rennke, J. L. Troy, and B. M. Brenner. 1981. Pathophysiology of altered glomerular function in aminoglycoside-treated rats. Kidney Int. 19:288–296.

28. Silverblatt, F. J., and C. Kuehn. 1979. Autoradiography of gentamicin uptake by the rat proximal tubule cell. Kidney Int. 15:335–345.

29. Smith, C. R. 1982. Review of studies evaluating the physiopathological effects of aminoglycosides in normal human volunteers, p. 833–835. In P. Periti and G. Gialdroni-Grassi (ed.), Current chemotherapy and immunotherapy. American Society for Microbiology, Washington, D.C.

30. Smith, C. R., J. J. Lipsky, O. L. Laskin, D. B. Hellmann, E. D. Mellits, J. Longstreth, and P. Lietman. 1980. Double blind comparison of the nephrotoxicity and auditory toxicity of gentamicin and tobramycin. N. Engl. J. Med. 302:1106–1109.

31. Tulkens, P., and A. Trouet. 1978. The uptake and intracellular accumulation of aminoglycoside antibiotics in lysosomes of cultured fibroblasts. Biochem. Pharmacol. 27:415–424.

32. Van Urk, H., D. Malamud, L. Soler-Montesinos, and R. A. Malt. 1978. Compensatory hyperplasia with increasing loss of renal mass. Lab. Invest. 38:674–676.

33. Wedeen, R. P., V. Batuman, C. Cheeks, E. Marquet, and H. Sobel. 1983. Transport of gentamicin in rat proximal tubule. Lab. Invest. 48:212–223.

34. Wyllie, A. H. 1981. Cell death: a new classification separating apoptosis from necrosis, p. 9–34. In I. D. Bowen and R. A. Lockskin (ed.), Cell death in biology and pathology. Chapman and Hall, London.