EFFECT OF D-GLUCARATES ON BASIC ANTIBIOTIC-INDUCED RENAL DAMAGE IN RATS

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Dehydrated rats regularly develop acute renal failure following single injection of aminoglycoside antibiotics combined with dextran or of antibiotics only. Oral administration of 2,5-di-O-acetyl-D-glucaro-1,4-6,3-dilactone protected rats against renal failure induced by kanamycin-dextran. The protective effect was prevalent among D-glucarates, and also to other saccharic acid, hexauronic acids and hexaaldonic acids, although to a lesser degree, but not to a hexaaldose, sugar alcohols, substances in the TCA cycle and other acidic compounds. D-Glucarates were effective against renal damage induced by peptide antibiotics as well as various aminoglycoside antibiotics. Dose-responses were observed in the protective effect of D-glucarates. With a D-glucarate of a fixed size of dose, approximately the same degree of protection was obtained against renal damages induced by different basic antibiotics despite large disparities in administration doses of different antibiotics. D-Glucarates had the ability to prevent renal damage but not to cure it. Rats excreted acidic urine when they were spared from renal lesions by monosaccharides. The reduction effect of D-glucarates against nephrotoxicity of basic antibiotics was discussed.

Aminoglycoside antibiotics, used effectively in the treatment of gram-negative bacterial infections or tuberculosis, frequently cause renal insufficiency in human.[1,2] However, the rat is quite resistant to the nephrotoxicity of aminoglycoside antibiotics which made it difficult to bring about a definite renal failure in normal animals even by continuous administration of large doses of antibiotics over a long period.[3,4] Recently K. HIRATA, one of the authors, found that dehydrated rats regularly and rapidly develop extensive renal failure when single injection of an aminoglycoside antibiotic is administered with a plasma expander. According to a biochemical and pathological study by his groups,[5] this renal failure has been attributed to necrosis of epithelial cells of proximal convoluted tubules and has also been observed at subcellular levels where there were massive formations of phagolysosomes accompanied by frazilization. Experiments were performed to study the effect of D-glucarates on prevention of renal damage induced by aminoglycoside antibiotics.

Materials and Methods

Reagents: Antibiotics were marketed products: kanamycin sulfate, aminodeoxy kanamycin sulfate and streptomycin sulfate (Meiji Seika Co., Ltd.); neomycin sulfate (Nippon Kayaku Co., Ltd.); gentamicin sulfate (Schering Co., Ltd.); paromomycin sulfate (Kyowa Hakko Co., Ltd.); capreomycin sulfate (Eli Lilly Co., Ltd.); colistin sodium methanesulfonate (Kayaku Antibiotic

Research Co., Ltd.); and viomycin sulfate (Pfizer Taito Co., Ltd.). D-Glucarates were synthesized in the Chugai Pharmaceutical Co., Ltd. Other monosaccharides were of reagent grade.

Experimental procedures: Male Wistar-Imamichi rats weighing 300~400 g were maintained without water for 48 hours. These dehydrated rats were then injected intramuscularly with 150 mg as base/kg of kanamycin-sulfate, followed simultaneously by an intraperitoneal injection of 10 w/v % dextran (MW *ca.* 40,000) in a dose of 30 ml/kg. The dehydrated rats were also injected with an aminoglycoside or peptide antibiotic. Antibiotics were dissolved in physiological saline solution in a dose volume of 0.5 ml per animal. Monosaccharides were administered intraperitoneally 5 minutes before antibiotics injection, except for 2,5-di-O-acetyl-D-glucaro-1,4-6, 3-dilactone which was administered orally one hour before antibiotic injection. After antibiotic injection, the rats were kept in cages, ten animals per cage, under standard conditions with free access to water and food. The rats were placed in individual metabolic cages when urine was collected. Ordinarily the rats were sacrificed 24 hours after antibiotic injection by drawing the blood from the inferior vena cava under anesthesia and then the kidneys were excised. In some experiments, blood samples were collected at defined times from the cervical aorta. Concentration of blood urea nitrogen (BUN) was measured by a Unigraph (Warner-Chilcott) and urinary occult blood by Labstix (Ames division, Miles-Sankyo). Urine pH was determined with a pH meter. The rate of kidney edema was calculated from the following equation:

$$\text{Kidney edema rate } (\%) = \left(\frac{\text{Kidney weight}}{\text{Body weight}}\right) \times 100 .$$

Results

1. Reduction of kanamycin-dextran-induced renal damage by 2,5-di-O-acetyl-D-glucaro-1,4-6,3-dilactone

Administration of kanamycin combined with dextran produced intensive renal failure in dehydrated rats (Fig. 1). Injection with 150 mg as base/kg of kanamycin and 30 ml/kg of 10 % dextran concurrently caused almost no urinary excretion for 24 hours, occult blood in the urine for 72 hours, and kidney swelling with elevated BUN even 72 hours later in dehydrated rats. In contrast, dehydrated rats receiving 800 mg/kg of 2,5-di-O-acetyl-D-glucaro-1,4-6,3-dilactone one hour before kanamycin-dextran injection showed only slight symptoms of renal dysfunction.

2. Effect of various monosaccharides on renal damage induced by kanamycin-dextran

Various monosaccharides were tested for their effectiveness against kanamycin-dextran-induced renal damage in order to delineate the relationship between activity and chemical

Fig. 1. Effect of 2,5-di-O-acetyl-D-glucaro-1,4-6,3-dilactone on kanamycin-dextran-induced renal damage in rats.

Renal failure was induced in rats maintained without water for 48 hours, by intramuscular injection of kanamycin (150 mg/kg) and intraperitoneal injection of 10% dextran (30 ml/kg). 2,5-Di-O-acetyl-D-glucaro-1,4-6,3-dilactone was given orally in a dose of 800 mg/kg one hour before kanamycin-dextran injection.

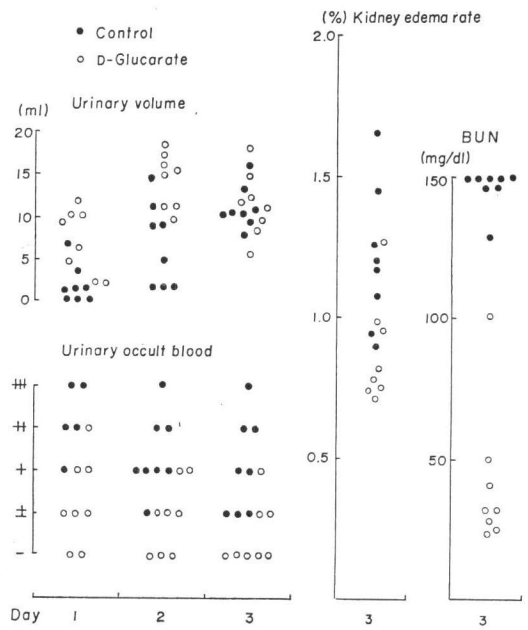


Table 1. Effect of various monosaccharides on kanamycin-dextran induced renal damage in rats.

Compound	Dose (mg/kg)	BUN (mg/dl)	Kidney edema rate
Sodium D-glucaro-1,4-lactone	600	27± 3	0.74±0.03
Sodium D-glucaro-6,3-lactone	600	32± 3	0.76±0.02
D-Glucaro-1,4-6,3-dilactone	600	29± 5	0.72±0.03
D-Mannaro-1,4-6,3-dilactone	600	136±11	0.88±0.09
Sodium tartaric acid	600	96±18	0.93±0.08
Sodium D-glucuronic acid	800	43± 7	0.78±0.03
Sodium D-galacturonic acid	800	54±11	0.78±0.06
D-Glucurono lactone	800	67±15	0.86±0.07
D-Mannurono lactone	800	75±20	0.89±0.07
Sodium L-gulonic acid	800	54±17	0.84±0.07
Sodium L-idonic acid	800	68±13	0.84±0.05
L-Gulono lactone	800	94±24	1.02±0.10
D-Glucono lactone	800	77±28	0.90±0.12
D-Glucose	800	157±16	1.07±0.05
D-Mannitol	800	163±15	1.06±0.07
Xylitol	800	161±10	1.16±0.04
Sodium citric acid	600	156±17	1.06±0.16
Sodium succinic acid	600	147±29	1.12±0.06
Sodium glutaric acid	600	156±11	1.10±0.06
Sodium oxalic acid	600	162±14	1.11±0.08

Monosaccharides were given intraperitoneally 5 minutes before the injection of kanamycin-dextran. Animals were sacrificed 24 hours after kanamycin-dextran injection.
Other experimental conditions were the same as in Fig. 1. Each value is the mean±S.D. of more than three animals.

structure (Table 1). BUN and the kidney edema rate were used as indicators of the degree of renal dysfunction. D-Glucarates, such as sodium D-glucaro-1,4-lactone, sodium D-glucaro-6,3-lactone and D-glucaro-1,4-6,3-dilactone, which are metabolites of 2,5-di-O-acetyl-D-glucaro-1,4-6,3-dilactone, were the most potent of the compounds examined. D-Mannaro-1,4-6,3-dilactone caused renal impairment by itself. Among the other saccharic acids, sodium tartaric acid was slightly effective. Hexauronic acids such as sodium D-glucuronic acid, sodium D-galacturonic acid, D-glucurono lactone and D-mannurono lactone; and hexaaldonic acids such as sodium L-gulonic acid, sodium L-idonic acid, L-gulono lactone and D-glucono lactone, were also active, although to a lesser degree than the D-glucarates. Acid types of D-glucuronic acid and L-gulonic acid were more active than the corresponding lactone types. However, hexaaldose such as D-glucose; sugar alcohols such as D-mannitol, and xylitol; members of the TCA cycle such as sodium citric acid and sodium succinic acid; and other acidic compounds such as sodium glutaric acid and sodium oxalic acid, had no effect.

3. Protective effect of D-glucarates against renal damages induced by aminoglycoside and peptide antibiotics

D-Glucaro-1,4-lactone was subsequently examined for its capacity to protect against renal damage induced by various aminoglycoside and peptide antibiotics.

Peptide antibiotics such as colistin, capreomycin and viomycin produced renal failure similar

to that induced by aminoglycoside antibiotics, *i.e.* characterized by urinary depletion, occult blood in the urine, and high level of BUN accompanied by swelling of the kidneys.

The results concerning intensity of nephrotoxicity of those antibiotics coincide well with the clinical evaluation.

D-Glucaro-1,4-lactone was capable of preventing renal damage induced in rats by all aminoglycoside or peptide antibiotics, just as in the case of other D-glucarates (Table 2). At this time, however, no D-glucarate protection against lethal toxicity of antibiotics was observed.

Table 2. Protective effect of sodium D-glucaro-1,4-lactone on basic antibiotic-induced renal damage in rats.

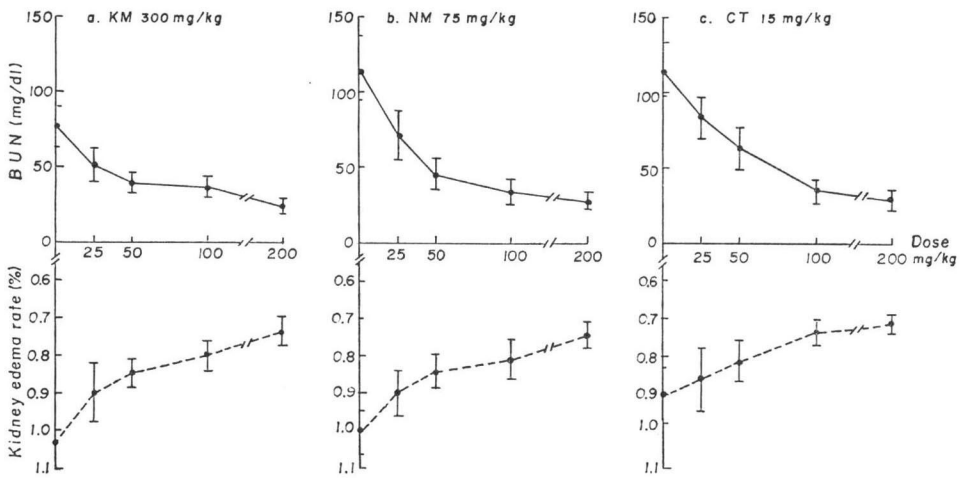
Compound	Dose (mg/kg)	BUN (mg/dl)		Kidney edema rate (%)	
		None	D-Glucarate	None	D-Glucarate
Neomycin	50	85±21	30±4	0.92±0.15	0.72±0.03
Gentamicin	100	58±16	26±3	0.92±0.01	0.77±0.03
Paromomycin	100	110± 9	26±4	0.96±0.06	0.73±0.02
Aminodeoxy-kanamycin	100	85±12	30±3	1.02±0.06	0.76±0.03
Streptomycin	200	52±10	27±3	0.77±0.05	0.71±0.03
Kanamycin	300	89±16	26±2	0.91±0.05	0.71±0.02
Colistin	15	122±18	26±5	0.94±0.02	0.71±0.04
Viomycin	100	146±16	30±5	1.03±0.07	0.79±0.05
Capreomycin	100	108± 9	31±6	0.94±0.09	0.77±0.06

Sodium D-glucaro-1,4-lactone was administered in a dose of 300 mg/kg 5 minutes before antibiotic injection.
BUN and kidney edema rates were measured 24 hours after antibiotic injection.
Each value is the mean±S.D. of more than four animals.

Fig. 2. Effect of various doses of D-glucaro-1,4-lactone on kanamycin, neomycin and colistin-induced renal damage in rats.

Dehydrated rats, receiving various doses of D-glucaro-1,4-lactone were injected with kanamycin (KM), neomycin (NM), and colistin (CT) in doses of 300, 75 and 15 mg/kg, respectively. BUN and kidney edema rate were determined 24 hours after antibiotic injection.

Each value is the mean±S.D. of four animals.



4. Dose response relation of D-glucarate as a protective drug against renal damage induced by antibiotics

With neomycin and kanamycin as the most and least potent nephrotoxic agents, respectively, among aminoglycoside antibiotics and colistin as the peptide antibiotics, studies were performed on the reducing effect of various doses of sodium D-glucaro-1,4-lactone against renal damage (Fig. 2). Kanamycin, neomycin and colistin were injected in dehydrated rats in a dose of 300, 75 and 15 mg as base/kg, respectively. Administration of doses of sodium D-glucaro-1,4-lactone higher than 25 mg/kg decreased BUN and kidney edema rate 24 hours after injection of any antibiotic irrespective of marked disparities in administered doses of the three antibiotics. Increased doses of sodium D-glucaro-1,4-lactone were also accompanied by similar reduction curves of renal damage among the three drugs.

5. Protection against renal damage as a function of the time interval between administration of D-glucarate and of the antibiotics

The effects of sodium D-glucaro-1,4-lactone administered 5, 30, 60, 90 and 180 minutes before and 10, 20 and 40 minutes after kanamycin-dextran injection are shown in Table 3. Sodium D-glucaro-1,4-lactone was the most potent in reducing renal damage when given 5~30 minutes before antibiotic injections and less effective when administered earlier. When administered after antibiotic injection, the drug lost its protective efficiency as time passed and failed to reduce renal damage when given 40 minutes after antibiotic injection.

Table 3. Protection against renal damage as a function of the time interval between administration of sodium D-glucaro-1,4-lactone and kanamycin-dextran.

Time (min.)	BUN (mg/dl)	Kidney edema rate (%)
180 (before)	130±17	1.10±0.08
90 (〃)	52± 7	0.88±0.07
60 (〃)	35±12	0.78±0.06
30 (〃)	28± 3	0.76±0.03
5 (〃)	27± 2	0.74±0.03
10 (after)	44±10	0.84±0.06
20 (〃)	64±12	0.92±0.08
40 (〃)	160±20	1.04±0.12

400 mg/kg of D-glucaro-1,4-lactone was given before and after kanamycin-dextran injection.

Animals were sacrificed 24 hours after kanamycin-dextran injection.

Other experimental conditions were the same as in Fig. 1. Each value is the mean±S.D. of four animals.

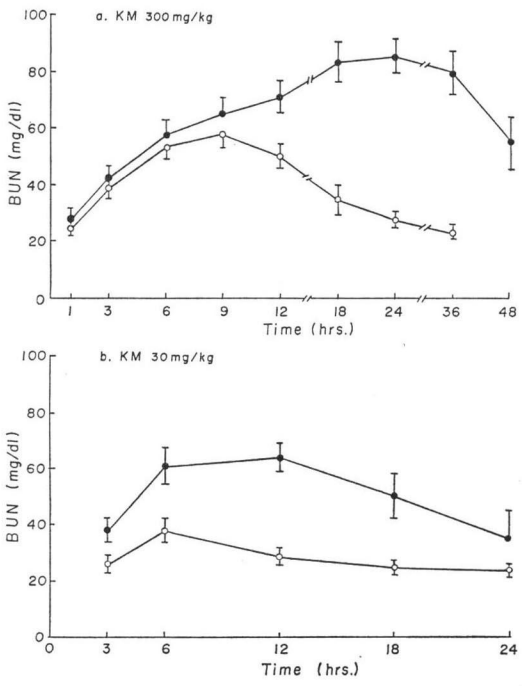
6. Progressive renal damage following antibiotic injection

Fig. 3 illustrates the changes in BUN after administration of kanamycin at high or chemo-

Fig. 3. Change of blood urea nitrogen in dehydrated rats following kanamycin injection in large or chemotherapeutical doses.

Kanamycin was injected in doses of 300 and 30 mg/kg into dehydrated rats receiving D-glucaro-1,4-lactone in a dose equivalent to that of kanamycin.

Each value is the mean±S.D. of five animals.



therapeutic doses in dehydrated rats receiving a vehicle or sodium D-glucaro-1,4-lactone. When rats were injected with kanamycin in a dose of 300 mg as base/kg, BUN began to increase 3 hours after injection and continued to rise for about a day. It reached a maximum after 18 hours and remained at a plateau for 36 hours, returning to normal gradually thereafter. On the other hand, BUN in rats receiving 300 mg/kg of sodium D-glucaro-1,4-lactone with antibiotic increased to the same degree as that in control rats for 9 hours, but began to decrease after 12 hours and reached the normal value after 24 hours. The changes in BUN were similar to those observed in control rats receiving one-tenth of dose of kanamycin. When rats were injected with kanamycin in a dose of 30 mg as base/kg, BUN increased to the same degree as in rats injected with high doses of kanamycin during the first 6 hours and then remained at a plateau up to 12 hours. This was followed by decrease to the normal value after 24 hours. BUN in rats receiving 30 mg/kg of sodium D-glucaro-1,4-lactone with antibiotic increased slightly after 6 hours but returned nearly to normal after 12 hours. No increase of BUN was observed in rats receiving 60 mg/kg of sodium D-glucaro-1,4-lactone.

7. Effect of monosaccharides on acidification of urine

Administration of D-glucarates in dehydrated rats resulted in excretion of marked acidic urine. It was considered that acidification of urine may have had some connection with the action of monosaccharides against renal damage. Data in Table 4 show pH value of urine excreted by dehydrated rats for the first 9 hours and 9 to 24 hours following administration of various monosaccharides. Rats receiving sodium D-glucaro-1,4-lactone, sodium D-glucuronic acid, D-glucurono lactone, sodium L-gulonic acid, L-gulono lactone and D-glucono lactone excreted acidic urine approximately in parallel with their ability to reduce renal damage. However, acidic urine was also observed in rats receiving sodium citric acid or sodium glutaric acid, which had no effect on renal damage.

Table 4. Effect of monosaccharides on acidification of urine.

Compound	Dose (mg/kg)	pH of urine	
		0~9 hours	9~24 hours
None	—	6.6±0.1	6.6±0.2
Sodium D-glucaro-1,4-lactone	400	5.0±0.1	6.0±0.2
Sodium D-glucuronic acid	800	5.5±0.2	6.1±0.3
D-Glucurono lactone	800	6.1±0.2	6.3±0.1
Sodium L-gulonic acid	800	5.6±0.2	6.3±0.2
L-Gulono lactone	800	6.1±0.1	6.4±0.1
D-Glucono lactone	800	6.1±0.1	6.6±0.1
Sodium citric acid	600	5.9±0.2	6.7±0.3
Sodium glutaric acid	600	5.7±0.1	6.3±0.2

Urine was collected during the first 9 hours and from 9 to 24 hours following administration of compounds to dehydrated rats. Each value is the mean±S.D. of four to seven animals.

Discussion

The susceptibility of kidneys to basic antibiotics depends greatly on the state of renal function. It is well known that basic antibiotic-induced renal insufficiency can occur in human suffering

from renal function disorders.[6,7] A heavy burden is imposed on the kidneys during thirst. In this experiment, acute renal failure was induced without failure in rats which were dehydrated for 48 hours by the administration of chemotherapeutic doses of basic antibiotics while consecutive administration of large doses for three weeks running did not produce renal failure in rats kept under normal conditions (unpublished observation).

Renal failure induced in rats was prevented most potently by D-glucarates among various species of monosaccharides tested. 2,5-Di-O-acetyl-D-glucaro-1,4-6,3-dilactone is an orally absorbable drug of the D-glucarate group and is easily deacetylated to form D-glucaro-1,4-lactone, D-glucaro-6,3-lactone and D-glucaro-1,4-6,3-dilactone *in vivo*. The protective effect of D-glucarates was dose-related for renal damage induced by any of the basic antibiotics tested.

It was considered that monosaccharides, which had carbonyl group, alleviated the nephrotoxicity of basic antibiotics by acylating the amino group of them. However, the lactone types of D-glucuronic acid and L-gulonic acid which are apt to react with amino groups to form amides were less effective in reducing renal damage than the corresponding acid types. And the lethal toxicity as opposed to nephrotoxicity of antibiotics was not reduced by D-glucarates. These results may be interpreted to show that alleviation of nephrotoxicity is not based on acylation of the amino group of basic antibiotics. In addition, while a larger dose rate of D-glucarate to basic antibiotics would be expected to result in more acylation, approximately the same degree of protection was observed despite the great differences in the doses of the three antibiotics, kanamycin, neomycin and colistin (Fig. 2).

D-Glucarate protection against antibiotic-induced renal damage was found to be very high with pretreatment of D-glucarates and was gradually lost when administration followed the injection of antibiotics. This suggests that D-glucarates alleviate basic antibiotic kidney injuries by reaching the kidney faster than the antibiotics. According to HIRATA *et al.*,[5] incorporation of antibiotics into the epithelial cells of proximal convoluted tubules resulted in renal failure. We found that D-glucarates inhibit the distribution of an antibiotic in the kidney and resulted in a more rapid excretion of antibiotic into the urine (unpublished observation). A marked decrease of urinary pH was associated with protection against renal damage by D-glucarates and, in the case of hexauronic and hexaaldonic acids, the urinary acidification effect was approximately compatible with the ability to reduce nephrotoxicity of antibiotics. It might be that a mechanism such as that of the non-ionic diffusion theory (*i.e.*, reabsorption of weak electrolytes from renal tubules depends on their degree of ionization) is responsible for some monosaccharides reducing the nephrotoxicity of basic antibiotics. In support of this assumption, it was observed that aminoglycoside antibiotics are bound to the mycelium of the producing organism and released into the fermentation filtrate by adjustment of the whole broth to a low pH.[8] Therefore, it is possible that some monosaccharides, which increase ionization of amino groups of basic antibiotics by lowering the pH in renal tubular fluid, cause a decrease of absorption of antibiotics through the membranes. However, this hypothesis can not provide an explanation of the observation that dehydrated rats receiving sodium citric acid or sodium glutaric acid developed renal failure despite the discharge of acidic urine. It will be necessary to investigate the process by which basic antibiotics produce renal failure to elucidate the mechanism of preventive effect of D-glucarates.

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References

1) WILFERT, J. N.; J. P. BURKE, H. L. BLOOMER & C. B. SMITH: Renal insufficiency associated with gentamicin therapy. J. Infect. Dis. 124: Suppl. S148~S153, 1971
2) FINEGOLD, S. M.: Toxicity of kanamycin in adults. Ann. N.Y. Acad. Sci. 132: 942~956, 1966

3) FLANDRE, O. & M. DAMON: Experimental study of the nephrotoxicity of gentamicin in rats. In Gentamicin: First International Symposium, pp. 47~61, Basal, Schwabe & Co., 1967
4) FALCO, F. G.; H. M. SMITH & G. M. ARCIERI: Nephrotoxicity of aminoglycosides and gentamicin. J. Infect. Dis. 119: 406~409, 1969
5) HIRATA, K.; T. UEDA, K. MORITA, H. HIRATA, C. TAKAMATSU, K. GOTO, H. HIRATA, R. ARAMAKI, M. KAI & T. ISHIKAWA: Studies on pathogenesis of acute renal failure after administration of antibiotics with plasma expander. Jap. J. Nephrol. 15: 371~391, 1973
6) KUNIN, C. M.: Absorption, distribution, excretion and fate of kanamycin. Ann. N.Y. Acad. Sci. 132: 811~818, 1966
7) FINLAND, M.: The symposium on gentamicin. J. Infect. Dis. 119: 537, 1969
8) REIBLEIN, W. J.; P. D. WATKINS & G. H. WAGMAN: Binding of gentamicin and other aminoglycoside antibiotics to mycelium of various actinomycetes. Antimicr. Agents & Chemoth. 4: 602~606, 1973