AMINOGLYCOSIDE NEPHROTOXICITY. I.

EFFECTS OF AMINOGLYCOSIDE ANTIBIOTICS ON IODOHIPPURATE ACCUMULATION IN RABBIT RENAL CORTICAL SLICES

JØRGEN DAHLAGER and NILS MILMAN

Division of Nephrology, Department of Medicine P and
Division of Urology, Department of Surgery D, Rigshospitalet
(Copenhagen University Hospital), Copenhagen, Denmark

(Received for publication May 8, 1977)

The effects of aminoglycoside antibiotics on the accumulation of O-^{125}I-hippurate (OIH) in rabbit renal cortical slices were assessed in an attempt to establish an *in vitro* model for aminoglycoside nephrotoxicity. Accumulation of OIH was measured after incubation of cortex slices in media containing aminoglycosides in different concentrations. All aminoglycosides depressed OIH accumulation in the following minimum concentrations: Dihydrostreptomycin and kanamycin, 2,000 μg/ml ($P<0.01$); streptomycin and neomycin, 1,000 μg/ml ($P<0.05$ and $P<0.01$); amikacin and tobramycin, 300 μg/ml ($P<0.05$); gentamicin, 100 μg/ml ($P<0.05$). A concentration of 2,000 μg/ml caused the following reduction in OIH accumulation: Dihydrostreptomycin, 19.3%; streptomycin, 28.9%; kanamycin, 23.8%; neomycin, 62.5%; gentamicin, 68.0%; amikacin and tobramycin, 100%. Changes in pH of the incubation media after addition of aminoglycosides were only partially responsible for the observed depression of OIH accumulation and there was no evidence of substrate competition between aminoglycosides and OIH. The *in vitro* model described here appears to be inadequate as a sole predictor of aminoglycoside nephrotoxicity, but may provide a supplementary tool in the investigation of aminoglycoside proximal tubular cell toxicity.

The aminoglycoside antibiotics have established their position as highly potent and valuable drugs in the treatment of infections with a wide variety of Gram-negative and Gram-positive bacteria. However, all aminoglycosides possess nephrotoxic properties and usually a narrow range exists between the therapeutic and toxic serum concentrations. The nephrotoxicity has been documented both in animal experiments[1~8] and in clinical reports[2,9~16]. It is characterized by progressive proximal tubular damage, impairment of renal concentrating ability and eventual decrease in the glomerular filtration rate[3~5,14]; however, the causative factors remain obscure. In the present study the influence of clinically relevant aminoglycosides on the accumulation of O-^{125}I-hippurate (OIH) in rabbit renal cortical slices has been assessed in an attempt to establish an *in vitro* model for aminoglycoside nephrotoxicity.

Materials and Methods

Female Danish white rabbits weighing 2.8~3.0 kg and having normal serum creatinine, were used in the studies. The animals had free access to water until the experiments started and were anaesthetized with pentobarbital 20 mg/kg intravenously before the kidneys were removed.

Measurement of O-^{125}I-hippurate accumulation: The technique employed is a modification of the method described by CROSS and TAGGART[17]. Immediately after removal the kidneys were decapsulated and placed in chilled CROSS-TAGGART medium[17]. Slices of the kidney cortex, 0.2~0.4 mm thick and weighing 9~12 mg were cut by hand with a razor blade and kept in chilled CROSS-TAGGART medium until use. Slices from both kidneys were mixed and distributed evenly in sixteen 50-ml

Erlenmeyer incubation vessels, each containing 90~110 mg of slices, 10 ml CROSS-TAGGART medium (pH 7.4) and 20 μCi O-^{125}I-hippurate/l (Institute for Atomic Energy, Kjeller, Norway) corresponding to 3 μmol/l. Hereafter the slices were incubated for 60 minutes at 25°C with 100% oxygen in the gas space, while being shaken at 100 cycles/minute. The content was then rapidly chilled and the slices in each vessel blotted on filter paper, weighed and placed in a counting vial containing 1 ml 5% trichloroacetic acid. Both the slices and aliquots of 1 ml of the incubation media were counted for 60 seconds in a well type gamma scintillation counter (Selektronik, Copenhagen, Denmark). The OIH accumulation in the kidney slices was calculated as the ratio of the counts in 1 g tissue to those in 1 ml incubation medium, *i.e.* the slice to medium ratio (S/M ratio).

Aminoglycoside studies: Before incubation the following aminoglycosides in concentrations varying from 10 to 2,000 μg (as base)/ml were added to the incubation media: Streptomycin sulfate (714 μg base/mg), dihydrostreptomycin sulfate (700 μg/base mg), kanamycin sulfate (780 μg base/mg), neomycin sulfate (648 μg base/mg), gentamicin sulfate (571 μg base/mg), amikacin base (895 μg base/mg) and tobramycin base (925 μg base/mg). Incubation was then performed as described above. All experiments were made 6~8 times and each experimental setup included 4 control vessels without aminoglycoside. The OIH accumulation (S/M ratio) in the aminoglycoside-containing vessels was expressed in per cent of the accumulation in the controls. The per cent decrease in OIH accumulation was then calculated by subtraction of these values from 100.

pH studies: The OIH accumulation in cortical slices is a pH-dependent process[17]. Therefore pH of the incubation media were measured after addition of aminoglycosides, and it became evident that these drugs induced changes in pH. In order to distinguish between the effects of aminoglycosides and of pH changes on the OIH accumulation, the influence of pH in itself on accumulation was assessed in separate experiments. The pH was measured with a pH meter (Radiometer, Copenhagen, Denmark). Before incubation the pH was adjusted to 6.0~9.0 by means of 5 N hydrochloric acid or 5 N sodium hydroxide. All experiments were performed at least ten times and the OIH accumulation expressed in per cent of the accumulation at pH 7.4.

Substrate competition studies: In order to elucidate whether the aminoglycoside inhibition of OIH accumulation was attributable to substrate competition, experiments were performed with sodium cephalothin alone added to the incubation media in concentrations ranging from 10 to 3,000 μg/ml. In these experiments pH varied from 7.2 to 7.4.

Statistical evaluation was performed with the Student *t*-test and the actual S/M ratio values were used in these calculations.

Results

The effects of aminoglycosides on the OIH accumulation in renal cortical slices are summarized in Table 1 and Figs. 1 and 2.

Streptomycin: OIH accumulation was distinctly depressed by concentrations of 1,000 μg/ml or higher; at 2,000 μg/ml accumulation decreased to 71.1±2.4 (S.E.M.)%.

Dihydrostreptomycin: OIH accumulation was unaffected up to concentrations of 1,500 μg/ml; however, 2,000 μg/ml depressed accumulation to 80.7±1.9%.

Kanamycin: Accumulation was apparently stimulated by concentrations up to 1,000 μg/ml, but the increase was not significant and within the error of the investigation; at 2,000 μg/ml accumulation decreased to 76.2±3.5%.

Neomycin: OIH accumulation was significantly depressed by concentrations of 1,000 μg/ml or higher; at 2,000 μg/ml accumulation was 37.5±1.1%.

Gentamicin: Accumulation decreased already from concentrations of 100 μg/ml and 2,000 μg/ml reduced accumulation to 32.0±1.7%.

Table 1. Effects of different concentrations of aminoglycoside antibiotics on the accumulation of ^{125}I-hippurate in rabbit renal cortical slices.

Values are expressed in per cent of the accumulation in aminoglycoside free controls (mean ±S.E.M.)

Amino-glycoside (as base) (μg/ml)	Accumulation of ^{125}I-hippurate						
	Strepto-mycin	Dihydro-strepto-mycin	Kanamycin	Neomycin	Gentamicin	Amikacin	Tobra-mycin
0	100	100	100	100	100	100	100
10	96.9±2.8	99.4±4.8	106.3±3.2	101.9±4.1	89.5±1.7	101.4±5.0	96.8±6.0
100	91.5±2.3	94.2±3.8	103.1±4.9	103.4±4.9	85.6±1.7*	106.7±6.4	97.1±5.3
300	87.6±1.6	94.0±2.7	112.6±5.7	95.1±1.5	82.2±0.6**	86.0±6.9*	86.6±4.1*
500	84.1±2.8	94.5±2.9	110.9±5.1	92.6±4.7	79.5±2.4**	48.7±3.3**	25.5±2.4**
1,000	84.2±2.6*	93.0±1.8	104.3±5.6	59.8±1.5**	51.3±1.1**	2.2±0.8**	2.1±0.7**
1,500	75.5±1.4*	91.1±1.7	95.6±4.7	49.4±2.0**	41.0±2.7**	0.4±0.2**	0.4±0.4**
2,000	71.1±2.4**	80.7±1.9**	76.2±3.5**	37.5±1.1**	32.0±1.7**	0±0**	0±0**

* $P<0.05$, ** $P<0.01$.

Amikacin: Accumulation was depressed from concentrations of 300 μg/ml or higher and at 2,000 μg/ml there was no measurable accumulation.

Tobramycin: Accumulation decreased sharply from concentrations of 300 μg/ml and was unmeasurable at 2,000 μg/ml.

When arranged according to their effects on OIH accumulation the order became: Tobramycin, amikacin, gentamicin, neomycin, streptomycin, kanamycin and dihydrostreptomycin.

pH Studies

The addition of aminoglycosides changed the pH of the incubation media as shown in Table 2. Streptomycin, dihydrostreptomycin and kanamycin caused only minor changes in pH. Neomycin and gentamicin induced a shift in the acid direction to a minimum pH of 6.3 at 2,000 μg/ml while amikacin and tobramycin changed pH in the alkaline direction to a maximum pH of 9.3 at 2,000 μg/ml.

Fig. 1. Effects of different concentrations in the incubation media of streptomycin, dihydrostreptomycin and kanamycin on ^{125}I-hippurate accumulation in rabbit renal cortical slices.

Decrease is expressed in per cent of the accumulation in aminoglycoside-free controls.

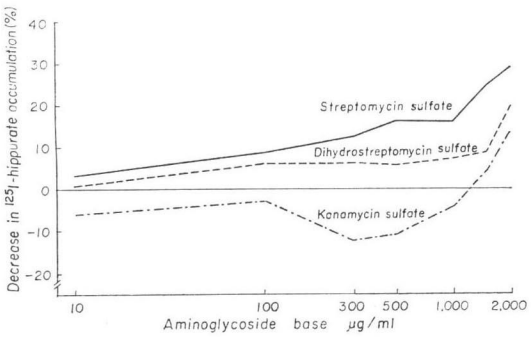


Fig. 2. Effects of different concentrations in the incubation media of amikacin, tobramycin, gentamicin and neomycin on ^{125}I-hippurate accumulation in rabbit renal cortical slices.

Decrease is expressed in per cent of the accumulation in aminoglycoside-free controls.

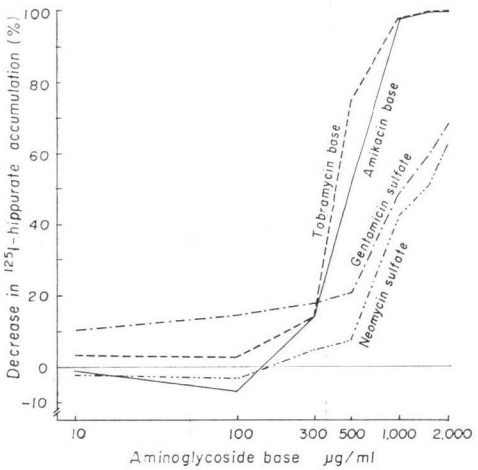


Fig. 3. Influence of pH variations in the incubation media on the accumulation of ^{125}I-hippurate in rabbit renal cortical slices.

Accumulation is expressed in per cent of the values at pH 7.4 (mean ± S.E.M).

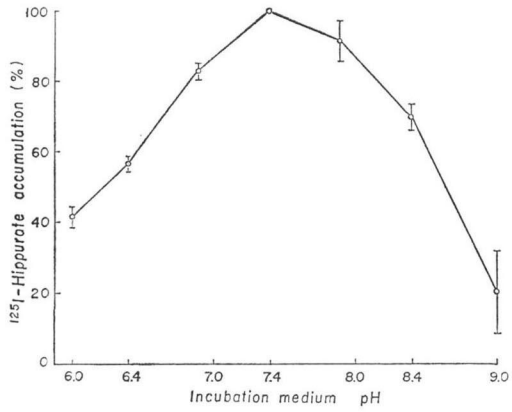
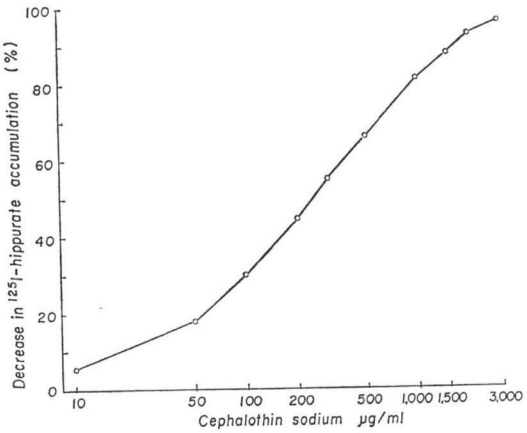


Fig. 4. Effects of different concentrations of sodium cephalothin in the incubation media on the accumulation of ^{125}I-hippurate in rabbit renal cortical slices.

Decrease is expressed in per cent of the accumulation in cephalothin-free controls.



The accumulation of OIH in renal cortical slices was sensitive to variations in the pH as shown in Table 3 and Fig. 3. Maximum accumulation was observed at pH 7.4 and a change in this value, both in the acid and in the alkaline directions, decreased accumulation considerably. The decrease in accumulation was greatest at alkaline pH.

Substrate Competition Studies

Cephalothin caused a gradual decrease in the OIH accumulation with increasing concentrations; concentrations of 3,000 μg/ml decreased accumulation to 3.9% (Fig. 4).

Discussion

The nephrotoxicity of aminoglycoside antibiotics was first recognized in animal experiments[1~8]

Table 2. Effects of different concentrations of aminoglycoside antibiotics on pH of the incubation medium.

Aminoglycoside (as base) (μg/ml)	pH						
	Streptomycin	Dihydrostreptomycin	Kanamycin	Neomycin	Gentamicin	Amikacin	Tobramycin
0	7.4	7.4	7.4	7.4	7.4	7.4	7.4
10	7.4	7.4	7.4	7.4	7.4	7.5	7.5
100	7.4	7.4	7.4	7.3	7.3	7.6	7.6
300	7.4	7.4	7.5	7.1	7.0	7.9	8.1
500	7.3	7.3	7.5	7.0	6.9	8.1	8.3
1,000	7.3	7.3	7.6	6.7	6.7	8.7	8.8
1,500	7.2	7.3	7.6	6.6	6.5	8.9	9.0
2,000	7.2	7.2	7.6	6.5	6.3	9.3	9.1

Table 3. Influence of pH in the incubation medium on the accumulation of ^{125}I-hippurate in rabbit renal cortical slices. Values are expressed in per cent of the accumulation at pH 7.4 (mean ±S.E.M.)

pH	6.0	6.4	6.9	7.4	7.9	8.4	9.0
Accumulation of ^{125}I-hippurate (%)	41.3±3.0	56.5±2.1	82.6±2.1	100	91.5±5.7	69.3±3.6	20.3±11.6

and later confirmed by experiences from the human clinic[2,9~16]. However, species-dependent differences in sensitivity[1,2] impede direct application of the animal experimental results to human subjects.

Renal damage caused by aminoglycosides takes the shape of various degrees of tubular injuries, which are most prominent in the proximal tubules, and may eventually lead to local or extensive tubular necrosis[1~6,9,13,14].

The active uptake and accumulation of *p*-aminohippurate and OIH is a metabolic characteristic of the proximal tubular cell[18]. This process is effectuated through a cellular transport system, common to numerous organic acids[19] and seems to be energy requiring and closely connected with oxygen utilization[20]. Several drugs, such as cephalothin, penicillins and probenecid are also transported by this system and show mutual competitive substrate inhibition of the transport capacity[19].

In the present study all aminoglycosides depressed OIH accumulation in high concentrations. Where significant effects were seen, the concentrations of streptomycin, dihydrostreptomycin, kanamycin and neomycin were above normal therapeutic levels, whereas gentamicin, amikacin and tobramycin depressed accumulation distinctly in concentrations which may normally appear in the urine[21]. Other investigators have accordingly found that gentamicin decreases *p*-aminohippurate accumulation in rat cortical slices[22].

The urinary concentration of gentamicin (and other aminoglycosides) is directly related to the serum concentration and inversely to the urine volume[23]; the urine/serum ratio of gentamicin in hydropenic dogs may be as high as 200~300[21] and urine concentrations of 500~1,000 μg/ml have been measured in human subjects[21].

The aminoglycoside-induced inhibition of OIH accumulation is partially due to pH changes resulting from addition of antibiotics to the incubation media (Table 2). However, when comparing the pH-related changes in OIH accumulation (Fig. 3 and Table 3) with the changes in pH and accumulation after addition of aminoglycosides to the incubation media (Table 2 and Table 1), it is evident that the alterations in pH alone cannot be fully responsible for the observed decrease in OIH accumulation.

The relevance of the presented *in vitro* model for aminoglycoside nephrotoxicity to the *in vivo* use of aminoglycosides remains to be established. Pretreatment of rats with subnephrotoxic doses of neomycin and gentamicin for short periods apparently stimulate *p*-aminohippurate accumulation in cortical slices[22,24,25]. This effect is possibly related to an increase in the active transport of *p*-aminohippurate as well a decrease in the efflux across the peritubular membrane of proximal tubular cells[25]. In contrast, we have been unable to demonstrate any effect of low-dose gentamicin and tobramycin treatment on OIH accumulation in rabbit cortical slices, whereas high, nephrotoxic doses, consistently produce a decrease in accumulation (MILMAN and DAHLAGER, unpublished results), as earlier demonstrated in rats[22].

The reported results are not entirely consistent with the known *in vivo* nephrotoxicity of aminoglycosides, where the order of increasing toxicity is dihydrostreptomycin-streptomycin, kanamycin-amikacin, gentamicin-tobramycin and neomycin[10,26], whereas we found increasing effect on OIH accumulation in the order of dihydrostreptomycin, kanamycin, streptomycin, neomycin, gentamicin, amikacin and tobramycin.

At present the mechanism of the aminoglycoside-induced inhibition of OIH accumulation is unexplained. There seems to be no specific affinity for these drugs to the *p*-aminohippurate transport system, since the administration of probenecid has no influence on the urinary excretion of gentamicin and amikacin[27,28]; furthermore, the entirely different shapes of the dose response OIH accumulation curves with aminoglycosides (Figs. 1 and 2) and cephalothin (Fig. 4) argue against competitive inhibition between aminoglycosides and OIH. Hypothetically, the aminoglycosides might affect the cell membrane of the proximal tubular cell, causing either decreased influx or increased efflux of OIH. Also they might penetrate intracellularly and impair metabolic processes involved with OIH accumulation, as it has been demonstrated that aminoglycosides are firmly bound to intracellular substances obtained from homogenization of kidney tissue[29~31].

The bactericidal effect of the aminoglycosides is probably mediated through impaired protein synthesis on the ribosomal level with misreading of the m-RNA code[32,33]. A similar mechanism might also be responsible for the toxicity in the proximal tubular cells, but so far no evidence has been

presented in favour of this hypothesis.

Being the principal route of excretion, the kidneys are particularly exposed to high concentrations of aminoglycosides. Elimination occurs mainly through glomerular filtration[1,34], but the extent of transtubular passage is unclarified. Tubular reabsorption and/or secretion of gentamicin have been suggested by some investigators[35,36] while others have demonstrated that urinary excretion of gentamicin is independent of urinary pH, indicating the absence of tubular ionic diffusion trapping[34]. However, micropuncture studies have not been performed in order to solve these problems more accurately.

The proximal tubular cells seem to possess a special affinity for, and ability to bind gentamicin. Several authors[31,37,38] have demonstrated a distinct accumulation of gentamicin in the renal cortex and have found evidence of an intracellular distribution of the drug. Intracellularly bound gentamicin is probably slowly released and responsible for the continued urinary excretion of the drug for a long time after it has been withdrawn[14,38]. These findings could explain why the proximal tubular cells are most extensively damaged by gentamicin although the highest intratubular concentrations occur in the distal and collecting tubules.

In conclusion, the above-mentioned discrepancies between the reported results and the *in vivo* toxicity of the various aminoglycosides suggest that the described *in vitro* model is inadequate as a sole predictor of nephrotoxicity. However, it may provide a supplementary tool in the investigation of aminoglycoside proximal tubular cell toxicity.

Acknowledgements

The authors wish to express their thank to Bristol-Myers Co. for providing amikacin, Eli Lilly & Co. for providing tobramycin, Lundbeck & Co. Ltd. for providing kanamycin, Novo Ltd. for providing streptomycin, dihydrostreptomycin and neomycin and to Schering Corp. U.S.A. for providing gentamicin.

References

1) Black, J.; B. Calesnick, D. Williams & M. J. Weinstein: Pharmacology of gentamicin, a new broad spectrum antibiotic. Antimicr. Agents & Chemoth. -1963: 138～147, 1964
2) Falco, F. G.; H. M. Smith & G. M. Arcieri: Nephrotoxicity of aminoglycosides and gentamicin. J. Infect. Dis. 119: 406～409, 1969
3) Flandre, O. & M. Damon: Experimental study of the nephrotoxicity of gentamicin in rats. In gentamicin: First Int. Symp., pp. 47～61, Schwabe & Co., Basel, 1967
4) Kosek, J. C.; R. I. Mazze & M. J. Cousins: Nephrotoxicity of gentamicin. Lab. Invest. 30: 48～57, 1974
5) Patel, V.; F. C. Luft, M. N. Yum, B. Patel, W. Zeman & S. A. Kleit: Enzymuria in gentamicin-induced kidney damage. Antimicr. Agents & Chemoth. 7: 364～369, 1975
6) Reiffenstein, J. C.; S. W. Holmes, G. H. Hottendorf & M. E. Bierwagen: Ototoxicity studies with BB-K8, a new semisynthetic aminoglycoside antibiotic. J. Antibiotics 26: 94～100, 1972
7) Tisch, D. E.; J. B. Huftalen & H. L. Dickison: Pharmacological studies with kanamycin. Ann. N.Y. Acad. Sci. 76: 44～65, 1958
8) Welles, J. S.; J. L. Emmerson, W. R. Gibson, R. Nickanser, N. V. Owen & R. Anderson: Preclinical toxicity studies with tobramycin. Toxicol. Appl. Pharmacol. 25: 398～409, 1973
9) Farrington, R. F.; H. Hull-Smith, P. A. Bunn & W. McDermott: Streptomycin toxicity. J. Amer. Med. Assoc. 134: 679～688, 1947
10) Kovnat, P.; E. Labovitz & S. P. Levison: Antibiotics and the kidney. Med. Clin. North Am. 57: 1045～1063, 1973
11) Kunin, C. M.: Nephrotoxicity of antibiotics. J. Amer. Med. Assoc. 202: 204～208, 1967
12) Kuntz, E.: Klinische Untersuchungen zur Nephrotoxicität von Kanamycin. Klin. Wschr. 40: 834～840, 1967
13) Milman, N.: Gentamicin nephropathy. Ugeskr. Laeg. 135: 1861～1865, 1973
14) Milman, N.: Renal failure associated with gentamicin nephropathy. Acta Med. Scand. 196: 87～91, 1974
15) Stone, H. H.; L. D. Kolb, C. E. Geheber & C. A. Currie: Treatment of Gram-negative infections in

surgical patients with a new aminoglycoside—tobramycin. *In* Tobramycin: Proc. VIIIth Int. Congr. Chemother., pp. 102～109, Excerpta Medica, Amsterdam, 1974
16) WAISBREN, B. A. & W. W. SPINK: A clinical appraisal of neomycin. Ann. Intern. Med. 33: 1099～1119, 1950
17) CROSS, R. J. & J. V. TAGGART: Renal tubular transport: Accumulation of *p*-aminohippurate by rabbit kidney slices. Am. J. Physiol. 161: 181～190, 1950
18) WEDEEN, R. P. & B. WEINER: The distribution of *p*-aminohippuric acid in rat kidney slices. I. Tubular localization. Kidney Intern. 3: 205～213, 1973
19) LANT, R. F.: Renal excretion and nephrotoxicity of drugs. pp. 591～614. *In* D. BLACK(*ed.*), Renal disease. Blackwell Scientific Publications, London, 1972
20) TAGGART, J. V. & R. P. FORSTER: Renal tubular transport: Effect of 2,4-dinitrophenol and related compounds on phenol red transport in the isolated tubules of the flounder. Am. J. Physiol. 161: 167～172, 1950
21) RIFF, L. J. & J. G. JACKSON: Pharmacology of gentamicin in man. J. Inf. Dis. 124 (Suppl): 98～105, 1971
22) HIRSCH, G. H.; A. J. BAYNE & A. P. PAKUTS: Gentamicin: Nephrotoxicity and effects on renal organic ion transport. Fed. Proc. 34: 803, 1975
23) JAO, R. L. & G. G. JACKSON: Gentamicin sulfate, new antibiotic against Gram-negative bacilli. J. Amer. Med. Assoc. 189: 817～822, 1964
24) COHEN, L.; R. LAPKIN & G. J. KALOYANIDES: Effect of gentamicin on PAH uptake by rat kidney slices. J. Pharmacol. Exp. Ther. 193: 264～270, 1975
25) LAPKIN, R.; H. ENSER & G. J. KALOYANIDES: Neomycin stimulates PAH transport in rat kidney slices. Fed. Proc. 34: 408, 1975
26) WEINSTEIN, L.: Antimicrobial agents. Streptomycin, gentamicin and other aminoglycosides. pp. 1167～1182. *In* L. S. GOODMAN & A. GILMAN (*ed.*), The pharmacological basis of therapeutics. MacMillan Publishing Co., New York, 1975
27) BERGAN, T.; L. WESTLIE & E. K. BRODWALL: Influence of probenecid on gentamicin pharmacokinetics. Acta Med. Scand. 191: 221～224, 1972
28) CABANA, B. E. & J. G. TAGGART: Comparative pharmacokinetics of BB-K8 and kanamycin in dogs and humans. Antimicr. Agents & Chemoth. 3: 478～483, 1973
29) KUNIN, C. M.: Binding of antibiotics to tissue homogenates. J. Infect. Dis. 121: 55～64, 1970
30) KUNIN, C. M.; W. A. CRAIG, M. KORNGUTH & R. MONSON: Influence of binding on the pharmacologic activity of antibiotics. Ann. N.Y. Acad. Sci. 226: 214～224, 1973
31) WHELTON, A. & W. G. WALKER: Intrarenal antibiotic distribution in health and disease. Kidney Intern. 6: 131～137, 1974
32) HAHN, F. E. & S. G. SARRE: Mechanism of action of gentamicin. J. Infect. Dis. 119: 364～369, 1969
33) LUZZATO, L.; D. APIRION & D. SCHLESSINGER: Mechanism of action of streptomycin in *E. coli*: Interruption of the ribosome cycle at the initiation of protein synthesis. Proc. Nat. Acad. Sci. 60: 873～880, 1968
34) MARIEL, C.; P. VEYSSIER, J. C. PECHERE & E. DE CERNER: Urinary pH and excretion of gentamicin. Brit. Med. J. 1972- 2: 406, 1972
35) BERGAN, T.; E. K. BRODWALL & A. ØYRI: Renal excretion of gentamicin in chronic pyelonephritis. Acta Med. Scand. 189: 1～5, 1971
36) NEDDEN, R.; T. FUCHS, K. SCHRÖDER & W. WUNDT: Die renale Ausscheidung von Gentamicin beim Menschen. Med. Wschr. 97: 1496～1503, 1972
37) ALFTHAN, O.; O. V. RENKONEN & A. SIVONEN: Concentration of gentamicin in serum, urine and urogenital tissue in man. Acta Path. Microbiol. Scand. Section B 241 (Suppl): 92～94, 1973
38) WAHLIG, H.; A. METALLINOS, W. HAMEISTER & R. BERGMANN: Gentamicin-Konzentrationen in Geweben und Körperflüssigkeiten von Versuchstieren. Int. J. Clin. Pharmacol. 10: 212～229, 1974