IN VITRO INTERACTION OF AMINOGLYCOSIDES AND BETA-LACTAM PENICILLINS

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ABSTRACT

The aminoglycoside antibiotics are often used in combination with a β-lactam antibiotic, to provide either a wider spectrum of activity against gram-negative bacilli or a synergistic antimicrobial effect against Pseudomonas aeruginosa and various enterobacteria.

In 1971, MacLaughlin & Reeves found that the combined use of gentamicin and carbenicillin resulted in an interaction and loss of activity of both antibiotics. Since then more studies, in vitro and in vivo, have been performed to study the effect of medium, temperature, concentration, time, pH and different penicillin-aminoglycoside combinations on the interaction.

The purpose of this study was to investigate the kinetics of the interaction in vitro. Four different concentrations of aminoglycosides (A) (5, 10, 15 & 20 µg/mL of gentamicin or tobramycin) and penicillins (P) (100, 200, 400 & 600 µg/mL of carbenicillin or ticarcillin) were incubated in plasma at 37°C for 3 days. Samples taken at 12 h intervals were analyzed for both aminoglycoside and penicillin by radioimmunoassay and high pressure liquid chromatography, respectively.

Degradation of all four antibiotics in controls were first order reactions. The degradation of penicillins was faster than the aminoglycosides, with only 50% of the

original concentration remaining at 24 h. In incubation mixtures, the rate of loss of penicillins was not significantly different from the controls and still appeared as a first order reaction. The interaction did not contribute significantly to the loss of penicillin. However, the rate of loss of aminoglycosides was greater than in controls and appeared as a second order reaction dependent on the concentration of both penicillin and aminoglycoside. The loss of aminoglycoside was due to its degradation in plasma and its interaction with penicillin.

The degradation constants of penicillins (K_P) were calculated as $dP/dt = -K_P P$ and averaged 1.8×10^{-2} h^{-1} for carbenicillin and 2.6×10^{-2} h^{-1} for ticarcillin in controls and averaged 2.2×10^{-2} h^{-1} for carbenicillin and 3.0×10^{-2} h^{-1} for ticarcillin in antibiotic mixtures. In both controls and mixtures, the time required for loss of 50% of initial analyzed concentration (t_{50}) was 30 & 55% larger, for carbenicillin and ticarcillin respectively, at higher penicillin concentrations of 400 & 600 µg/mL compared to lower penicillin concentrations of 100 & 200 µg/mL.

The degradation constants of aminoglycosides (K_A) in controls were calculated as $dA/dt = -K_A A$ and averaged 0.9×10^{-3} h^{-1} for gentamicin and 1.2×10^{-3} h^{-1} for tobramycin. The degradation constants of aminoglycosides in antibiotic mixtures and the interaction rate constants (K_i) were determined by computer fitting of the aminoglycoside

concentrations in incubation mixtures to a model incorporating a second order loss of aminoglycoside and a first order loss of penicillin from the mixtures. The degradation constants of aminoglycosides in antibiotic mixture were less than 1×10^{-8} h^{-1}. The t_{50} values of aminoglycosides in antibiotic mixtures were shorter than in controls (> 25 days) and were related to the concentration of penicillin. The t_{50} values of aminoglycosides were longer than 72 h at a penicillin concentration of 100 µg/mL. As the concentration of penicillin became higher, the t_{50} values became shorter and were less than 10 h for a penicillin concentration of 600 µg/mL.

The interaction rate constants averaged 2.2×10^{-4} mL/µgxh and 1.6×10^{-4} mL/µgxh for both carbenicillin and ticarcillin interactions with gentamicin and tobramycin, respectively. The "effective" interaction rate constants ($K_i \times P$) were larger for the higher penicillin concentrations. Examination of both the t_{50} values of aminoglycosides and the K_i indicated that there was no significant difference between the interaction rate produced by carbenicillin and ticarcillin and gentamicin was inactivated more by carbenicillin and ticarcillin than tobramycin.

The effect of the interaction in vivo was examined by computer simulation using the kinetic parameters determined in vitro. The interaction of penicillin and aminoglycoside would be significant in patients with impaired renal function and might be significant in patients with normal renal function when the concentration of penicillin is very high.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

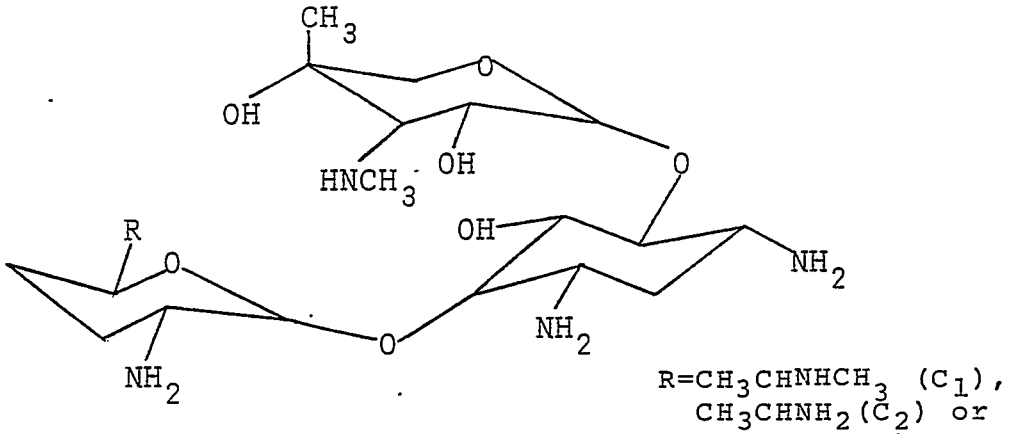
LITERATURE SURVEY

1. Aminoglycosides

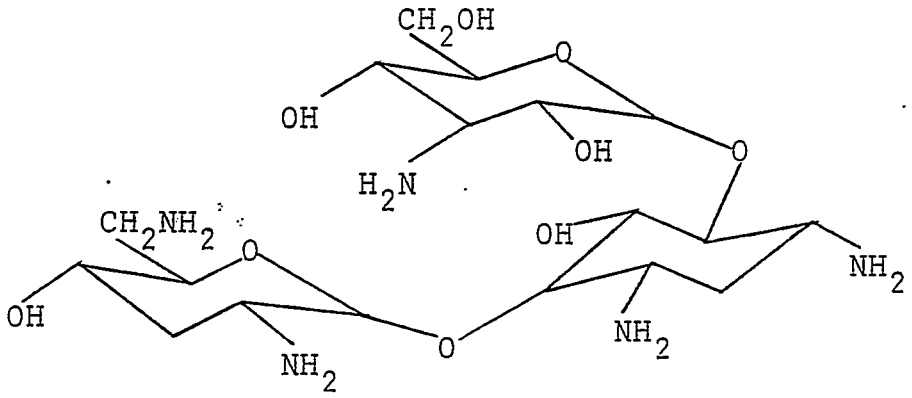
The aminoglycosides are broad-spectrum antibiotics which are produced from species of Streptomyces or Micromonospora. Streptomycin was the first to be discovered (1944), and was followed by neomycin (1949), paromomycin (1956), kanamycin (1957), gentamicin (1958), tobramycin (1967), netilmicin (1970) and amikacin (1972) (Barza, M. & Scheife, R. T., 1977). By convention, the names of aminoglycosides derived from species of Streptomyces are spelled with the letter "y" -- kanamycin, tobramycin -- whereas those from Micromonospora species have an "i" -- gentamicin, netilmicin (Appel, G. B. & Neu, H. C., 1978). The aminoglycosides each contain one or more amino-sugars, such as glucosamine or neosamine linked by glycoside linkages to a basic (amino or guanidino) 6-membered ring (e.g. streptidine or streptamine) (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Scheife, R. T., 1977) (Figure 1). Gentamicin contains three major components -- C_1, C_2 & C_{1a} -- that differ in the degree of methylation of the amino group at the 6' carbon of the amino-hexose (purpurosamine) and two minor components, C_{2a} & C_{2b} (Figure 1) (Kraisintu, K., et al., 1982; White, L. O., et al., 1983).

1.1. Spectrum of Activity

The aminoglycosides are broad-spectrum antibiotics



Gentamicin



Tobramycin

Figure 1: Chair form structures of gentamicin and tobramycin

with activity against gram-positive and gram-negative organisms and variable activity against mycoplasma and mycobacteria. They are especially effective against gram-negative aerobic and facultatively anaerobic bacilli (Barza, M. & Scheife, R. T., 1977; Whelton, A. & Neu, H. C., eds., 1982). The spectra of activity of all aminoglycosides are similar, although streptomycin and kanamycin have no effect against some gram-negative bacteria, especially Pseudomonas aeruginosa (Knoben, J. E. & Anderson, P. O., 1983). Staphylococcus aureus and Staphylococcus epidermidis are generally susceptible to parenterally-administered aminoglycosides (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Scheife, R. T., 1977). Mycoplasma, including Mycoplasma pneumoniae are inhibited by kanamycin, gentamicin and tobramycin (Waitz, J. A., et al., 1972b). Mycobacterium tuberculosis is susceptible in vitro to streptomycin, gentamicin and amikacin (Miller, R. R. & Greenblatt, D. J., eds., 1979). Listeria is readily inhibited by aminoglycosides (Barza, M. & Scheife, R. T., 1977).

The antimicrobial spectra of gentamicin and tobramycin are very similar. However, in all in vitro studies tobramycin has proved to be twice as active as gentamicin against Staphylococcus aureus, four times as active against Pseudomonas aeruginosa, slightly more active against Escherichia coli and Enterobacter species, and slightly less active against Klebsiella pneumoniae and

isolates of Serratia species (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Scheife, R. T., 1977; Levison, M. E., et al., 1972; Whelton, A. & Neu, H. C., eds., 1982). There is considerable cross-resistance between gentamicin and tobramycin for most enterobacteria. Pseudomonas species other than Pseudomonas aeruginosa and Pseudomonas stutzeri are often resistant to both gentamicin and tobramycin (Whelton, A. & Neu, H. C., eds., 1982). Despite the fact that strains highly resistant to gentamicin are usually resistant to tobramycin, tobramycin is still active against about 50% of gentamicin-resistant Pseudomonas aeruginosa (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Scheife, R. T., 1977; Brodgen, R. N., et al., 1976).

The aminoglycosides are not effective against anaerobic bacteria (including Bacteroides fragilis, clostridia and anaerobic cocci), meningococci, most strains of streptococci (including group A β-hemolytic streptococci and viridans streptococci), enterococci and pneumococci (Barza, M. & Scheife, R. T., 1977; Miller, R. R. & Greenblatt, D. J., eds., 1979; Waitz, J. A., et al., 1972b; Whelton, A. & Neu, H. C., eds., 1982).

1.2. Mechanism of Action

While many antibiotics which interfere with protein synthesis are bacteriostatic, the aminoglycoside antibiotics are bactericidal. They need contact with a cell only long enough for the compound to enter the bacterium,

bind to the ribosome and cause cell death as a "single hit phenomenon". The aminoglycosides accumulate within sensitive cells by a complex series of steps, one of which involves an aerobically-generated active transport system. They bind irreversibly to specific sites on the 30s subunit of bacterial ribosomes, blocking the "recognition" step in translation (protein synthesis). A "misreading" of the genetic code of messenger RNA results in defective incorporation of amino acids and the formation of "false" (nonfunctional) proteins. The ribosome separates from messenger RNA, and cell death ensues (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Lauermann, M., 1978).

Anaerobic bacteria are resistant to aminoglycosides because the transport of aminoglycosides into the bacterial cell is oxygen-dependent (DeTerres, O. H., 1981; Miller, R. R. & Greenblatt, D. J., eds., 1979). Another possible reason is the existence of a permeability mutant which does not transport the aminoglycosides across the bacterial cell wall (Appel, G. B. & Neu, H. C., 1978). Some gram-positive organisms such as streptococci are resistant to aminoglycosides because of the thick cell wall of gram-positive bacteria. This can be overcome by the addition of inhibitors of cell wall synthesis such as penicillins (Moellering, R. C., 1977). Most resistance among gram-negative organisms is due to transferable R-factor mediated enzymatic inactivation (Miller, R. R. & Greenblatt, D. J., eds., 1979). The

enzymes produced include acetylating enzymes, phosphorylating enzymes and nucleotidylating enzymes which acetylate, phosphorylate or adenylate aminoglycosides (DeTorres, O. H., 1981; Moellering, R. C., 1977; Whelton, A. & Neu, H. C., eds., 1982).

1.3. Therapeutic Use

The aminoglycoside antibiotics are used primarily to treat infections due to gram-negative aerobic and facultatively anaerobic bacilli. Of the available aminoglycosides, gentamicin, tobramycin and amikacin are the most clinically useful (Barza, M. & Scheife, R. T., 1977; DeTorres, O. H., 1981; Moellering, R. C., 1977; Pien, F. D. & Ho, P. W. L., 1981). The development of resistance has limited the clinical utility of streptomycin and kanamycin and to a lesser extent gentamicin and tobramycin (Whelton, A. & Neu, H. C., eds., 1982). Amikacin has the lowest overall rate of resistance of all aminoglycosides (Pien, F. D. & Ho, P. W. L., 1981; Whelton, A. & Neu, H. C., eds., 1982). Many institutions prefer to reserve amikacin for the treatment of infections with organisms resistant to other aminoglycosides, in order to prevent development of resistance to this drug (Pien, F. D. & Ho, P. W. L., 1981).

Gentamicin and tobramycin are drugs of first choice for infections due to aerobic gram-negative bacilli which are resistant to penicillins or cephalosporins, for peritonitis, non-gonococcal pelvic inflammatory disease

and for most Pseudomonas infections. Gentamicin is also the drug of first choice against most Serratia species. In addition, it is an excellent choice for use in sepsis from a suspected urinary tract source except in areas of the country where the frequency of gentamicin-resistant species is high (Appel, G. B. & Neu, H. C., 1978). Gentamicin is also useful in the therapy of meningitis due to aerobic gram-negative bacilli. However, except in neonates, it has to be given intrathecally or intraventricularly because of the poor penetration of gentamicin into the brain even in the presence of inflammation (Barza, M. & Scheife, R. T., 1977). Although the amino-glycosides are not drugs of choice, because of their toxicity they are often used in the initial stages of treatment after specimens have been collected for culture and before the organisms of infection can be detected and more effective and safer therapy can be selected. They will generally "cover" certain other organisms such as Mycoplasma pneumoniae, Staphylococcus aureus and Staphylococcus epidermidis, Salmonella and Haemophilus influenzae until a specific diagnosis is made (Barza, M. & Scheife, R. T., 1977). They are also used in the treatment of postoperative infection in patients after renal transplant (Noone, P., et al., 1978). Since tobramycin has less accumulation within the renal cortex, many clinicians feel that tobramycin is the aminoglycoside of choice for patients with documented or presumed Pseudomonas aeruginosa infections due to sensitive

organisms. Likewise, many would prefer to use tobramycin if prolonged aminoglycoside therapy is indicated, such as in the treatment of osteomyelitis or bacterial endocarditis (Appel, G. B. & Neu, H. C., 1978).

1.4. Pharmacokinetics of the Aminoglycosides

1.4.1. Absorption

The aminoglycosides are highly polar molecules and are relatively insoluble in lipids. As a result, patients must receive aminoglycosides by either the intramuscular or intravenous route because the drugs are minimally absorbed from the gastrointestinal tract (Appel, G. B. & Neu, H. C., 1978; Evans, W. E., et al., eds., 1980).

Peak serum concentrations are reached at the termination of infusion (usually over 30 to 60 minutes for intermittent intravenous infusion) and about one hour (30 to 90 minutes) after an intramuscular injection (Barza, M. & Scheife, R. T., 1977; Miller, R. R. & Greenblatt, D. J., eds., 1979).

Intermittent intravenous infusion is the preferred administration route for most aminoglycosides (Powell, S. H., et al., 1983). Continuous intravenous infusion has been used to maintain constant suprainhibitory plasma concentrations, primarily in granulocytopenic patients (Bodey, G. P., 1975; Issell, B. F., 1979). In general, it should not be recommended unless it can be shown more effective in the management of life threatening

infections since animal studies show that it is more nephrotoxic than intermittent injections (Reiner, N. E., et al., 1978). Rapid bolus injections should be avoided since they may cause greater toxicity (Mendelson, J., et al., 1976). Intramuscular administration is seldom a problem in ambulatory patients since the intramuscular absorption is quite complete. However, the reliability of intramuscular absorption after repeated injections into the same site might be questioned if peak blood levels are lower than expected. Intramuscular administration, therefore, is never completely reliable in critically ill patients and is best avoided (Evans, W. E., et al., eds., 1980).

1.4.2. Distribution

Following intravenous administration, the aminoglycosides rapidly distribute to highly perfused organs and within extracellular water. The volume of distribution is about 0.20 to 0.30 L/Kg (Barza, M. & Lauermann, M., 1978; Barza, M. & Scheife, R. T., 1977; Gyselynck, A. M., et al., 1971; Whelton, A. & Neu, H. C., eds., 1982). This rapid distributive phase has a half-life of about 5 to 15 minutes and is then followed by a second (α) phase of serum concentration decline. In adults with normal renal function, the half-life of this second (α) phase is approximately 2 hours but increases in proportion to decline in creatinine clearance (Cl_{cr}). Although a one

compartment model is usually chosen to describe the disposition of the aminoglycosides, two processes are occuring during this second phase. As serum levels decline, most of the drug is being excreted unchanged by glomerular filtration, while the remainder is being taken up by body tissues (Evans, W. E., et al., eds., 1980; Mangione, A. & Schentag, J. J., 1980; Schentag, J. J., 1977). A third (β) phase of serum level decline begins at 8 to 24 hours after either a single dose or the final dose in a multiple-dose regimen. In all age groups, the half-life of this phase normally exceeds 100 hours (Kahlmeter, G., et al., 1978; Schentag, J. J., et al., 1977) (Figure 2). During the third (β) phase, intracellularly bound aminoglycoside is released and excreted unchanged in urine (Schentag, J. J. & Jusko, W. J., 1977). Because release of tightly bound intracellular aminoglycoside is slow, and since any circulating drug that is available to the kidney is rapidly excreted, the rate-limiting step in the terminal phase is drug release from tissues (Mangione, A. & Schentag, J. J., 1980).

The aminoglycosides are minimally plasma protein bound, about 0 to 10% for gentamicin and tobramycin (Gorden, R. C., et al., 1972; Myers, D. R., et al., 1978). They penetrate the blood-brain barrier and blood-ocular barriers poorly. The aminoglycoside concentrations achieved in the cerebrospinal fluid and aqueous humor are subtherapeutic (Mangione, A. & Schentag, J. J., 1980). Effective therapy

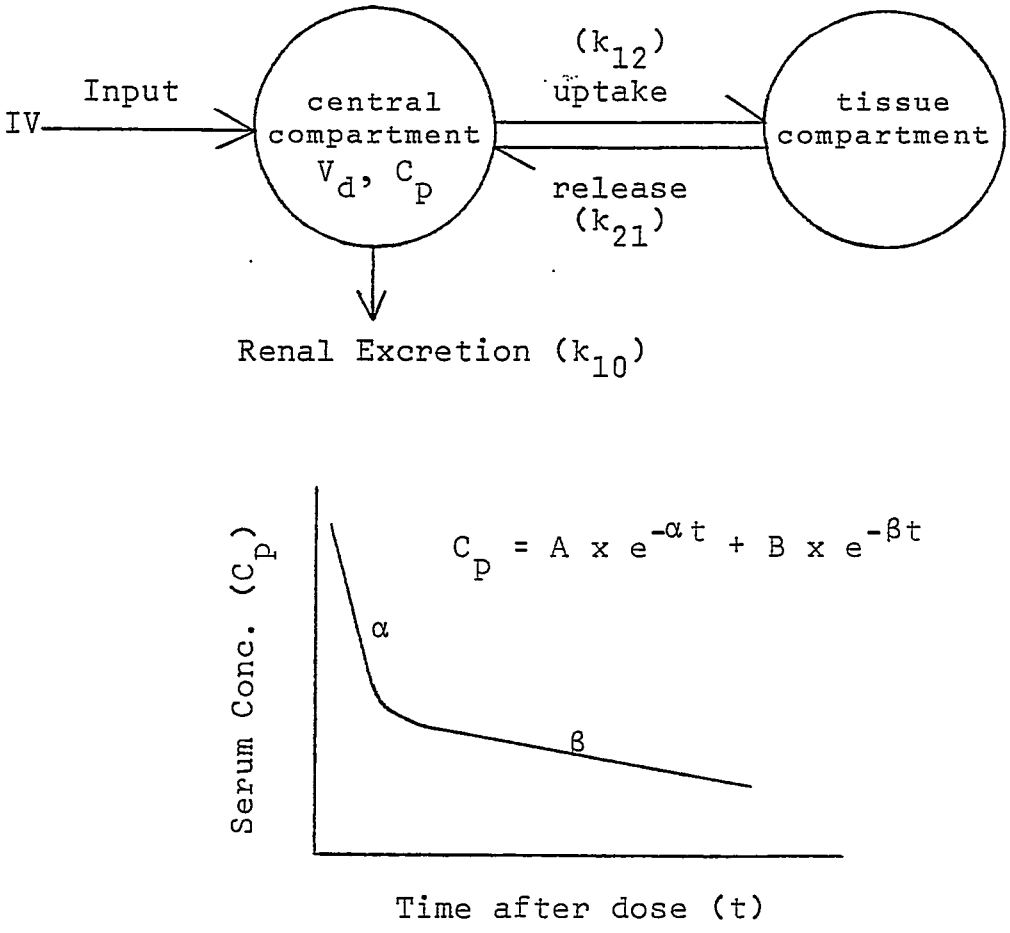


Fig 2: Two-compartment pharmacokinetic model characterizing the second (α) and third (β) phase of the aminoglycoside distribution and elimination (the rapid distributive phase right after IV administration is too short to be shown above) (Mangione, A. & Schentag, J. J., 1980)

of bacterial endophthalmitis requires peri-ocular injections of the aminoglycosides, and intrathecal or intraventricular administration is necessary to achieve adequate cerebrospinal fluid concentrations for the treatment of meningitis, although neurotoxic side effects may occur (Kaiser, A. B. & McGee, Z. A., 1975; Miller, R. R. & Greenblatt, D. J., eds., 1979).

In addition to measuring aminoglycoside levels in serum, the aminoglycosides are also measurable in nonobstructed bile, in synovial fluid, in renal lymph fluid, in sputum and bronchial secretions and in pleural fluid (Chisholm, G. D., et al., 1968; Mangione, A. & Schentag, J. J., 1980). Bronchial inflammation may enhance the penetration of aminoglycosides through the blood-bronchus barrier (Alexander, M. R., et al., 1982; Whelton, A. & Neu, H. C., eds., 1982). However, the aminoglycosides can be partially inactivated by purulent bronchial secretions (Bryant, R. E. & Hammond, D., 1974). Because of the poor penetration of aminoglycosides into the bronchial secretions (~20%), endotracheal administration can be used as an adjunct to intermittent infusion of aminoglycosides for therapy of severe bronchial infections due to gram-negative bacteria (Klastersky, J., et al., 1981; Whelton, A. & Neu, H. C., eds., 1982). The aminoglycosides are detectable in all body tissues. Highly perfused organs such as liver, kidney and lung usually have concentrations above those in serum, while muscle, fat and hard bone usually have concentrations lower

than those in serum (Schentag, J. J., et al., 1977; Schentag, J. J., et al., 1978). The aminoglycosides have a marked affinity for renal cortical tissue, accumulating in concentrations which are 10 to 50 times those in serum. Much lower levels of drug are found in the renal medulla. At similar serum concentrations less tobramycin accumulates within the renal cortex than gentamicin (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Lauermann, M., 1978; Barza, M. & Scheife, R. T., 1977; Schentag, J. J., et al., 1978).

The extracellular water compartment in neonates comprises approximately 40% of body weight, as compared to 20 to 30% in adults. Although there is a rapid loss of excess fluid during the first few weeks after birth, the ratio of extracellular water to body weight may not attain adult values until late infancy or early childhood. As a result, peak aminoglycoside concentrations in neonates, infants and young children are generally lower than peak concentrations obtained when the same dose on a weight basis is administered to adults (Yee, G. C. & Evans, W. E., 1981).

Obesity affects aminoglycoside distribution. Since aminoglycosides do not distribute well into fat tissue, ideal body weight or lean body weight rather than total body weight is used in calculating a weight-related volume of distribution (Hull, J. H. & Sarubbi, F. A., 1976; Sawchuk, R. J., et al., 1977). Recent studies have found that the

aminoglycosides also distribute to some extent into the extracellular fluid contained in adipose tissue and the drug's distribution volume increases with increasing excess weight (the difference between total body weight and ideal body weight). In obese patients, the contribution to the volume of distribution per unit of excess weight is less than the contribution per unit of lean body weight, i.e., adipose tissue contributes less volume per kilogram than does nonadipose tissue (Schwartz, S. N., et al., 1978). The volume of distribution in obese patients is smaller when standardized to total body weight but larger when standardized to ideal body weight. As a result, calculating the weight-related volume of distribution and dosage using total body weight will overestimate the volume of distribution and overdose the patient, while using ideal body weight will underestimate and underdose the patient. It is especially hazardous to markedly obese patients (Bauer, L. A., et al., 1983; Sketris, I., et al., 1981). A dosing weight, which takes into consideration the excess weight, should be used to calculate the volume of distribution in obese patients (patients whose total body weight exceeds ideal body weight by >30%). It is calculated by adding 40% of the excess weight to the ideal body weight (Bauer, L. A., et al., 1983; Korsager, S., 1980; Schwartz, S. N., et al., 1978; Yee, G. C. & Evans, W. E., 1981).

Patients with high fever, liver disease or

ascites may have a larger than normal central volume of distribution which will result in lower serum level (Barza, M. & Lauermann, M., 1978; Gill, M. A. & Kern, J. W., 1979; Pennington, J. E., et al., 1975). Age has no effect on the volume of distribution. Altered urine pH and changes in urine ion concentrations influence both therapeutic effect and nephrotoxicity of aminoglycosides. In rats, acidic urine increases the aminoglycoside renal tissue levels, decreases the effectiveness of the drug on bacteria and increases the risk of nephrotoxicity. Alkaline urine has precisely the opposite effects on renal tissue accumulation, bactericidal effect and nephrotoxicity (Chiu, P. J. S., et al., 1979; Minuth, J. N., et al., 1976). An increased urine sodium concentration will both decrease the tissue accumulation and decrease the severity of nephrotoxicity in rats (Bennett, W. M., et al., 1976). The protective effect of the sodium ion comes from the competition for binding sites between aminoglycosides and positively charged sodium ions on the renal tubular membrane (Evans, W. E., et al., eds., 1980).

Finally, the effects of renal disease on aminoglycoside distribution and tissue accumulation are minor. In renal impairment, the central compartment volume is about 10% larger and the rate of tissue uptake is lower (Schentag, J. J., et al., 1977). The aminoglycoside renal tissue concentration may reduce dramatically because severe chronic renal disease eliminates 40% of the aminoglycoside

binding sites. At identical serum levels, individuals with renal failure have a lower aminoglycoside steady state volume of distribution, lower total body load and a greater percentage of total body aminoglycoside in skeletal muscle and fat (Evans, W. E., et al., eds., 1980; Schentag, J. J., et al., 1977; Schentag, J. J., et al., 1978). In patients undergoing dialysis, the volume of distribution tends to be lower after dialysis and increases steadily until the next dialysis procedure (Matzke, G. R., et al., 1983).

1.4.3. Elimination

The aminoglycosides are not metabolized but are eliminated from the kidney predominantly by renal glomerular filtration. There is also some tubular reabsorption but no evidence for renal secretion. Probenecid does not alter the renal clearance of aminoglycosides (Barza, M. & Lauermann, M., 1978; Barza, M. & Scheife, R. T., 1977; Bergan, T., et al., 1973; Gyselynck, A. M., et al., 1971).

After a single dose, 40 to 65% of gentamicin or 65 to 80% of tobramycin is recovered in the urine during the first twenty-four hours, and ultimately almost 80 to 90% of the drug is excreted. Complete recovery of the dose can be achieved only if urine is collected for periods as long as 20 to 30 days. The accumulation of drug in the kidney accounts for the delayed recovery of the total dose in the urine (Schentag, J. J. & Jusko, W. J., 1977). Renal clearance

is 73 to 85 mL/min for gentamicin and 80 to 90 mL/min for tobramycin in normal individuals (Barza, M. & Scheife, R. T., 1977). The half-lives are about 2 to 3 hours (1.7 to 2.3 hours for gentamicin and 2 to 2.7 hours for tobramycin) in patients with normal renal function (creatinine clearance = 100 mL/min x 1.73 m^2) but may be 40 to 120 hours in patients with chronic renal failure (Wilson, T. W., et al., 1973). Changes in renal function influence the elimination rate constant and half-life of aminoglycosides. There is a direct correlation between the prolongation of the serum half-life and the decline in creatinine clearance (Appel, G. B. & Neu, H. C., 1978; Pechere, J. C. & Dugal, R., 1979).

A recent study suggests that there is considerable interpatient variation in the elimination and clearance of gentamicin, tobramycin and amikacin even in patients with normal serum creatinine or creatinine clearance. However, this interpatient variation seems greater in patients being treated for gram-negative sepsis than in normal volunteers and it may also be greater in the initial phases of treatment rather than later in the treatment course when patients have stabilized (Evans, W. E., et al., 1980).

The relationship between the half-life and distribution volume is significant with aminoglycosides. As the distribution volume of the drug increases, the elimination rate constant decreases and the half-life of the drug

increases (Evans, W. E., et al., eds., 1980).

Certain physiological conditions and disease states associated with volume expansion and/or increased urine flow may lead to increased aminoglycoside clearance and alter the association between body clearance and the glomerular filtration rate. One must be careful in predicting body clearance from the glomerular filtration rate, especially when an increased volume of distribution is present. Actual renal clearance of drug should be determined by measuring urinary drug excretion. Aminoglycoside dosing nomograms which correlate elimination half-life or decreasing body clearance with decreasing creatinine clearance must be interpreted differently when the volume of distribution is known to have increased (Riviere, J. E., 1982).

There is a significant correlation of the half-life of gentamicin and the reciprocal of hematocrit. However, the degree of the association is low since it only explains 1.4% of variance in half-life. There is no apparent relation between the half-life and the level of serum albumin or between the hematocrit and the apparent volume of distribution (Barza, M., et al., 1975; Evans, W. E., et al., eds., 1980).

The elimination half-life is longer in neonates than in adults, and is related to post-natal age, gestational age and birthweight. This is probably caused by

the limited capacity of the maturing kidney, during the first four weeks of life, to eliminate renally excreted drugs like the aminoglycosides. Birthweight also affects the elimination half-life. The glomerular filtration process matures within the first six months of life. However, the renal elimination of aminoglycosides in older infants and young children seems to still be partially dependent on age (Yee, G. C. & Evans, W. E., 1981).

The elimination rate constants of the aminoglycosides continually decrease with increasing age. The average half-life in older patients is approximately twice as long as the average half-life in patients less than 30 years of age and the clearance is markedly decreased with increasing age. Cardiac output, renal blood flow and glomerular filtration decrease with increasing age and drugs which are primarily eliminated by glomerular filtration are affected by these physiological changes (Evans, W. E., et al., eds., 1980; Miller, R. R. & Greenblatt, D. J., eds., 1979).

Obesity has no effect on the elimination rate and half-life but increases the clearance of aminoglycosides (Bauer, L. A., et al., 1983; Schwartz, S. N., et al., 1978).

Fever seems to affect both the serum concentrations and elimination of aminoglycosides. Physiologically, fever changes the elimination rate of the

drug by increasing heart rate and cardiac output and thereby increasing renal blood flow and glomerular filtration. Thus, patients with high fevers may have higher elimination rates for the aminoglycosides (Pennington, J. E., et al., 1975).

The half-life of aminoglycosides is shortened in patients with cystic fibrosis, burns and leukemia (especially substantial in children and young adults (Siber, G., et al., 1975) and in obstetric patients during pregnancy. In the latter phases of pregnancy, the extracellular fluid compartment, total body water, cardiac output, renal blood flow and glomerular filtration are all increased. A two to five day period postpartum is required before a new equilibrium is re-established (Evans, W. E., et al., eds., 1980). In burn patients, hemodynamic changes secondary to the burn cause the patients to have an extremely rapid rate of elimination and shorter than normal half-life. However, an occasional burn patient who develops gram-negative sepsis early in the course of burn resuscitation may have a prolonged drug half-life because of the extremely high extracellular fluid compartment immediately post-injury (Loirat, P., et al., 1978).

Hemodialysis is highly effective in the removal of circulating aminoglycosides in patients with chronic renal failure, and 4 to 6 hours of hemodialysis may remove as much as half of the drug present in the blood (Danish, M., et al., 1976). The dialysis clearance is

26 to 48 mL/min for gentamicin and 50 to 60 mL/min for tobramycin (Barza, M. & Scheife, R. T., 1977). Hemodialysis, however, is less effective in the treatment of aminoglycoside nephrotoxicity because in such patients, most of the drug is bound to tissues and is inaccessible during hemodialysis. A slow redistribution from tissues to blood occurs after stopping dialysis, and may return serum concentrations almost to predialysis levels (Bauer, L. A., 1982; Christopher, T. G., et al., 1974). Peritoneal dialysis is generally less effective than hemodialysis, removing only about 25% of a dose in 48 to 72 hours (Barza, M. & Scheife, R. T., 1977; Gary, N. E., 1971). The peritoneal clearance is 5 to 10 mL/min for gentamicin and 15 mL/min for tobramycin (Barza, M. & Scheife, R. T., 1977). Hamann and his co-workers reported that there was a marked decrease in the elimination half-life and an increase in the total body clearance of gentamicin during peritoneal dialysis (Hamann, S. R., et al., 1982).

1.4.4. Dosing Regimens

The usual dosage of gentamicin or tobramycin for patients with normal renal function is 1.7 mg/Kg every eight hours by intramuscular injection or by slow intermittent intravenous infusion over 30 to 60 minutes. For patients with impaired renal function, there are various means of estimating an appropriate dosing schedule; i.e., with decreased dose and/or increased dosing interval. Two such methods which

alter dosing interval recommend a 2 mg/Kg loading dose of gentamicin or tobramycin followed by maintenance dose of 0.8 to 1.0 mg/Kg at a dosage interval equal to three to four times the serum creatinine concentration in mg/dl (half dose every half-life), or 1.5 to 2.0 mg/Kg doses at a dosage interval equal to eight times the serum creatinine concentration in mg/dl (full dose every two to three half-lives). The former method; i.e., a half dose every half-life, is preferable to the latter; i.e., a full dose every two to three half-lives, since the shorter dosing intervals give effective concentrations (>4 µg/mL) for a greater percent of the time (Barza, M. & Scheife, R. T., 1977).

Appropriate dosing regimens for gentamicin and/or tobramycin, which alter both dose and dosage interval according to the patient's decline in renal function, can be estimated from Hull and Sarubbi's dosing chart (Hull, J. H. & Sarubbi, R. A., 1976). Chan's nomogram alters the dose according to the patient's endogenous creatinine clearance (Chan, R. A., et al., 1972) and Ritschel's nomogram determines both the loading dose and maintenance dose according to the patient's body weight and serum creatinine to maintain the minimum steady state concentration at a desired "minimum inhibitory concentration" (MIC) with peak concentrations not exceeding 10 µg/mL (Ritschel, W. A., et al., 1980a; Ritschel, W. A., et al., 1980b).

Dosage regimens, to obtain a desired target

serum level, may also be chosen based on initial estimate of individual pharmacokinetic parameters. The estimates are obtained from the patient's body weight and renal function. It is more accurate than those methods previously discussed and appears to be the optimum approach to estimate initial dosage regimens for gentamicin and tobramycin (Cipolle, R. J., et al., 1980; Matzke, G. R., et al., 1983; Sawchuk, R. J., et al., 1977; Vakoutis, J., et al., 1981).

a) To determine an individual patient's pharmacokinetic parameters:

IBW_{male} = 50 Kg + 2.3 Kg /inch over 5 ft (Devine, B., 1974)

IBW_{female} = 45.4 Kg + 2.3 Kg /inch over 5 ft

DW = (TBW - IBW) x 0.4 + IBW

$Cl_{cr\ male}$ = TBW or DW x (140 - Age) / 72 x serum creatinine

$Cl_{cr\ female}$ = 0.85 x $Cl_{cr\ male}$

$K_{d\ gentamicin}$ = 0.015 + 0.00285 x Cl_{cr}

$K_{d\ tobramycin}$ = 0.010 + 0.0031 x Cl_{cr}

V_d = 0.2 x TBW or DW

b) To establish a dosing regimen for the patient:

IV infusion:

$$\text{Loading dose} = C_{p\ maxD} \times V_d \times K_d / (1 - e^{-K_d T})$$

Maintenance dose (R_o)

$$= C_{p\ maxD} \times V_d \times K_d \times (1 - e^{-K_d t}) / (1 - e^{-K_d T}) \quad \text{..Equation 1}$$

$$t = 1/K_d \times \ln (C_{p\ minD} / C_{p\ maxD}) + T \quad \text{...Equation 2}$$

c) To predict the serum levels of patients which will be achieved:

$$C'_{max} = R_o \times (1 - e^{-K_d T}) / K_d \times V_d$$

$$C'_{min} = R_o \times (1 - e^{-K_d T}) \times (e^{-K_d (t-T)}) / K_d \times V_d$$

$$= C'_{max} \times e^{-K_d (t-T)}$$

$$C^n_{max} = C'_{max} / (1 - e^{-K_d t})$$

$$C^n_{min} = C'_{min} / (1 - e^{-K_d t}) = C^n_{max} \times e^{-K_d (t-T)}$$

where
- TBW = Total body weight (Kg)
- IBW = Ideal body weight (Kg)
- DW = Dosing weight (Kg)
- K_d = Elimination rate constant (h^{-1})
- V_d = Volume of distribution (L/Kg)
- Cl_{cr} = Creatinine clearance (mL/min)
- $C_{p\ maxD}$ = Desired peak serum level (µg/mL)
- $C_{p\ minD}$ = Desired trough serum level (µg/mL)
- R_o = Rate of infusion (mg/h)
- T = Infusion duration (h)
- t = Dosing interval (h)
- C'_{max} = Peak serum level (µg/mL)
- C'_{min} = Trough serum level (µg/mL)
- C^n_{max} = Steady state peak serum level (µg/mL)
- C^n_{min} = Steady state trough serum level (µg/mL)

Note: 1) DW should be used instead of TBW if the patient is grossly obese (TBW exceeds IBW by >30%).

2) V_d ranges from 0.15 to 0.30 L/Kg, depending on the hydration state of the patient. It is 0.20 L/Kg for normal hydrated patients, 0.15 L/Kg for dehydrated patients (patients who have a high temperature or experience considerable vomiting or diarrhea and/or have been diuresed) and 0.30 L/Kg for edematous, overhydrated patients (patients who have evidence of pulmonary edema, uncontrolled congestive heart failure or peripheral edema).

3) $C_{p\ maxD}$ will vary depending on the site of infection, the severity of the infection and the organism involved. In general, serum levels of 4 - 8 µg/mL are necessary for most infections and serum levels of >8 µg/mL are required for Pseudomonas infections.

4) Once a reasonable t is approximated, it should be rounded to one of the following acceptable intervals: 4, 6, 8, 12, 24, 36 or 48 hours.

Since body surface area correlates more closely than body weight with extracellular fluid volume, it can also be used to calculate the aminoglycoside dosage. Lietman found that gentamicin dosage based on body surface area produced uniform peak serum gentamicin concentrations in both children and adults (Lietman, P. S., 1979). Evans

and his co-workers, however, suggest that there is no significant difference in variability in serum concentrations calculated from body surface area or body weight (Evans, W. E., et al., 1979).

The dosage regimens appropriate for patients undergoing dialysis procedures are difficult to predict. Aminoglycosides are removed by hemodialysis. Therefore, it may be necessary to administer a supplemental dose after each hemodialysis procedure. Approximately 1/2 and 3/4 of the loading dose should be administered to anuric patients and patients with some renal function, respectively, after a 4 to 6 hour hemodialysis (Barza, M. & Scheife, R. T., 1977; Danish, M., et al., 1974). Because the extent of dialyzability may be profoundly affected by the type of dialyzer coil used and the flow rate through it, the supplemental dose should be further modified according to the results of assays of serum concentrations of the drug (Christopher, T. G., et al., 1974; Danish, M., et al., 1976; Jaffe, G., et al., 1974; Hewitt, W. L., 1973).

1.4.5. Clinical Monitoring of Serum Aminoglycoside Concentrations

The aminoglycosides have a very low therapeutic index; i.e., the therapeutic level and toxic level are close. The recommended range for gentamicin or tobramycin serum levels is 4 to 8 µg/mL for the peak level and 1 to 2 µg/mL for

the trough level. Peak levels of 10 to 12 µg/mL and trough levels of 2 to 4 µg/mL have been claimed to be associated with a greater risk of toxicity (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Scheife, R. T., 1977; Hewitt, W. L., 1973). Peak concentrations of 5 to 8 µg/mL are necessary for maximum therapeutic efficacy in most types of severe gram-negative bacillary infections, and slightly higher levels (8 to 10 µg/mL) may be advisable for the treatment of pneumonias due to these organisms (Jackson, G. G. & Riff, L. J., 1971; Noone, P., et al., 1974). There is no evidence that concentrations above 10 µg/mL are more effective than lower levels (Barza, M. & Lauermann, M., 1978; Darrell, J. H. & Waterworth, P. M., 1967).

Since aminoglycosides have a relatively low therapeutic index, measurement of serum levels of aminoglycosides has become essential to avoid the risks of toxicity, yet maintain adequate serum concentrations for antibacterial activity (Edwards, D. J., 1981; Riff, L. J. & Thomason, J. L., 1982). Serum for measurement of peak levels may be drawn immediately after (Appel, G. B. & Neu, H. C., 1978; Mangione, A. & Schentag, J. J., 1980) or half an hour after the end (Matzke, G. R., et al., 1983) of a slow intravenous infusion or within 30 to 60 minutes of an intramuscular dose. Trough levels should be checked just before the administration of a subsequent dose (Appel, G. B. & Neu, H. C., 1978; Mangione, A. & Schentag, J. J., 1980;

Matzke, G. R., et al., 1983).

These serum levels (peak and trough) can be used to calculate the patient's elimination rate constant and volume of distribution. The elimination rate constant is obtained from the slope of the logarithmic concentration-time curve and the volume of distribution from the following formula:

$$V_d = R_o \times (1 - e^{-K_d T}) / K_d \times (C^n_{max} - C^n_{min} \times e^{-K_d T})$$

These calculated parameters are then used to adjust the dosage regimen using Equation 1 & 2 (p.23).

Usually only peak and trough serum levels (before and after the dose) are measured and used in adjusting dosage regimen. Hamilton and Evans suggested that there is less error in estimating pharmacokinetic parameters and adjusting gentamicin dosage in children and adolescents when three serial serum concentrations (0.5, 3 and 6 hours after a dose) (Hamilton, S. F. & Evans, W. E., 1981) are used.

Serum levels should be monitored in patients with high fever, liver disease or ascites -- conditions associated with expanded extracellular fluid volume and lower peak serum aminoglycoside levels (Sketris, I., et al., 1981); in pediatric patients with cystic fibrosis, burns and leukemia, in obstetric patients during pregnancy and in patients with high fever -- conditions associated with a rapid elimination rate and shortened half-life of

aminoglycoside; in neonates, infants and children in whom serum aminoglycoside levels may be low because of a larger volume of distribution; in elderly patients in whom the serum drug levels may be unexpectedly high because of decreased cardiac output, renal blood flow and glomerular filtration and in patients to whom a beta-lactam penicillin is given at the same time (Barza, M. & Lauermann, M., 1978; Evans, W. E., et al., 1980).

Obesity is also associated with expanded extracellular fluid volume. Although excess weight is considered in predicting the volume of distribution in obese patients, measuring serum concentrations to adjust dosage regimens is necessary to ensure therapeutic serum levels of aminoglycosides because of the substantial interpatient variation in the relationship between the volume of distribution and excess weight (Sketris, I., et al., 1981; Yee, G. C. & Evans, W. E., 1981).

Serum aminoglycoside levels should also be monitored in patients undergoing hemodialysis to obtain a suitable supplemental dose. Since a redistribution of aminoglycoside from tissue to blood occurs after the stopping of hemodialysis, a short period of time should be allowed before postdialysis aminoglycoside levels are obtained, especially in patients who have received multiple doses and as a result have substantial tissue accumulation. Failing to do this may result in the administration of excessive

replacement doses (Bauer, L. A., 1982). It is also important to monitor the serum level frequently in renal failure patients undergoing peritoneal dialysis because of the interpatient variation in the effect of peritoneal dialysis on the elimination of aminoglycoside (Hamann, S. R., et al., 1982).

Assays should be performed every few days in patients who develop symptoms and/or signs of ototoxicity and/or nephrotoxicity to ensure that the patient is neither being underdosed nor overdosed. In patients in whom the serum creatinine concentration is changing from day to day, dosage regimens based on a single creatinine concentration may be misleading and direct measurements of the serum level of antibiotic are necessary (Mangione, A. & Schentag, J. J., 1980). If the patient's creatinine clearance is <10 mL/min (or dosing interval >48 hours), serum samples should be obtained 2 hours after infusion, to ensure that the distributive phase is completed, and just prior to the next dose (Matzke, G. R., et al., 1983).

In those patients whose pathophysiological states are more stable, the clinician can expect a relatively constant serum level curve from day to day so that occasional measurements of the peak and trough concentrations are sufficient (Barza, M. & Lauermann, M., 1978; Barza, M. & Scheife, R. T., 1977; Mangione, A. & Schentag, J. J., 1980).

1.5. Adverse Effects of Aminoglycosides

Patients receiving aminoglycosides may have allergic reactions, such as eosinophilia, rash and fever. More serious reactions such as anaphylaxis, agranulocytosis and other blood dyscrasias are extremely rare. The most important adverse effects are nephrotoxicity and ototoxicity (Barza, M. & Scheife, R. T., 1977).

1.5.1. Nephrotoxicity

The aminoglycoside antibiotics are potentially nephrotoxic substances which may cause specific damage to the lining cells of renal proximal tubules (Dahlgren, J. G., et al., 1975; Schentag, J. J., et al., 1981). About 2% of patients experience severe nephrotoxicity from gentamicin and about 5 to 10% suffer milder damage (Appel, G. B. & Neu, H. C., 1978). The degree of nephrotoxicity varies with the aminoglycoside and may result from differences in renal accumulation. For example, gentamicin has a higher renal accumulation than tobramycin and is possibly more nephrotoxic than tobramycin (Feig, P. U., et al., 1982; Schentag, J. J., et al., 1978; Schentag, J. J., et al., 1981; Smith, C. R., et al., 1980).

Aminoglycoside nephrotoxicity is manifested functionally by decreased urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, mild glucosuria, decreased ammonium excretion, and depression of glomerular

filtration rate. Histopathologic lesions are confined primarily to the proximal tubule and consist of an increase in the number and size of secondary lysosomes and cytosegresomes containing myeloid bodies, disruption of brush border membranes, mitochondrial swelling, and frank tubular cell necrosis. Pathogenesis of aminoglycoside toxicity involves the accumulation of these drugs within renal proximal tubular epithelium and their propensity to interact with one or more intracellular metabolic pathways such as phospholipid metabolism which results in lysosomal dysfunction (Kaloyanides, G. J. & Pastoriza, E., 1980). Both renal morphologic and functional damage are generally reversible if the renal damage is discovered early and the drug is promptly withdrawn. Return of renal function tests to base-line status is characteristically slow and may take many weeks to months (Appel, G. B. & Neu, H. C., 1978).

Among the contributing factors of nephrotoxicity are hypotension, dehydration, concomitant administration of furosemide, methoxyflurane or cephalothin, previous renal damage and contracted intravascular fluid volume. Elderly debilitated patients also have a high risk of nephrotoxicity (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Lauermann, M., 1978; Prince, R. A., et al., 1980).

Broadly speaking, aminoglycoside nephrotoxicity is dose-related phenomenon. However, there is considerable uncertainty concerning the relative importance of the total

dose, duration of therapy and serum levels in the development of nephrotoxicity. Some studies ascribe toxicity primarily to the presence of excessively high peak levels in the serum, while others to the absence of a sufficiently low trough level. Since trough levels represent distribution equilibrium between blood and tissue, they are more useful than peak levels for prediction of tissue accumulation and for assessment of the risk of nephrotoxicity (Dahlgren, J. G., et al., 1975; Mangione, A. & Schentag, J. J., 1980). The occurence of high trough levels before the onset of nephrotoxicity may indicate accumulation of the drug (Appel, G. B. & Neu, H. C., 1978) and can be interpreted as an early sign of renal damage rather than the cause of it (Barza, M. & Lauermann, M., 1978; Mangione, A. & Schentag, J. J., 1980).

1.5.2. Otoxicity

The aminoglycosides cause ototoxicity and in man, as in animal models, damage to the vestibular system predominates over cochlear impairment (Appel, G. B. & Neu, H. C., 1978). About 10% of patients treated with gentamicin experience damage to the eighth cranial nerve often leading to some degree of permanent injury (Barza, M. & Scheife, R. T., 1977). Tobramycin has significantly less vestibulotoxicity than gentamicin (Fee, W. E., 1980).

Although the aminoglycosides do not have a particular affinity for the vestibular or cochlear apparatus,

they diffuse much more readily into the perilymph of the inner ear than into cerebrospinal fluid or the vitreous humor of the eye. In addition, their half-life in perilymph is much longer than in serum or ocular humors. This has been attributed to an electrochemical interaction between the positively charged aminoglycoside molecules and the negatively charged acid mucopolysaccharides in the inner ear (Barza, M. & Lauermann, M., 1978; Federspil, P., et al., 1976).

Symptoms of vestibular toxicity include vertigo, ataxia and nystagmus. These symptoms may progress to the point that the patient cannot walk unaided (Dayal, V. S., et al., 1974). Auditory damage is usually first manifested by high tone hearing loss and tinnitus but may progress to total deafness. Symptoms may not be apparent until several weeks after therapy has been discontinued. Damage to both the vestibular and auditory apparatus is usually bilateral. With early cessation of therapy, damage may be reversible; but with continued administration of the drug, damage is often permanent (Appel, G. B. & Neu, H. C., 1978).

The most prominent risk factor of ototoxicity appears to be renal impairment. Such patients suffer a greater frequency of eighth nerve damage than those with normal renal function (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Lauermann, M., 1978; Dayal, V. S., et al., 1974).

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Other risk factors of ototoxicity include coadministration of furosemide, ethacrynic acid, mannitol, and possibly other diuretics (Appel, G. B. & Neu, H. C., 1978; Mathog, R. M. & Klein, W. J., 1969). Ototoxicity is probably also increased by concomitant administration of other ototoxic antimicrobials, and perhaps by prior treatment with such agents (Barza, M. & Scheife, R. T., 1977). Advanced age, prolonged therapy and high daily dose are other factors associated with ototoxicity (Neu, H. C. & Bendush, C. L., 1976; Jackson, G. G. & Arcieri, G., 1971).

As is true of nephrotoxicity, some suggest that a high peak level might be a crucial determinant of ototoxicity, while others suggest that high trough levels of aminoglycosides predispose to eighth-nerve damage (Barza, M. & Lauermann, M., 1978; Barza, M. & Scheife, R. T., 1977; Powell, S. H., et al., 1983). Another study showed that it may be the "area under the serum level time curve" (AUC)

rather than the peak or trough level, which predisposes to adverse effects, particularly in patients with major renal impairment who are prone to have a larger AUC (Barza, M. & Lauermann, M., 1978).

Fee in his study found that all the risk factors mentioned above were not statistically associated with toxicity. Only fever, initial hematocrit and critical illness were significantly associated with toxicity. He also suggested that the incidence of ototoxicity is decreased if the total dose of the aminoglycoside can be limited to less than 2.0 g and duration of therapy to less than 10 days (Fee, W. E., 1980).

1.5.3. Neuromuscular Blockade

The neuromuscular blockade induced by the aminoglycosides appears similar to that produced by conventional blocking agents, such as d-tubocurarine or pancuronium, and may result in weakness of skeletal muscles and respiratory depression. Patients with myasthenia gravis or severe hypocalcemia and individuals who have recently received other neuromuscular blocking agents appear to be particularly sensitive to this adverse effect (Warner, W. A. & Sanders, E., 1971).

The relative potency of aminoglycosides to block neuromuscular transmission in decreasing order, is as follows: neomycin > streptomycin > kanamycin and amikacin >

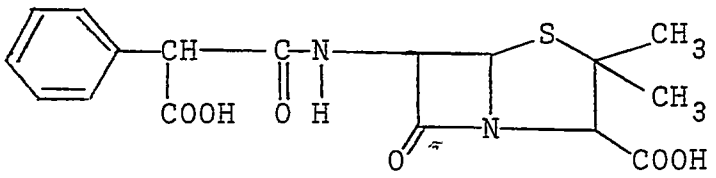
gentamicin and tobramycin (Kubikowski, P. & Szreniawski, Z., 1963).

Blockade induced by aminoglycosides can be partially or completely reversed by the administration of calcium salts intravenously. The efficacy of cholinomimetic agents (edrophonium, neostigmine) is highly variable (Barza, M. & Scheife, R. T., 1977; Kubikowski, P. & Szreniawski, Z., 1963; Pittinger, C. & Adamson, R., 1972).

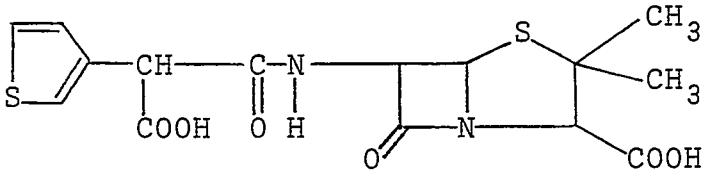
2. Penicillins

Penicillins are bactericidal antibiotics. They block cell wall synthesis by competitively inhibiting the activity of transpeptidase which crosslinks the peptidoglycan molecule of the cell wall into a stable monomer. Their activity, therefore, requires that the bacteria be growing (Miller, R. R. & Greenblatt, D. J., eds., 1979).

Carbenicillin and ticarcillin (Figure 3) are "broad-spectrum" semi-synthetic penicillins. They differ from the "natural" penicillins in that their spectra of activity expand from gram-positive bacteria, gram-negative cocci (meningococcus and gonococcus) and anaerobes (clostridia and anaerobic cocci) to include gram-negative bacilli such as Haemophilus influenzae and Escherichia coli but not Klebsiella and Serratia species. They also cover Pseudomonas species such as Pseudomonas aeruginosa and all enterobacteria which are resistant to other "broad-spectrum"



Carbenicillin



Ticarcillin

Figure 3: Structures of carbenicillin and ticarcillin

penicillins (ampicillin and amoxicillin) (Ervin, F. R. & Bullock, W. E., 1976; Marcy, S. M. & Kein, J. O., 1970). The spectra of carbenicillin and ticarcillin are similar but ticarcillin is 2 to 4 times more active against Pseudomonas aeruginosa while slightly less active against gram-positive organisms (Ervin, F. R. & Bullock, W. E., 1976; Klastersky, J., et al., 1974). Both of them can be destroyed by penicillinase and are therefore resistant to penicillin-resistant Staphylococcus aureus (Miller, R. R. & Greenblatt, D. J., eds., 1979).

The pharmacokinetics of carbenicillin and ticarcillin are similar. Both are acid-labile and are minimally absorbed by mouth (Price, K. E., 1969). Therefore, they are given intramuscularly or intravenously. The penicillins are well distributed into interstitial fluid, serosal cavities, synovial fluid and bone, and the placenta (Barza, M. & Weinstein, L., 1976). Because they are relatively insoluble in lipids, they penetrate the blood-brain and blood-ocular barriers poorly (Barza, M. & Weinstein, L., 1976). Inflammation improves their penetration into the central nervous system and eye. Protein binding is about 50% for carbenicillin and 55 to 65% for ticarcillin (Bergan, T., 1978; Libke, R. D., et al., 1975; Brogden, R. N., et al., 1980). The volume of distribution is 0.15 to 0.20 L/Kg for carbenicillin and 0.20 to 0.25 L/Kg for ticarcillin. Penicillins are primarily cleared by the kidney by glomerular filtration and tubular secretion.

Only a small amount is metabolized (Barza, M. & Weinstein, L., 1976). For carbenicillin, virtually 100% of a dose is excreted unchanged in the urine within 24 hours, producing extremely high urine concentrations after usual doses in patients with normal renal function (Libke, R. D., 1975). For ticarcillin, a total of 90 to 95% of a dose is recovered in the urine within 24 hours, with 10 to 14% as penicilloic acid and the remainder as unchanged drug (Brogden, R. N., et al., 1980). Because most penicillins are rapidly secreted into the urine, their half-lives in the serum are exceedingly short. The half-lives of carbenicillin and ticarcillin are 1 hour and 1.2 hours, respectively (Libke, R. D., 1975; Brogden, R. N., et al., 1980). Renal disease prolongs their half-lives substantially. For carbenicillin, the half-life increases to 10 to 15 hours in patients with impaired renal function and 20 hours or longer in patients with simultaneous renal and hepatic failure (Barza, M. & Weinstein, L., 1976). For ticarcillin, the half-life increases to 15 to 16 hours in patients with severely impaired renal function and about 30 hours in patients with simultaneous hepatic and renal failure (Brogden, R. N., 1980).

The dose of carbenicillin or ticarcillin depends on the site, type and severity of infection. The dose of carbenicillin administered intravenously for serious systemic infection due to Pseudomonas aeruginosa or anaerobic organisms is

400 to 500 mg/Kg/day (30 to 40 g/day) in divided doses or by continuous infusion; for serious urinary tract infection due to Escherichia coli, Proteus species, Enterobacter species, Enterococci or Pseudomonas aeruginosa, 200 mg/Kg/day (10 g/day) by continuous infusion. The intravenous dose of ticarcillin for serious systemic infections is 200 to 300 mg/Kg/day (about 16 to 24 g/day) in 3 to 8 divided doses; for complicated urinary tract infection, 150 to 200 mg/Kg/day (12 to 16 g/day) in 4 to 6 divided doses (Knoben, J. E. & Anderson, P. O., 1983).

Serum levels after intravenous dosage depends on time of infusion and dose size. For both carbenicillin and ticarcillin, 2 g administered by intravenous infusion over 30 minutes produces a plasma level of about 175 µg/mL at the end of the infusion (Bergen, T., 1978; Libke, R. D., 1975). Peak serum levels after intramuscular dosage are different. For carbenicillin, intramuscular injection of 1 g produces a peak plasma level of 15 to 20 µg/mL at 1 hour after administration (Knirsch, A. K., et al., 1973), and for ticarcillin, 22 to 33 µg/mL at 0.5 to 2.0 hours after administration (Brogden, R. N., et al., 1980).

3. Use of Aminoglycosides in Combination with Penicillins

Aminoglycosides may be used in combination with other antibiotics such as cephalosporins and/or penicillins. Such combinations have the advantage of a broader spectrum of

activity and often synergistic antimicrobial effects (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Scheife, R. T., 1977) and are, therefore, strongly recommended for the treatment of serious Pseudomonas infections, especially in immunosuppressed patients (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Scheife, R. T., 1977; Edwards, D. J., 1981; Pickering, L. K. & Rutherford, I., 1981). The mechanism of synergy proposed is that of penicillin-induced damage to the cell wall permitting the entry of the aminoglycoside (Appel, G. B. & Neu, H. C., 1978).

The combination of an aminoglycoside and an anti-pseudomonas penicillin such as carbenicillin or ticarcillin broaden the spectrum of activity of the regimen to include Klebsiella pneumoniae and other gram-negative bacilli. As well, the combination provides a synergistic effect in vitro against clinical isolates of Pseudomonas aeruginosa and strains of Serratia marcescens and in vivo against Pseudomonas bacteria (Chanbusarakum, P. & Murray, P. R., 1978; Pogwizd, S. M. & Lerner, S. A., 1976; Riff, J. L. & Jackson, G. G., 1972).

Other reasons for the use of carbenicillin and gentamicin in combination are the rapid development of bacterial resistance when carbenicillin is used alone (Noone, P. & Pattison, J. R., 1971; Riff, L. J. & Jackson, G. G., 1972) and the disappointing therapeutic results when gentamicin is used alone in patients with leukopenia or leukemia, who are

among the individuals that commonly acquire bacteremic infections with Pseudomonas aeruginosa (Riff, L. J. & Jackson, G. G., 1972).

The ticarcillin-gentamicin combination has been successfully used in the treatment of suspected infections in granulocytopenic cancer patients (Schimpff, S. C., et al., 1976). Pulmonary infections in patients with cystic fibrosis are often treated with a penicillin-aminoglycoside combination (Crozier, D. N. & Khan, S. R., 1976; Martin, A. J., et al., 1980). Antibiotic regimens for patients with granulocytopenia may combine a penicillin with activity against Pseudomonas aeruginosa such as ticarcillin or carbenicillin, an aminoglycoside and a cephalosporin (Schimpff, S. C., et al., 1976). In septic states, therapy is often initiated with gentamicin in combination with either carbenicillin or a cephalosporin. Such combinations have also been recommended for septic patients who are neutropenic (<500 neutrophils/mm^3) because it has been suggested that they are not cured when an aminoglycoside is used alone (Appel, G. B. & Neu, H. C., 1978).

4. Interaction of Aminoglycosides and Beta-lactam Penicillins

In 1971, McLaughlin and Reeves found that the combined use of gentamicin and carbenicillin resulted in an interaction and loss of activity of both antibiotics. Further in vitro, and later, in vivo studies were performed on the interaction

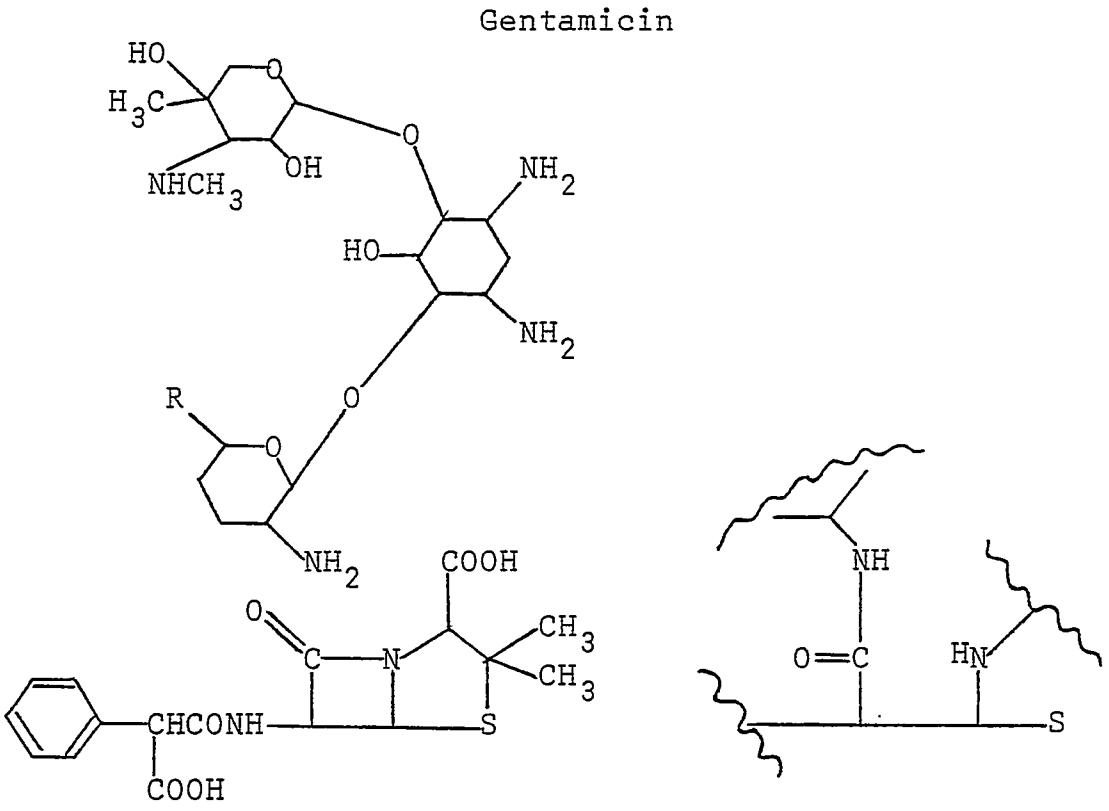
between different aminoglycosides (such as tobramycin, amikacin, netilmicin, etc.) and penicillins (such as ticarcillin, azlocillin, mezlocillin, mecillinam, oxacillin, methicillin, ampicillin, piperacillin, penicillin-G, etc.) (Chanbusarakum, P. & Murray, P. R., 1978; Davies, M., et al., 1975; Ebert, S. L. & Clementi, W. A., 1983; Edwards, D. J. & Schentag, J. J., 1981; Eykyn, S., et al., 1971; Glew, R. H. & Pavuk, R. A., 1983; Flournoy, D. J., 1978; Gupta, V. D. & Stewart, K. R., 1983; Hale, D. C., et al., 1980; Henderson, J. L., et al., 1981; Holt, H. A., et al., 1976; Janerich, D. T., 1971; Klastersky, J., et al., 1972; Konishi, H., et al., 1983; Kradjan, W. A. & Burger, R., 1980; Lau, A., et al., 1983; Levison, M. E. & Kaye, D., 1971; McLaughlin, J. E. & Reeves, D. S., 1971; Murillo, J., et al., 1979; Noone, P & Pattison, J. R., 1971; O'Bey, K. A., et al., 1982; Pickering, L. K. & Gearhart, P., 1979; Pickering, L. K. & Rutherford, I., 1981; Pieper, J. A., et al., 1980; Riff, L. J. & Jackson, G. G., 1972; Riff, L. J. & Thomason, J. L., 1982; Russo, M. E., 1980; Thompson, M. I. B., et al., 1982; Tindula, R. J., et al., 1983; Waitz, J. A., et al., 1972; Weibert, R., et al., 1976; Winters, R. E., et al., 1971).

The interaction of aminoglycosides and penicillins results in the loss of activity (or antimicrobial effect) of both antibiotics. The mechanism of the interaction is thought to be first a nucleophilic opening of the beta-lactam ring, which then combines with an amino group of the aminoglycoside,

leading to the formation of a biologically inactive amide (Appel, G. B. & Neu, H. C., 1978; Henderson, J. L., et al., 1981; Kradjan, W. A. & Burger, R., 1980; Riff, L. J. & Jackson, G. G., 1972; Russo, M. E., 1980) (Figure 4). Since the loss of activity is due to the opening of the beta-lactam ring, an intact beta-lactam ring is therefore essential for the inactivation of an aminoglycoside. The reaction between penicillins and aminoglycosides does not occur if the beta-lactam ring is destroyed (Kradjan, W. A. & Burger, R., 1980; Pickering, L. K. & Rutherford, I., 1981; Riff, L. J. & Jackson, G. G., 1972).

Visible precipitates are formed when mixing cloxacillin or cephalothin with gentamicin and their combination use is grossly incompatible. On the other hand, no visible precipitate can be seen when mixing other penicillins with aminoglycosides (Noone, P. & Pattison, J. R., 1971).

In vitro inactivation is medium, temperature, concentration and time dependent, but pH independent (Appel, G. B. & Neu, H. C., 1978; Henderson, J. L., et al., 1981; Pickering, L. K. & Gearhart, P., 1979; Pickering, L. K. & Rutherford, I., 1981; Riff, L. J. & Jackson, G. G., 1972; Russ, M. E., 1980). The drug interaction between penicillins and aminoglycosides is affected by the presence of other solutes and especially by proteins (Riff, L. J. & Jackson, G. G., 1972). The interaction is greater in solutions with lower salt concentrations and in deionized water as opposed to solutions with high salt



Carbenicillin

Figure 4: Nucleophilic opening of the beta-lactam ring of carbenicillin by amino group on a sugar of gentamicin, leading to the formation of a biologically inactive amide (Henderson, J. L., et al., 1981)

concentrations (such as phosphate buffer or phosphate-citrate buffer) or serum (Kradjan, W. A. & Burger, R., 1980; Pickering, L. K. & Rutherford, I., 1981). Increasing the temperature accelerates the rate of inactivation, and decreasing it slows the reaction (Riff, L. J. & Jackson, G. G., 1972). The inactivation of aminoglycosides is shown to be directly related to the concentration of penicillin or the penicillin-to-aminoglycoside concentration ratio (Pickering, L. K. & Gearhart, P., 1979; Pickering, L. K. & Rutherford, I., 1981; Riff, L. J. & Jackson, G. G., 1972) and the duration of the interaction (Kradjan, W. A. & Burger, R., 1980; Pickering, L. K. & Rutherford, I., 1981).

Of all the penicillins tested, ticarcillin and carbenicillin cause the greatest and piperacillin the smallest loss of activity of aminoglycosides (Edwards, D. J. & Schentag, J. J., 1981; Hale, D. C., et al., 1980; Riff, L. J. & Thomason, J. L., 1982). Of all the aminoglycosides studied, gentamicin and tobramycin are inactivated the most and amikacin the least by penicillins (Pickering, L. K. & Rutherford, I., 1981; Pieper, J. A., et al., 1980).

The kinetics of the interaction has not yet been fully investigated. The degradation of aminoglycosides in vitro due to interaction with penicillins appeared to be a first-order reaction in some studies (Edwards, D. J. & Schentag, J. J., 1980; O'Bey, K., et al., 1982; Pieper, J. A., et al., 1980) but a more complex reaction in others (Davies, M.,

et al., 1975; Glew, R. H. & Pavuk, R., 1983; Noone, P. & Pattison, J. R., 1971). Konishi, et al. (1983) suggest that the degradation of tobramycin shows a biexponential pattern of decay. O'Bey, et al. (1979) calculated the degradation rate constants of tobramycin alone and in combination with ampicillin, carbenicillin and penicillin-G at 0°C, 23°C and 37°C in pooled human serum. For penicillins, Gupta & Stewart (1983) calculated the degradation constants of carbenicillin alone and in combination with aminoglycosides. They suggest that the degradation of carbenicillin due to interaction with aminoglycosides is a first order reaction. Thompson, et al. (1982) calculated the in vivo interaction constants of gentamicin-carbenicillin and gentamicin-piperacillin in patients with chronic renal failure. Konishi, et al. (1983) calculated the in vivo interaction constants of tobramycin-carbenicillin, tobramycin-ticarcillin and tobramycin-piperacillin in subjects with normal renal function. In both studies, the in vivo interaction constants were calculated by subtracting the elimination rate constants of aminoglycosides in combination with penicillins from the elimination rate constants of aminoglycosides alone (Table 1).

The inactivation occurs predominantly if the drugs are mixed before or during their administration (Appel, G. B. & Neu, H. C., 1978; Riff, L. J. & Jackson, G. G., 1972). In most patients with normal renal function, the antibacterial effects of aminoglycosides and penicillins on bacteria are

Table 1: *In vitro* studies on the interaction of penicillins and aminoglycosides in serum

Authors	Temp	Amino[a] studied	Pen[b] studied	Assay[c] method	Results presented
Noone & Pattison, 1971	37°C	Gen (10µg/mL)	Carb, Amp, Pen-G, Meth & Clox (200µg/mL)	Micro	% remaining of Gen from 0-48h
Waitz, *et al.*, 1972	37°C	Gen	Carb & Pen-G	Micro	Potency of Gen at 0, 24, 48 & 72h
McLaughlin & Reeves, 1971	20°C, 35°C & 56°C	Gen (5µg/mL)	Carb (200µg/mL)	Micro	Conc of Gen from 0-145h
Riff & Jackson, 1972	25°C & 37°C	Gen (10µg/mL)	Carb (200, 500 & 1000 µg/mL)	Micro	Conc of Gen from 0-48h
Flournoy, 1978	4°C, 25°C, 35°C & 42°C	Net (10µg/mL)	Carb (125, 250, 500 & 1000 µg/mL)	Micro	% activity of Net at 24h
Pickering & Gearhart, 1979	37°C & -70°C	Gen, Tob & Net (5 & 10 µg/mL) Ami (10 & 20µg/mL)	Carb & Tica (100, 200, 300 & 600 µg/mL)	REA, RIA & Micro	% activity of Amino at 24 & 72h (37°C) and at 8 weeks (-70°C)
Hale, *et al.*, 1980	37°C	Gen & Tob (10µg/mL)	Carb & Pip (62.5, 125, 250, 500 & 1000µg/mL)	Micro & RIA	% recovery of Amino at 4 & 24h
Edwards & Schentag, 1981	Room temp & Fridge temp	Tob (10µg/mL)	Carb, Tica & Mezlo (200µg/mL) Amp & Moxa (100µg/mL) Pen-G (75 µg/mL)	RIA	Conc of Tob at 24, 48, 72 & 96h

Table 1 (Continued)

Authors	Temp	Amino[a] studied	Pen[b] studied	Assay[c] method	Results presented
Henderson, et al., 1981	27°C & 37°C	Gen, Tob & Net (5-8µg/mL)	Carb, Azlo & Mezlo (50, 250 & 500µg/mL)	RIA & REA	Conc of Amino at 0.3, 1, 3, 6 & 9 days
Riff & Thomason, 1982	4°C & 37°C	Gen, Tob & Net (10µg/mL) Ami & Kana (20µg/mL)	Pen-G, Amp, Meth, Oxa, Carb & Tica (250, 500 & 1000µg/mL)	Micro & RIA	% activity of Amino & Pen at 0, 24 & 48h
Pickering & Rutherford, 1981	37°C	Gen, Tob & Net (5 & 10µg/mL) Ami (10 & 20µg/mL)	Carb, Azlo, Meci, Pip & Mezlo (125, 250 & 500 µg/mL)	Micro & REA	% activity of Amino at 24 & 72h
O'Bey, et al., 1982	0°C, 23°C & 37°C	Tob (8µg/mL)	Carb, Amp & Pen-G (200µg/mL)	REA	Conc of Tob at 0, 4, 8, 12, 24 & 48h; Tob degradation rate constants & t_{90}
Tindula, et al., 1983	room temp & frozen	Gen & Tob (10µg/mL) Ami (35 µg/mL)	Pen-G, Amp, Naf, Carb & Tica (400 µg/mL)	RIA	% remaining of Amino at 24h
Glew & Pavuk, 1983	25°C, 4°C, -8°C & -70°C	Gen & Tob (5µg/mL) Ami (20 µg/mL)	Carb & Pip (200µg/mL) Moxa (100 µg/mL)	EMIT	% activity of Amino at 8 & 48h, 1 & 3 weeks

a Amino (Aminoglycoside): Ami (Amikacin), Gen (Gentamicin), Net (Netilmicin) & Tob (Tobramycin)

b Pen (Penicillin): Amp (Ampicillin), Azloc (Azlocillin), Carb (Carbenicillin), Clox (Cloxacillin), Meci (Mecillinam), Meth (Methicillin), Mezlo (Mezlocillin), Moxa (Moxalactam), Naf (Naficillin), Oxa (Oxacillin), Pen-G (Penicillin-G), Pip (Piperacillin) & Tica (Ticarcillin)

c Assay method: Micro (Microbiological assay), RIA (Radioimmunoassay) REA (Radioenzymatic assay) & EMIT (Homogenous enzyme immunoassay)

thought to occur prior to any significant loss of activity due to inactivation (Pickering, L. K. & Gearhart, P., 1979; Pieper, J. A., et al., 1980). The penicillin antibiotics are excreted at a rate faster than the interaction between the two drugs and the interaction is regarded as not clinically significant (Hoecher, et al., 1978; Pickering L. K. & Rutherford, I., 1981; Riff, L. J. & Jackson, G. G., 1972; Winters, R. E., et al., 1971). In addition, if the patient is given frequent multiple doses, a new supply of the drugs will be furnished at a much greater rate than their in vivo inactivation by the drug interaction (Riff, L. J. & Jackson, G. G., 1972). Therefore, in vivo inactivation of aminoglycosides by penicillins appears to be restricted to patients in whom the drugs can be expected to circulate for 24 hours or longer without excretion or replacement (Riff, L. J. & Jackson, G. G., 1972). In patients with severe renal failure where the excretion of both drugs is delayed but the penicillin dosage schedule is not modified, an accumulation of the penicillin may occur producing a longer contact time between the two drugs. This could result in a significant inactivation of the concurrently administered aminoglycoside antibiotic. Clinical evidence of such inactivation includes a distinct shortening of the half-life of the aminoglycoside and failure of larger aminoglycoside doses to produce therapeutic serum levels (Appel, G. B. & Neu, H. C., 1978; Davies, M., et al., 1975; Henderson, J. L., 1981; Kradjan, W. A. &

Burger, R., 1980; McLaughlin, J. E. & Reeves, D. S., 1971; Riff, L. J. & Jackson, G. G., 1972; Russo, M. E., 1980; Weibert, R., et al., 1976).

In vitro, the activity of gentamicin after inactivation by penicillin can be recovered by the addition of a reducing agent. It is suggested that the same could happen in vivo if the antibiotic conjugate or altered aminoglycoside is not excreted (Riff, L. J. & Jackson, G. G., 1972).

In order to avoid the interaction between aminoglycoside and penicillin, it is suggested that these antibiotics should not be mixed in a syringe or in an intravenous infusion bottle and they should be administered separately via different injection sites (Eykyn, S., et al., 1971; Riff, L. J. & Jackson, G. G., 1972; Winters, R. E., et al., 1971). When large doses of a penicillin are being administered by continuous intravenous infusion, it is recommended that the aminoglycoside be given by intramuscular or "bolus" intravenous injection (Noone, P. & Pattison, J. R., 1971). When aminoglycosides and penicillins are administered together in patients with severe renal failure, it is suggested that the dose of penicillin must be adjusted to renal function and serum levels of both drugs should be monitored to avoid a penicillin-to-aminoglycoside ratio of greater than 50:1 (Kradjan, W. A. & Burger, R., 1980; Weibert, R., et al., 1976). Among the aminoglycosides, amikacin is more resistant to inactivation by the penicillins;

and piperacillin, compared to the other penicillins, may be slightly less effective in inactivating aminoglylosides. Thus in patients who have markedly diminished renal function, and when the use of a combination of an aminoglycoside and a broad-spectrum penicillin is indicated, the use of amikacin with piperacillin may have a theoretical advantage over other combinations (Hale, D. C., et al., 1980; Pieper, J. A., et al., 1980). Table 2 is a summary of the previous in vivo studies on the interaction of penicillins and aminoglycosides.

5. Analytical Methods of Aminoglycosides

There are mainly three kinds of assay methods for the measurement of aminoglycoside concentrations in body fluids. They are: biological assays, biochemical assays and chemical assays.

5.1. Biological Assays

Microbiological assays have long been used for measuring antibiotic concentrations in body fluids and tissues. The basic procedure is an agar-diffusion method which is capable of a reasonable degree of accuracy, is simple and convenient, requires no unusual equipment or expertise and with modifications, can produce a result within 6 hours or less (the time required for conventional microbiological assays is 12 to 48 hours) (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Lauermann, M., 1978; Maitra,

Table 2: *In vivo* studies on the interaction of penicillins and aminoglycosides in patients

Authors	Patient's renal function	Amino[a] studied	Pen[b] studied	Assay[c] method	Results[d] presented
Winters, *et al.*, 1971	normal	Gen	Carb	Micro	Gen serum levels from 5 min-4 h postinfusion
Riff & Jackson, 1972	normal & impaired	Gen	Carb	Micro	Gen peak serum levels from 1-12 h post-infusion; Gen $t_{\frac{1}{2}}$
Davies, *et al.*, 1975	impaired (end-stage)	Gen	Carb & Tica	Micro	Gen, Carb & Tica serum levels from 1-48 h postinfusion; Gen $t_{\frac{1}{2}}$
Weibert, *et al.*, 1976	impaired	Gen & Tob	Carb	Micro	Carb, Gen & Tob serum levels from 3-24 h postinfusion; Tob $t_{\frac{1}{2}}$
Murillo, *et al.*, 1979	normal	Gen	Tica	Micro	Tica & Gen serum levels at 1 & 5 h postinfusion
Thompson, *et al.*, 1982	impaired (end-stage)	Gen	Carb & Pip	Micro	Gen serum levels from 1-48 h postinfusion; Gen K_{el} & $t_{\frac{1}{2}}$; K_i
Lau, *et al.*, 1983	normal	Tob	Pip	RIA	Tob serum levels from 0.5-6 h postinfusion; Tob K_{el} & $t_{\frac{1}{2}}$; V_d & $AUC^{0-\infty}$
Konishi, *et al.*, 1983	normal	Tob	Carb, Tica & Pip	EMIT	Tob peak serum levels from 2.5-24 h post-infusion; Tob K_{el} & $t_{\frac{1}{2}}$; K_i

a Amino (Aminoglycoside): Gen (Gentamicin) & Tob (Tobramycin)

b Pen (Penicillin): Carb (Carbenicillin), Pip (Piperacillin) & Tica (Ticarcillin)

c Assay method: Micro (Microbiological assay), RIA (Radio-immunoassay) & EMIT (Enzyme immunoassay)

d Results presented: K_{el} (Elimination rate constant), $t_{\frac{1}{2}}$ (Elimination half-life), K_i (Interaction rate constant), V_d (Volume of distribution) & AUC (Area under the curve)

S. K., et al., 1979; Matzke, G. R., et al., 1982). A number of combinations of test organisms and specific agars are reliable for the rapid assays. They are: Bacillus subtilis ATCC 6633 in heart infusion agar or in antibiotic medium no. 5 or 11, Bacillus globigii in brain heart infusion agar, Staphylococcus aureus ATCC 6538P in antibiotic medium no. 11, and Klebsiella NCTC 10896 in DST or sensitest agar. The usual approaches to circumvent interfering substances, especially other antibiotics in the sample, include the use of beta-lactamases (added to the specimen or the agar) to destroy penicillins and cephalosporins, the use of a multiple antibiotic-resistant test organism or a combination of these techniques, with appropriate controls to ensure that only the aminoglycoside is being detected. The reverse, inactivation of the aminoglycoside in order to assay concomitant agents can be accomplished by adding cellulose phosphate powder to the sample, or by incorporating 4 to 6% sodium chloride into the agar (Barza, M. & Lauermann, M., 1978; Maitra, S. K., et al., 1979).

5.2. Biochemical Assays

The biochemical assays include radioenzymatic assay (REA), radioimmunoassay (RIA), fluoroimmunoassay (FIA) and homogenous enzyme immunoassay (or enzyme-modified immunoassay) (EMIT).

The principle of radioenzymatic assay is based

on the specific enzymatic transfer of a radioactive modifying group to an aminoglycoside drug, yielding a radiolabelled product which will adhere, by virtue of electrostatic charge to the phospho-cellulose paper. The amount of radioactivity produced is proportional to the concentration of aminoglycoside antibiotic and can be counted directly (Barza, M. & Lauermann, M., 1978; Maitra, S. K., et al., 1979). These enzymes are present in organisms that carry resistant (R) factors which inactivate aminoglycosides by means of phosphorylation, acetylation or adenylation. The assay is simple, fast, accurate, highly specific and sensitive. It is not interfered with by antimicrobial agents of other classes. However, it requires special equipment, radioactive labelled substrate and specific bacterial enzyme necessary to catalyze the reaction between the substrate and the aminoglycoside. Other problems include high background radioactivity, instability and nonuniformity of the ingredients (Appel, G. B. & Neu, H. C., 1978; Barza, M. & Lauermann, M., 1978).

The radioimmunoassay system consists of a constant & limiting amount of specific antibody and radioactive aminoglycoside -- addition of serum sample containing the aminoglycoside competes with the radioactive tracer for antibody binding sites. The more aminoglycoside in the sample, the less radioactivity is bound. Addition of a second antibody allows separation of the bound and free aminoglycoside by centrifugation (Matzke, G. R., et al., 1982; Stobberingh,

E. E., et al., 1982). The assay is rapid, sensitive and specific. Commercial RIA kits are available. Each aminoglycoside usually requires a separate assay kit because of the specificity of the antibody thus increasing the cost per sample, particularly with small sample numbers (Barza, M. & Lauermann, M., 1978).

The principle of fluoroimmunoassay is similar to that of the RIA except that a fluorogenic reagent is used instead of radioactive tracer. The aminoglycoside in the serum sample competes with the fluorogenic aminoglycoside reagent (nonfluorescent under the conditions of the assay) for the antibody binding sites. The fluorogenic aminoglycoside reagent which is not bound to antibody is hydrolyzed by an enzyme to produce a fluorescent product. The fluorescence produced, therefore, is proportional to the aminoglycoside concentration in the serum samples (Stobberingh, E. E., et al., 1982). The assay is fast, sensitive and specific but less accurate at low concentrations (<4 µg/mL). Commercial kits are available, however the kits come without internal controls (Ngui-Yen, J. H., et al., 1981).

The homogenous enzyme immunoassay is a newer technique for microanalysis of aminoglycoside in biological fluids. The aminoglycoside in the sample competes with enzyme-labelled aminoglycoside for an antibody. The more aminoglycoside present in the serum, the more enzyme-aminoglycoside complex is free to act on the substrate of

that enzyme (Matzke, G. R., et al., 1982; Ngui-Yen, J. H., et al., 1981). The assay is fast, simple and accurate. However, the reagents are expensive and the method works well only if the whole EMIT system including equipment is provided (Mannisto, P. T., 1982).

5.3. Chemical Assays

The high-pressure liquid chromatography methods involve extraction procedures followed by precolumn or postcolumn derivatization, separation by chromatography and measurement of the fluorescent product. The Anhalt technique involves extraction of gentamicin from serum by using a C-M cephadex ion-exchange column, separation by reverse phase ion-pair chromatography, and fluorescence detection by continuous-flow, postcolumn derivatization with o-phthaldehyde. The method separates the three gentamicin components and is applicable to other aminoglycosides (Anhalt, J. P., 1977; Anhalt, J. P., et al., 1978; Anhalt, J. P. & Brown, S. D., 1978). The Maitra technique differs from that of Anhalt mainly in the use of precolumn derivatization with o-phthaldehyde, followed by reverse phase separation of the reaction product of the three gentamicin components (Maitra, S. K., et al., 1977). Peng and his co-workers employ precolumn derivatization with dansyl chloride but C_1 and C_2 components are not separated by that method (Peng, G. W., et al., 1977). Both pre- and post-column

derivatization techniques require careful development of the assay conditions (Maitra, S. K., et al., 1977).

5.4. Comparison of Assay Methods

Many studies have compared the assay methods for the aminoglycosides, with respect to their simplicity, rapidity, accuracy, sensitivity, reproducibility, cost-effectiveness and their usefulness in hospital and commercial laboratories (Mannisto, P. T., 1982; Matzke, G. R., et al., 1982; Ngui-Yen, J. H., et al., 1981; Oeltgen, P. R., et al., 1980; Rotschafer, J. C., 1982; Stobberingh, E. E., et al., 1982). The microbiological assay is the easiest method to perform. It does not require specialized equipment or highly qualified technicians (Stobberingh, E. E., et al., 1982). The biochemical assays are faster to run, more specific and generally more sensitive than microbiological techniques (Matzke, G. R., et al., 1982). The microbiological assay usually needs an overnight incubation but the results of radioimmunoassay are ready in a few hours, and the results of enzyme immunoassay or fluoroimmunoassay in a few minutes (Mannisto, P. T., 1982). The homogenous enzyme immunoassay appears to be the easiest and most accurate for determination of serum aminoglycoside concentration. Besides, it does not require specially trained personnel for its performance, disposal of radioactive waste or dedicated instrumentation which are needed by radioimmunoassay and radioenzymatic assay (Oeltgen, P. R., et al., 1980).

However, its relatively high cost may limit its widespread acceptance (Matzke, G. R., et al., 1982). In general, the cost of supplies is greater for the RIA and REA than for the microbiological assay. Cost per assay for the RIA is greater than for either the EMIT or FIA. However, the final cost per assay based on 10 analyses by the RIA is less expensive than the other two methods (Ngui-Yen, J. H., et al., 1981). The RIA technique is comparable in precision, accuracy and sensitivity to the EMIT technique (Matzke, G. R., et al., 1982). The accuracy of HPLC favors its use for research purposes, especially for pharmacokinetic studies (Stobberingh, E. E., et al., 1982). In fact, each assay technique possesses advantages and disadvantages (Table 3) and the choice of the system depends mainly on the local conditions and needs (Mannisto, P. T., 1982).

Table 3: Comparison of different aminoglycoside serum assay techniques

Methods	Techniques	Advantages	Disadvantages	Aminoglycosides assayed
Biological assay	Agar diffusion plate assay	Simple, versatile & inexpensive High capacity & sufficient sensitivity No highly qualified technicians needed & no special equipment needed	Too slow (4-18h) Poor precision, poor specificity (interference of co-administered antibiotics), day-to-day variation & large sample size required	All
Biochemical assay	Radioenzymatic assay (REA).	Simple, accurate, sufficiently fast (2-3 h) & small sample size needed High capacity & sufficient sensitivity	Expensive reagents & equipment (γ-counter) Radioisotopes required (^{3}H, ^{14}C) Interference (tetracycline by adenylating technique and amikacin by acetylating technique)	gentamicin, tobramycin, kanamycin, amikacin & sisomicin
	Radioimmunoassay (RIA)	Simple, accurate, sufficiently fast (a few hours) & small sample size needed High capacity, high specificity & extremely high sensitivity	Expensive equipment (γ-counter) Radioisotopes required (^{3}H, ^{125}I) High bias (∿20%), not the best method for clinical use	gentamicin, tobramycin sisomicin, amikacin & netilmicin

Table 3 (Continued)

Methods	Techniques	Advantages	Disadvantages	Aminoglycosides assayed
	Fluoroimmunoassay (FIA)	Accurate, rapid (0.5-1h) & small sample size needed Sufficient specificity, high capacity & sufficient sensitivity	Expensive equipment (fluorometer) Less accurate at low concentrations (<4 µg/mL)	gentamicin, tobramycin kanamycin amikacin, sisomicin & netilmicin
	Homogenous enzyme immunoassay (EMIT)	Simple, accurate, rapid (1-15 min) & small sample size needed High capacity & high sensitivity	Expensive reagents & equipment (spectrophotometer)	gentamicin tobramycin amikacin & netilmicin
Chemical assay	High pressure liquid chromatography (HPLC)	Accurate, versatile & small sample size needed Extremely high specificity & sufficient sensitivity	Expensive equipment (chromatograph) Limited capacity Specialized personnel required	All

STATEMENT OF PROBLEM

Most of the in vitro studies of the interaction between aminoglycosides and penicillins have investigated the relationship between the extent of inactivation of aminoglycosides by penicillins and the concentration of beta-lactam antibiotics, the incubation time, the temperature of incubation and the composition of the medium (Edwards, D. J. & Schentag, J. J., 1981; Holt, H. A., et al., 1976; Konishi, H., et al., 1983; McLaughlin, J. E. & Reeves, D. S., 1971; Noone, P. & Pattison, J. R., 1971; O'Bey, K. A. et al., 1982; Riff, L. J. & Jackson, G. G., 1972; Riff, L. J. & Thompson, J. L., 1982). Others have determined the degree of inactivation of different aminoglycosides by beta-lactam antibiotics and the abilities of different beta-lactam antibiotics to inactivate aminoglycosides (Chanbusarakum, P. & Murray, P. R., 1978; Glew, R. H. & Pavuk, R. A., 1983; Hale, D. C., et al., 1980; Henderson, J. L., et al., 1981; Riff, L. J. & Thomason, J. L., 1982; Tindula, R. J., et al., 1983). Only O'Bey, et al. (1979) calculated the degradation rate constants for tobramycin alone and in combination with ampicillin, carbenicillin and penicillin-G. In fact, few of these experiments have generated sufficient data -- either too few drug concentrations or inactivation measured at too few times after mixing -- to determine the order of the inactivation reaction or reaction rate constants. In

addition, most studies measured only the degradation and/or inactivation of the aminoglycosides but not that of the beta-lactam antibiotics (Table 1).

The assay methods used in those studies include microbiological assay, radioimmunoassay, radioenzymatic assay and a homogenous enzyme immunoassay. Among these assay methods, the extent of inactivation reported in those microbiological assays may be inaccurate and misleading since degradation may continue during the incubation period and recent evidence indicated that the addition of penicillinase slows but does not stop the inactivation (Pieper, J. A., et al., 1980).

The purpose of this study is to investigate the kinetics of the interaction between two aminoglycosides -- gentamicin and tobramycin -- and two beta-lactam penicillins -- carbenicillin and ticarcillin. The extent of degradation (controls and mixtures) of both aminoglycosides and beta-lactam penicillins will be measured by radioimmunoassay and high pressure liquid chromatography, respectively. Data will be collected and examined by various graphical techniques to determine the order of the degradation and interaction. The degradation and interaction rate constants will also be calculated. In addition, a mathematical method will be developed to describe the change of drug concentrations with time.

Although the interaction of the two antibiotics can be

avoided by not pre-mixing these two drugs in a same syringe or in a same intravenous infusion bottle, some inactivation will still occur in the body if these two antibiotics have to be administered together even by different administration routes. The inactivation is generally regarded as clinically insignificant in patients with normal renal function and clinically significant in patients with impaired renal function. However, recent studies by Murillo, et al. (1979) and Konishi, et al. (1983) indicated that the interaction between aminoglycosides and penicillins might be of potential clinical significance even in patients with normal renal function. If the antibiotic dosage is not changed, especially in those patients with acute renal failure, serum aminoglycoside levels may be sub-therapeutic because of the inactivation. Increasing the aminoglycoside dosage in order to raise the serum concentration to therapeutic level will expose the patient to a greater risk of nephrotoxicity and ototoxicity. With the in-vitro information on the order and rate of the interaction, the extent and significance of the in-vivo inactivation of these two antibiotics can be predicted; and together with the existing kinetic information of the two antibiotics, a suitable combined dosage schedule can be designed to minimize the in-vivo interaction.

EXPERIMENTAL

1. Assay Techniques

1.1. High Pressure Liquid Chromatography Analysis of Penicillins (Kwan, R. H., et al., 1982)

1.1.1. Mobile Phase

The mobile phase consists of acetonitrile-aqueous 0.06 M sodium biphosphate (50.5 : 100) with the pH of the mobile phase adjusted to 2.05 using 85% phosphoric acid.

1.1.2. Standard Solutions and Internal Standard

Stock solutions of carbenicillin or ticarcillin (5000 µg/mL of base) were diluted to 100, 200, 400 and 600 µg/mL as the standard solutions. A stock solution of penicillin-G (5000 µg/mL) was diluted to 400 µg/mL and was used as the internal standard.

1.1.3. Plasma Extraction Procedure

Carbenicillin or ticarcillin solution (50 µL) was added to serum (150 µL), followed by penicillin-G solution (50 µL)(as internal standard) and 1 M sulfuric acid (50 µL). The tube contents were vigorously agitated with a mechanical mixer for 5 seconds. Then ethyl acetate (1.0 mL) was added and the tube capped and shaken for 2 minutes prior to centrifugation for another 2 minutes. The supernate was

transferred to another tube and evaporated to dryness under nitrogen. Methylene chloride (200 µL) and 0.04 M sodium biphosphate (pH 6.8, 300 µL) were added to the extract residue. The tube contents were gently agitated for 1 minute and centrifuged for 2 minutes. The top aqueous layer (∿75%) was accurately transferred to another test tube and briefly shaken mechanically in a water bath at 35°C to remove traces of methylene chloride. And 50 µL aliquot of this final sample solution was injected into the µ-Bondapak C_{18} column. Duplicate injections were performed for each sample.

1.1.4. Standard Curves and Extraction Efficiency

The analysis of water and plasma carbenicillin and ticarcillin standard solutions were carried out following the procedure of Kwan et al. (1982) except that plasma extraction steps were excluded from water penicillin standard solutions. The peak heights of both carbenicillin or ticarcillin standards and penicillin-G were calculated. Standard curves with concentrations of carbenicillin or ticarcillin versus peak height ratio were plotted for both water and serum standards.

The extraction efficiency of carbenicillin or ticarcillin was calculated by comparing the water and serum standard curves for each concentration of carbenicillin or ticarcillin. A mean extraction efficiency and standard deviation were calculated from two samples of duplicate

injections.

1.1.5. Calculation of Penicillin Concentrations of Samples

A standard curve was prepared each day along with the analysis of incubation samples for both carbenicillin and ticarcillin. Their peak heights and peak height ratio were measured and calculated. The concentration of carbenicillin or ticarcillin of the incubation samples were read from that day's standard curve.

1.2. Radioimmunoassay (RIA) of Aminoglycosides (New England Nuclear, RIA Instruction Manual)

1.2.1. Procedure

Standards, control serum I & II (from Gentamicin or Tobramycin RIA kits), and samples were all diluted 1 : 101 for gentamicin (add 0.010 mL of each solution to 1.0 mL of distilled water and mix well) or 1 : 201 for tobramycin (add 0.010 mL of each solution to 2.0 mL of distilled water and mix well) in polystyrene tubes. Then 50 µL of the diluted solution was pipetted into another similar tube. A 500 µL aliquot of the blue tracer solution and then 500 µL of the Gentamicin or Tobramycin Antiserum Complex were added. The tube contents were mixed for 5 seconds and incubated for 10 minutes at room temperature, followed by another 10 minutes' centrifugation at 1000xg

at 4°C. The supernatants were decanted by gently inverting all tubes once, preferably at the same time and the supernatants were discarded into a radioactive waste container. The tubes, still inverted, were placed on absorbent paper for blotting. To facilitate removal of remaining droplets, the rims of the tubes were tapped on the paper while they drained, about 30 seconds. The outside of each tube was wiped and counted in a gamma counter with a counting time of one minute for each tube.

1.2.2. Standard Curves and Calculation of Aminoglycoside Concentrations of Samples

Average counts bound, net counts bound and normalized % bound of the standards were calculated from their counts bound. A standard curve with concentration of gentamicin or tobramycin versus normalized % bound was plotted on a Rianen assay system graph paper. The higher the concentration of gentamicin or tobramycin, the lesser the radioactivity bound because more will be replaced by unlabelled gentamicin or tobramycin in standards.

Average counts bound, net counts bound and normalized % bound of the samples were also calculated and their concentrations were read from the standard curves.

2. Interaction Experiments

Stock solutions of gentamicin and tobramycin were diluted to 15, 30, 45 and 60 µg/mL and stock solutions of carbenicillin

and ticarcillin were diluted to 300, 600, 1200 and 1800 µg/mL, respectively. Equal volumes of the diluted gentamicin or tobramycin solutions, diluted carbenicillin or ticarcillin solutions and human plasma were mixed together to yield final concentrations of 5, 10, 15 and 20 µg/mL of gentamicin or tobramycin and final concentrations of 100, 200, 400 and 600 µg/mL of carbenicillin or ticarcillin in gentamicin-carbenicillin, gentamicin-ticarcillin, tobramycin-carbenicillin and tobramycin-ticarcillin mixtures. The control of each antibiotic mixture was the same concentration of gentamicin or tobramycin and carbenicillin or ticarcillin as a single component. These control solutions, as well as the antibiotic mixtures were kept at 37°C in a constant-temperature incubator. Samples were taken at a 12-hour interval for three days. All samples were frozen immediately at -20°C until the day of analysis. Duplicate experiments were performed on each antibiotic combination. The concentration of carbenicillin or ticarcillin was analyzed by high pressure liquid chromatography while the concentration of gentamicin or tobramycin was analyzed by Radioimmunoassay. A standard curve was constructed each day for the analysis of the samples for both assay methods.

3. Kinetic Calculations

The degradation constants of penicillins (K_P) and aminoglycosides (K_A) (control samples) were calculated from

the slopes of the logarithmic-linear regression curves, with % remaining of penicillin or aminoglycoside versus incubation time (Equation 3 & 4).

	Penicillins	Aminoglycosides
First order differential equations:	$dP/dt = -K_P \times P$	$dA/dt = -K_A \times A$
Integrated equations:	$\ln(P/P_o) = -K_P \times t$ ...Eq 3	$\ln(A/A_o) = -K_A \times t$ ...Eq 4
	Slope = $-K_P$	Slope = $-K_A$
Where:	P = Concentration of penicillin at time t (µg/mL)	A = Concentration of aminoglycoside at time t (µg/mL)
	P_o = Concentration of penicillin at time zero (µg/mL)	A_o = Concentration of aminoglycoside at time zero (µg/mL)
	K_P = Degradation constant of penicillin (h^{-1})	K_A = Degradation constant of aminoglycoside (h^{-1})
	t = Incubation time (h)	t = Incubation time (h)

The degradation constants of penicillins in antibiotic mixtures (K_P) were also calculated from the slopes of the logarithmic-linear regression curves, with % remaining of penicillin versus incubation time (Equation 6).

The time required for loss of 50% of initial analyzed concentration (or 50% remaining) (t_{50}) of penicillins in controls and antibiotic mixtures were calculated from: $t_{50} = 0.693/K_P$. The t_{50} of aminoglycosides in control samples was also calculated from: $t_{50} = 0.693/K_A$ while the t_{50} of aminoglycosides in antibiotic mixtures was estimated

from the loagrithmic degradation curves of aminoglycosides.

The degradation constants of aminoglycosides in antibiotic mixtures (K_A) and the interaction constants (K_i) were obtained by computer fitting of aminoglycoside concentrations with time (Equation 7), using the MLAB programme and DEC-20 computer. For computer fitting, aminoglycoside concentrations were weighted to $1/C^2$ (Boxenbaum, H. G., et al., 1974).

The initial estimates of the degradation constants of aminoglycosides in antibiotic mixtures (K_A) and the interaction constants (K_i) were calculated from the slope and intercept of the terminal portion of the semi-logarithmic plot of % aminoglycoside remaining versus incubation time (Equation 8). These calculations and the following equations were based on three assumptions: a) the interaction was a second order reaction dependent on aminoglycoside and penicillin concentration, i.e., Interaction rate = $K_i \times A \times P$ b) in aminoglycoside-penicillin combinations, the decrease in concentration of each antibiotic was due to both degradation (as in controls) and the interaction and c) the contribution of the interaction to the decrease in penicillin concentration was negligible, i.e., $K_P \times P >> K_i \times A \times P$

Aminoglycosides: $dA/dt = -K_A \times A - K_i \times A \times P$ ... Equation 5

Penicillins: $dP/dt = -K_P \times P - K_i \times A \times P$

Assuming: $K_P \times P >> K_i \times A \times P$

Then: $dP/dt = -K_P \times P$

$\ln(P/P_o) = -K_P \times t$ ... Equation 6

$P = P_o \times e^{-K_P t}$

Therefore: $dA/dt = -K_A \times A - K_i \times A \times (P_o \times e^{-K_P t})$

$dA/dt = -(K_A + K_i \times P_o \times e^{-K_P t}) \times A$

Integrating: $\int_o^A dA/A = -\int_o^t (K_A + K_i \times P_o \times e^{-K_P t}) \times dt$

$= -K_A \int_o^t dt - K_i \times P_o \int_o^t e^{-K_P t} \times dt$

$\ln(A/A_o) = -K_A \times t - K_i \times P_o \times (e^{-K_P t}/(-K_P) - 1/(-K_P))$

$= -K_A \times t + K_i \times P_o \times e^{-K_P t}/K_P - K_i \times P_o/K_P$

$A = A_o \times \exp(-K_A \times t + K_i \times P_o \times e^{-K_P t}/K_P - K_i \times P_o/K_P)$

... Equation 7

For initial estimates, when $t \to \infty$

$\ln(A/A_o) = -K_i \times P_o/K_P - K_A \times t$ ... Equation 8

Slope $= -K_A$

Intercept (b) $= -K_i \times P_o/K_P$

$K_i = -b \times K_P/P_o$

Where:

A = Concentration of aminoglycoside at time t (µg/mL)
A_o = Concentration of aminoglycoside at time zero (µg/mL)
P = Concentration of penicillin at time t (µg/mL)
P_o = Concentration of penicillin at time zero (µg/mL)
K_A = Degradation constant of aminoglycoside (h^{-1})
K_P = Degradation constant of penicillin (h^{-1})
K_i = Interaction constant ($mL \times \mu g^{-1} \times h^{-1}$)
t = Incubation time (h)

Values for P_o and K_p in Equation 8 were determined from log-linear regression of penicillin concentrations analyzed in the same incubation mixture (Equation 6).

Values for kinetic parameters (K_P, K_A & K_i) were then used in computer simulations to confirm that assumption (c), above, was valid, to demonstrate that the differential equation (Equation 5) and the integrated equation (Equation 7) gave comparable results (APPENDIX X) and to examine the effect of the interaction of aminoglycosides and penicillins in patients with impaired renal function.

4. Statistical Analysis of Data

Slopes and intercepts of assay standard curves and semi-logarithmic plots of antibiotic degradation in controls and antibiotic mixtures were determined using a least squares linear regression programme.

Statistical analyses were performed using a Factorial design. One-way analysis of variance was performed on the degradation constants of penicillins in controls and on the t_{50} values of penicillins and aminoglycosides in controls, to determine the effect of the concentration of the antibiotics on these parameters. Two-way analysis of variance was performed on the degradation constants of penicillins, the interaction rate constants and the t_{50} values of aminoglycosides, to determine the effect of the concentration of penicillin and the concentration of

aminoglycoside on these parameters in the antibiotic mixtures. Three-way analysis of variance was performed on the t_{50} values of aminoglycosides to determine the difference in the ability of carbenicillin and ticarcillin to inactivate the two aminoglycosides -- gentamicin and tobramycin -- and the difference in the degree of gentamicin and tobramycin degradation produced by the two penicillins -- carbenicillin and ticarcillin. Three-way analysis of variance was also performed on the interaction constants to determine their differences in the four antibiotic mixtures. Differences were considered statistically significant if $p<0.05$. Multiple comparison of means was performed using a Newman-Keuls multiple range test.

RESULTS AND DISCUSSION

1. Preliminary Evaluation of Analytical Methods

Spectrofluorometry and high pressure liquid chromatography (HPLC) were initially tested as possible analytical methods for the aminoglycosides. Both were unsatisfactory and radioimmunoassay (RIA) was therefore selected for analysis of samples.

1.1. Spectrofluorometry

Derivatization of gentamicin with dansyl chloride and detection of fluorescence with a spectrofluorometer was first tried (Frei, R. W. & Lawrence, J. F., eds., 1981; Lawrence, J. F. & Frei, R. W., eds., 1976; Thoma, J. J., et al., eds., 1977). The results showed that the fluorescence intensity of the dansyl derivative of gentamicin was not related to the concentration of gentamicin. Therefore, the method could not be used for quantitative analysis of aminoglycosides. Another derivatizing agent -- fluorescamine -- was used (Frei, R. W. & Lawrence, J. F., eds., 1981; Lawrence, J. F. & Frei, R. W., eds., 1976). Fluorescamine is by itself nonfluorescent but reacts with primary amines to form intensely fluorescent substances. However, the fluorescamine we used showed intense fluorescence when mixed with sodium borate buffer (pH 9.0) and some fluorescence when mixed with water, even in the absence of gentamicin. Because of this, water was used to dissolve gentamicin instead of

buffer. A wide range of concentrations of gentamicin (0 to 30 µg/mL) was tested. The fluorescence intensity of the fluorescamine derivative of gentamicin was directly proportional to the concentration of gentamicin only when the concentration of gentamicin was low (<10 µg/mL). When the concentration of gentamicin was high, the fluorescence intensity of the fluorescamine derivative of gentamicin was still related but no longer directly proportional to the concentration of gentamicin; i.e., the standard curve was not linear. Gentamicin in plasma was also tested. The fluorescence intensity of the fluorescamine derivative of gentamicin in plasma was not related to the concentration of gentamicin. In addition, fluorescamine in plasma showed intense fluorescence even in the absence of gentamicin. Gentamicin and carbenicillin water solutions were mixed together and incubated at 37°C in a water bath. Samples were taken for two days. The fluorescence intensity of the fluorescamine derivative of gentamicin in the samples increased with increasing time of incubation and also the concentration of carbenicillin, showing that the interaction product of gentamicin and carbenicillin might react with fluorescamine to produce fluorescence (carbenicillin alone was not tested but theoretically carbenicillin should not react with fluorescamine since it does not have any amine group). As a result, spectrofluorometry could not be used for the quantitative analysis of aminoglycosides in the

study of the interaction with penicillins.

1.2. High Pressure Liquid Chromatography

Two HPLC methods involving pre-column derivatization of aminoglycosides were tried. The first used derivatization of the aminoglycoside with o-phthaldehyde and detection of fluorescence with a fluorometer. The chromatographic conditions used by Maitra, S. K., et al. (1977) included methanol : water (79 : 21) as the mobile phase and tobramycin as the internal standard. Problems we encountered included interfering peaks from the blank, difficulty in determing the optimum amount of o-phthaldehyde to be used, and difficulty in separating the peak of tobramycin from the peak of gentamicin. Different ratios of gentamicin to o-phthaldehyde were tested and the volume of gentamicin (0 to 20 µg/mL), o-phthaldehyde (0.1 g/mL methanol) and water with a ratio of 5 : 1 : 9 seemed to be the best. The mobile phase was modified by changing the ratio of methanol to water but there was no improvement in the separation of the peak of gentamicin from that of tobramycin. Moreover, there was little difference between the peak heights for the different concentrations of gentamicin. Another chromatographic condition using methanol : water : acetonitrile (62 : 35.1 : 2.9) as the mobile phase was also unsuccessful. Again, the peak of tobramycin could not be separated from the peak of gentamicin. When fluoranthene was used in place of tobramycin as the internal

standard, a linear curve was obtained for gentamicin. However, the peak height ratio was reduced to one-half with the second (duplicate) injection (approximately half an hour after the first injection).

The second method involved derivatization of aminoglycoside with fluorescamine and detection of fluorescence with a fluorometer. Walker, S. E. (1981) used water : acetonitrile : acetic acid (300 : 700 : 1) as the mobile phase and tobramycin as the internal standard. Problems encountered included interference peak from the blank which increased with increasing amount of fluorescamine used, tobramycin eluting at the same time as gentamicin, an interfering peak with high concentrations of carbenicillin and interfering peaks from amines in the plasma. The mobile phase was modified by changing the ratio of water to acetonitrile to acetic acid but there was no improvement either in the separation of the peak of gentamicin from the peak of tobramycin or the peak of gentamicin from the interfering peaks in plasma. Although the latter method could have been adopted for analysis of samples with gentamicin only, the interference by carbenicillin made it unsuitable for the interaction experiment.

2. Analytical Methods for Interaction Experiments

2.1. High Pressure Liquid Chromatography of Penicillins

The retention time of carbenicillin, ticarcillin and penicillin-G (as the internal standard) were 4.8 min, 4.5 min and 7.8 min, respectively. The aminoglycosides (gentamicin and tobramycin) did not show any peak and the interaction products, with a retention time of 2.8 min, did not interfere with the peaks of carbenicillin, ticarcillin or penicillin-G (Figure 5).

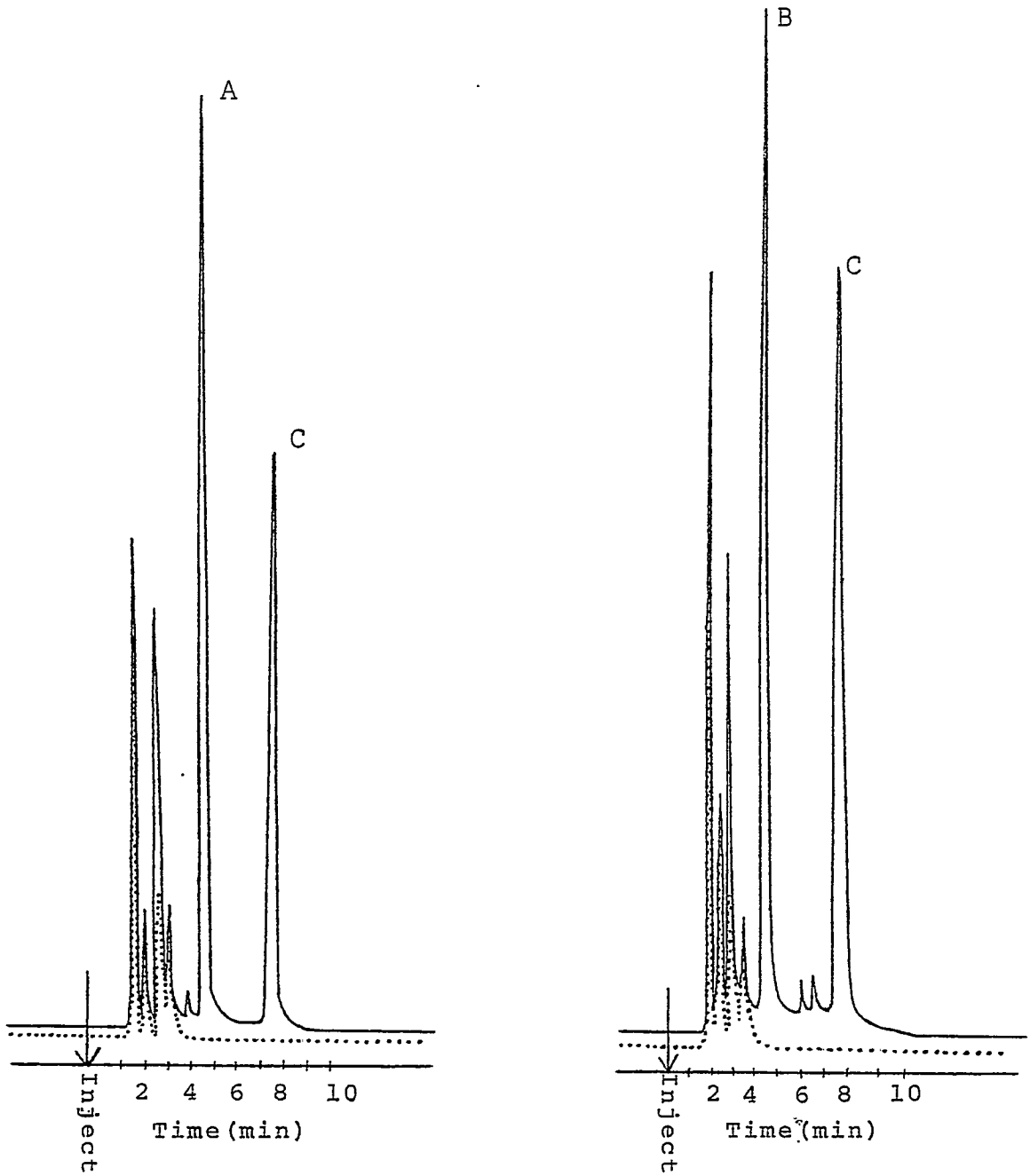
The standard curves of both carbenicillin and ticarcillin, with peak height ratio versus concentrations of penicillin, were linear (Figure 6).

The extraction efficiency of carbenicillin was 82.0% (±0.9% S.D.) and ticarcillin, 88.3% (±0.8% S.D.) (Table 4).

2.2. Radioimmunoassay

The standard curves of both gentamicin and tobramycin, with normalized % bound versus concentrations of aminoglycoside, were linear (plotted on Rianen assay system graph paper) (Figure 7).

Various antibiotics have been tested for cross reactivity by the manufacturer of the RIA kits (New England Nuclear). Among them disodium carbenicillin has shown no cross reactivity to tobramycin, at least in sample concentrations of 1 mg/mL which is considerably higher than would be expected therapeutically; and less than 0.1% cross reactivity to gentamicin (New England Nuclear, RIA Instruction Manual).



Sample I

Conc of Carb(A): 600µg/mL
Conc of Gen: 10µg/mL
Conc of Pen-G(C): 400µg/mL
Time of incubation: 12h

Sample II

Conc of Tica(B): 600µg/mL
Conc of Tob: 15µg/mL
Conc of Pen-G(C): 400µg/mL
Time of incubation: 12h

Figure 5: Chromatograms of carbenicillin (A), ticarcillin (B) and penicillin-G (C) in extracted plasma incubation samples I & II (the dotted lines above represent plasma without antibiotics)

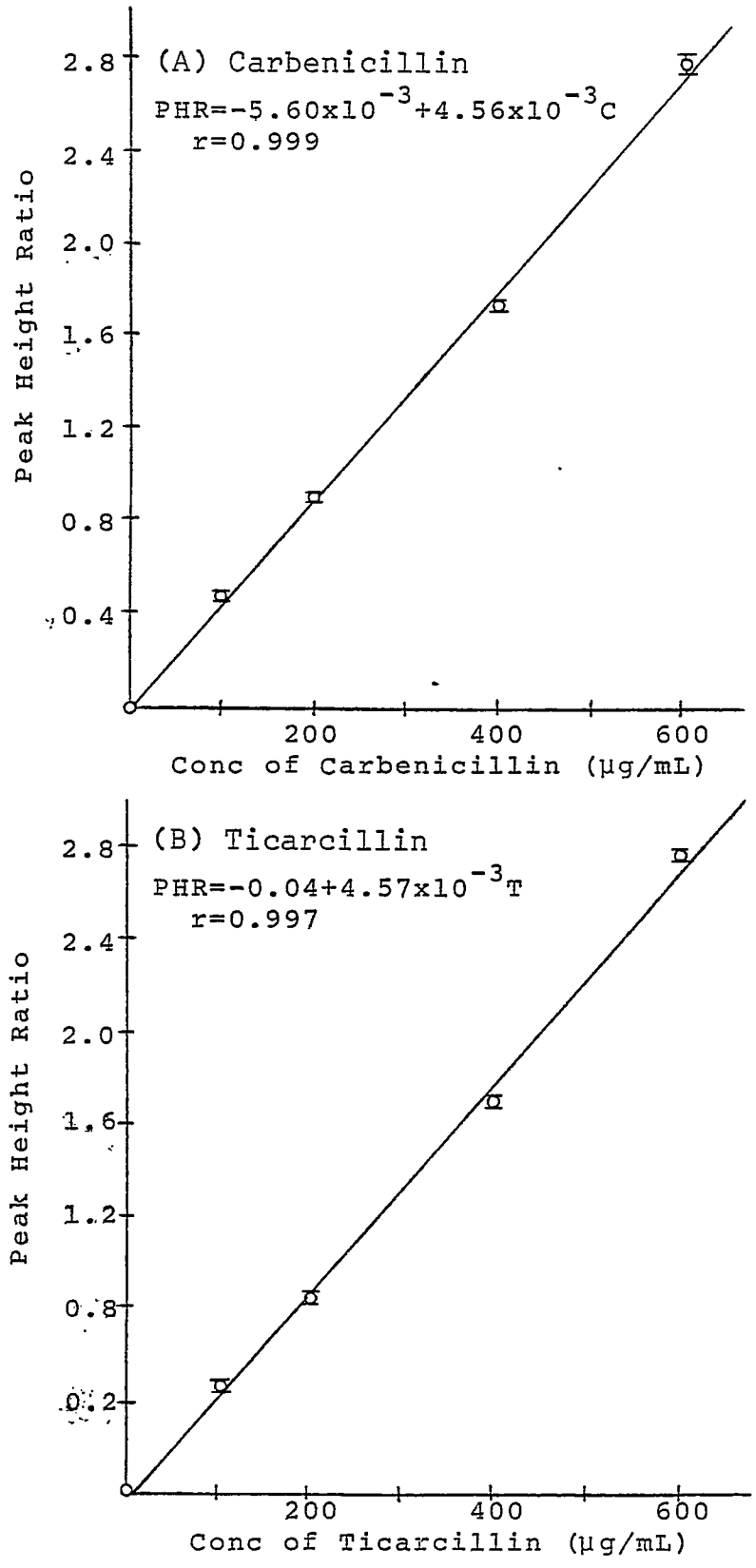


Figure 6: High Pressure Liquid Chromatography standard curves of carbenicillin (A) and ticarcillin (B)

Table 4: Extraction efficiency of the HPLC method for carbenicillin and ticarcillin

Penicillin	Concentration of penicillin (μg/mL)	% Extraction efficiency ± S.D.	Mean ± S.D.
Carbenicillin	100	82.3 ± 5.3	82.0 ± 0.9
	200	83.0 ± 4.2	
	400	81.3 ± 4.2	
	600	81.2 ± 3.5	
Ticarcillin	100	89.5 ± 9.2	88.3 ± 0.8
	200	88.0 ± 4.2	
	400	87.9 ± 2.3	
	600	87.9 ± 1.3	

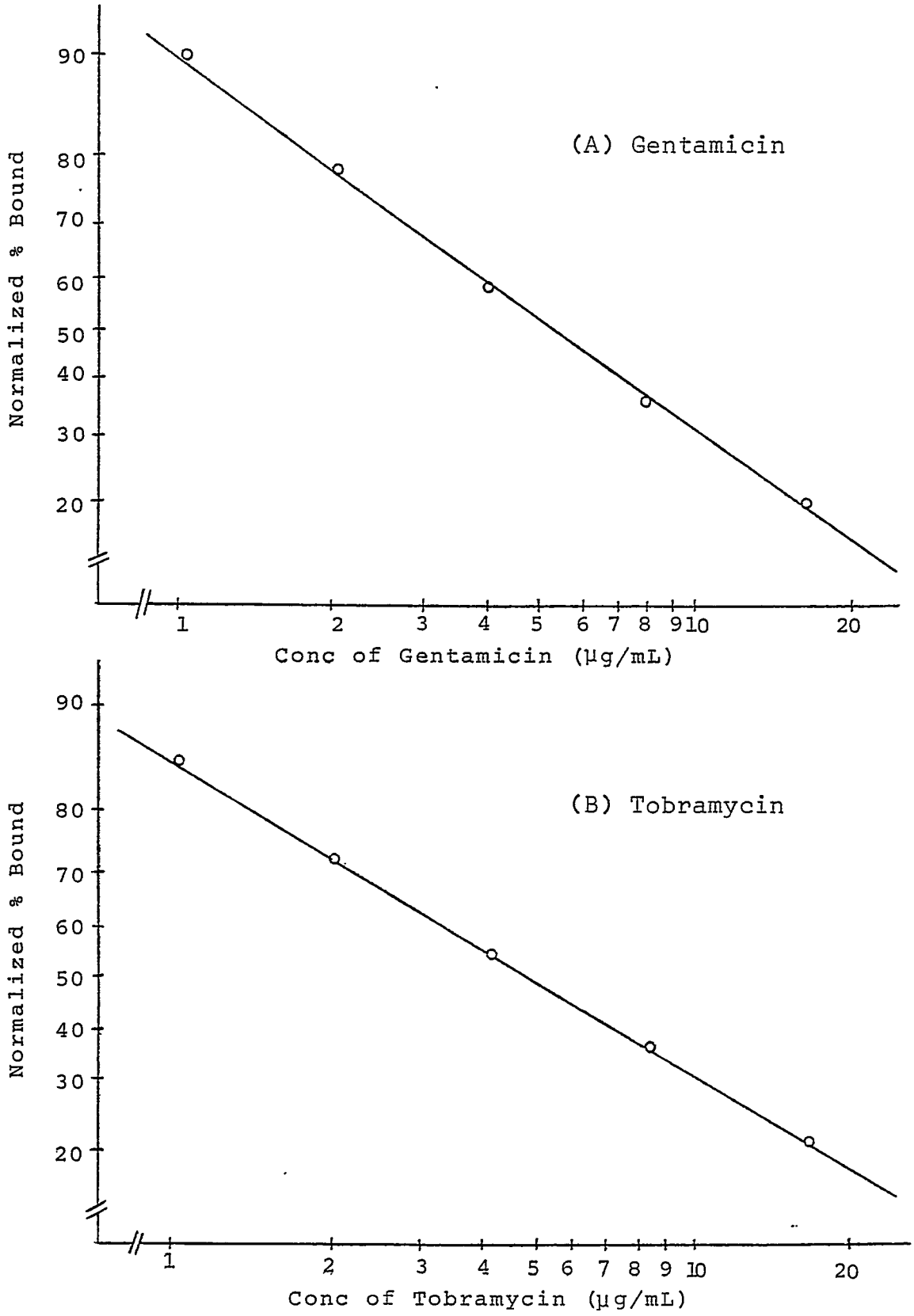


Figure 7: Radioimmunoassay standard curves of gentamicin (A) and tobramycin (B)

The newer penicillin -- ticarcillin -- was checked in our study for cross reactivity to gentamicin. Various concentrations of ticarcillin (100 to 400 µg/mL) were tested and all analyzed as less than 1 µg/mL, indicating that ticarcillin had no cross reactivity to gentamicin.

3. Interaction Experiments

In vitro inactivation of aminoglycosides and penicillins is medium, temperature, concentration and time-dependent, but pH independent (Edwards, D. J., 1981; Holt, H. A., et al., 1976; McLaughlin, J. E. & Reeves, D. S., 1971; Noone, P. & Pattison, J. R., 1971; O'Bey, K. A., *et al.*, 1982; Pickering, L. K. & Gearhart, P., 1979; Riff, L. J. & Jackson, G. G., 1972; Riff, L. J. & Thomason, J. L., 1982). In our experiments, serum was chosen as the medium and antibiotic controls and mixtures were incubated at 37°C to mimic the conditions for the interaction *in vivo*. Samples were taken at a 12-hour interval for three days; i.e., long enough for penicillin concentrations to decrease to ∿25% of their initial concentrations. The two antibiotics were mixed together to yield concentrations of 5, 10, 15 and 20 µg/mL of aminoglycoside (gentamicin or tobramycin) and concentrations of 100, 200, 400 and 600 µg/mL of penicillin (carbenicillin or ticarcillin) and give different penicillin-to-aminoglycoside concentration ratios (Table 5). These concentrations were selected to cover the normal concentration ranges of

Table 5: Penicillin-to-aminoglycoside concentration ratios in study

Concentration of penicillin (µg/mL)	Concentration of aminoglycoside (µg/mL)			
	5	10	15	20
100	20:1	10:1	6:1	5:1
200	40:1	20:1	13:1	10:1
400	80:1	40:1	26:1	20:1
600	120:1	60:1	40:1	30:1

penicillin and aminoglycoside in serum. The pH of the antibiotic mixtures were measured and were in a relatively narrow range (APPENDIX V).

In each experiment, samples were taken and frozen immediately at -20°C until the day of analysis. The analysis of carbenicillin and ticarcillin was carried out within a month after the sampling and the analysis of gentamicin within a week. Since Riff and Thomason (1980) reported that most samples of aminoglycoside stored with a penicillin at -20°C for seven to fourteen days had relatively stable values but tobramycin lost 15 to 20% of its initial activity after one to three days and 30 to 40% after seven to fourteen days, the analysis of tobramycin was carried out the same day or the second day of the sampling.

4. Degradation and Interaction Curves

The concentrations of penicillins (carbenicillin and ticarcillin) and aminoglycosides (gentamicin and tobramycin) during their 3 days' incubation together in plasma at 37°C were determined (APPENDIX VI) and expressed as percentages of their concentrations at time zero -- % remaining (APPENDIX VII).

Based on the control curves of carbenicillin and ticarcillin, both penicillins decayed rapidly in human plasma at 37°C even when present as a single component. Only 50% of the original concentration remained after 24 hours (Figure 8).

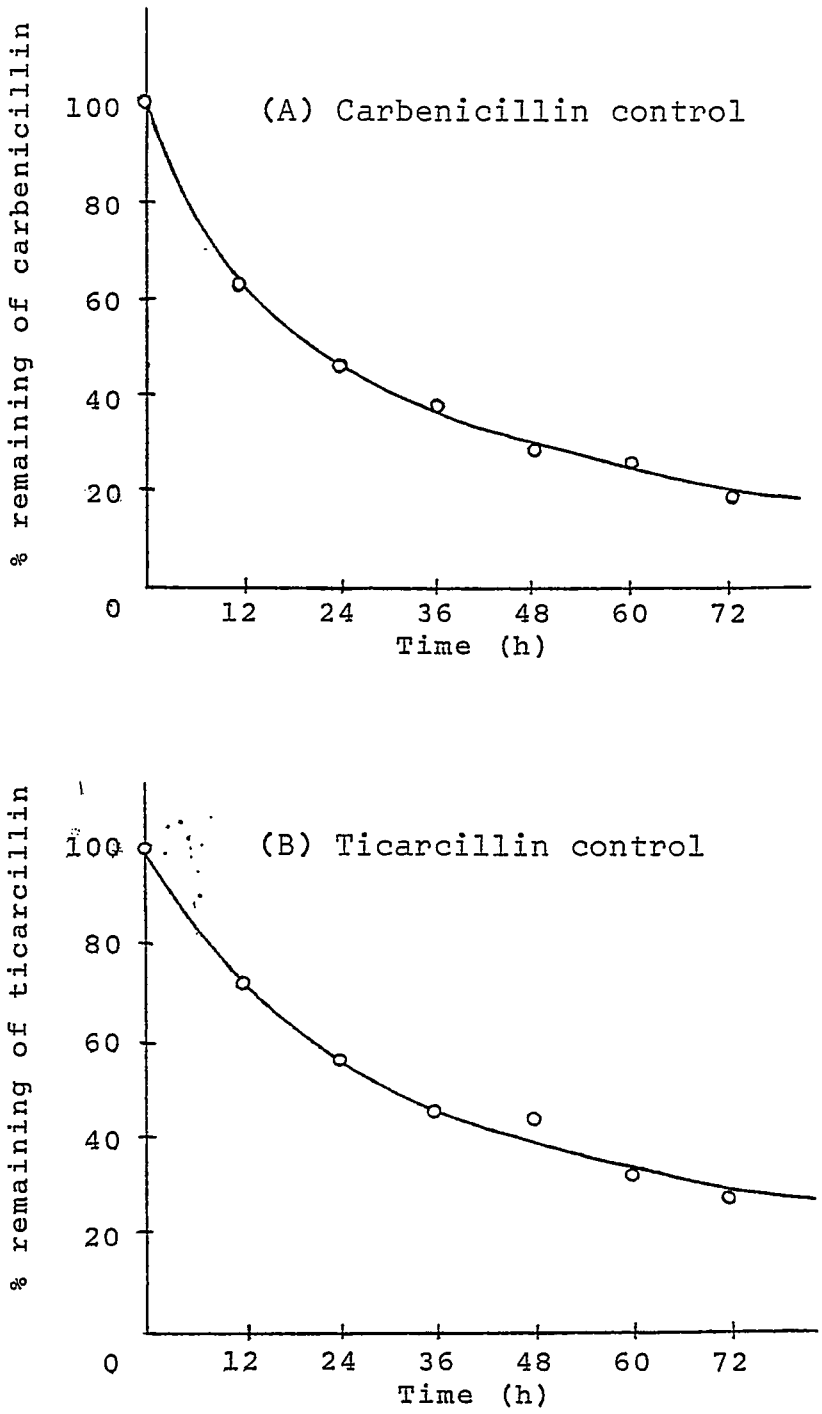


Figure 8: Degradation of carbenicillin (A) (200µg/mL) and ticarcillin (B) (200µg/mL) in plasma during 3 days' incubation at 37°C (controls) (the above is one of the duplicate results of the study)

On the contrary, the control curves of gentamicin and tobramycin showed that both of the aminoglycosides were quite stable in serum during 3 days' incubation at 37°C. There was only a minor degradation with time and the percentage loss at 24 hours was less than 10% (Figure 9). When plotted on semi-logarithmic graph paper, both the control curves of penicillins (carbenicillin and ticarcillin) (Figure 10) and aminoglycosides (gentamicin and tobramycin) (Figure 11) appeared as straight lines, indicating first-order reactions.

The concentrations of both penicillins and aminoglycosides decreased significantly with time when incubated together in plasma at 37°C for 3 days (Figure 12-15). The degradation curves for penicillins in control samples were very close to their degradation curves in incubation mixtures, indicating that the loss of penicillin due to the interaction was not significant (Figure 12-15A). On the other hand, the loss of aminoglycoside due to the interaction was significant. The inactivation appeared faster before 24 hours than after, probably because of the substantial degradation of the penicillin (Figure 12-15B). When plotted on semi-logarithmic graph paper, the degradation curves of penicillins in incubation mixtures were close to straight lines, indicating that the degradation of penicillin with aminoglycoside was essentially a first order reaction (Figure 16-19A). However, the degradation curves of aminoglycosides in incubation

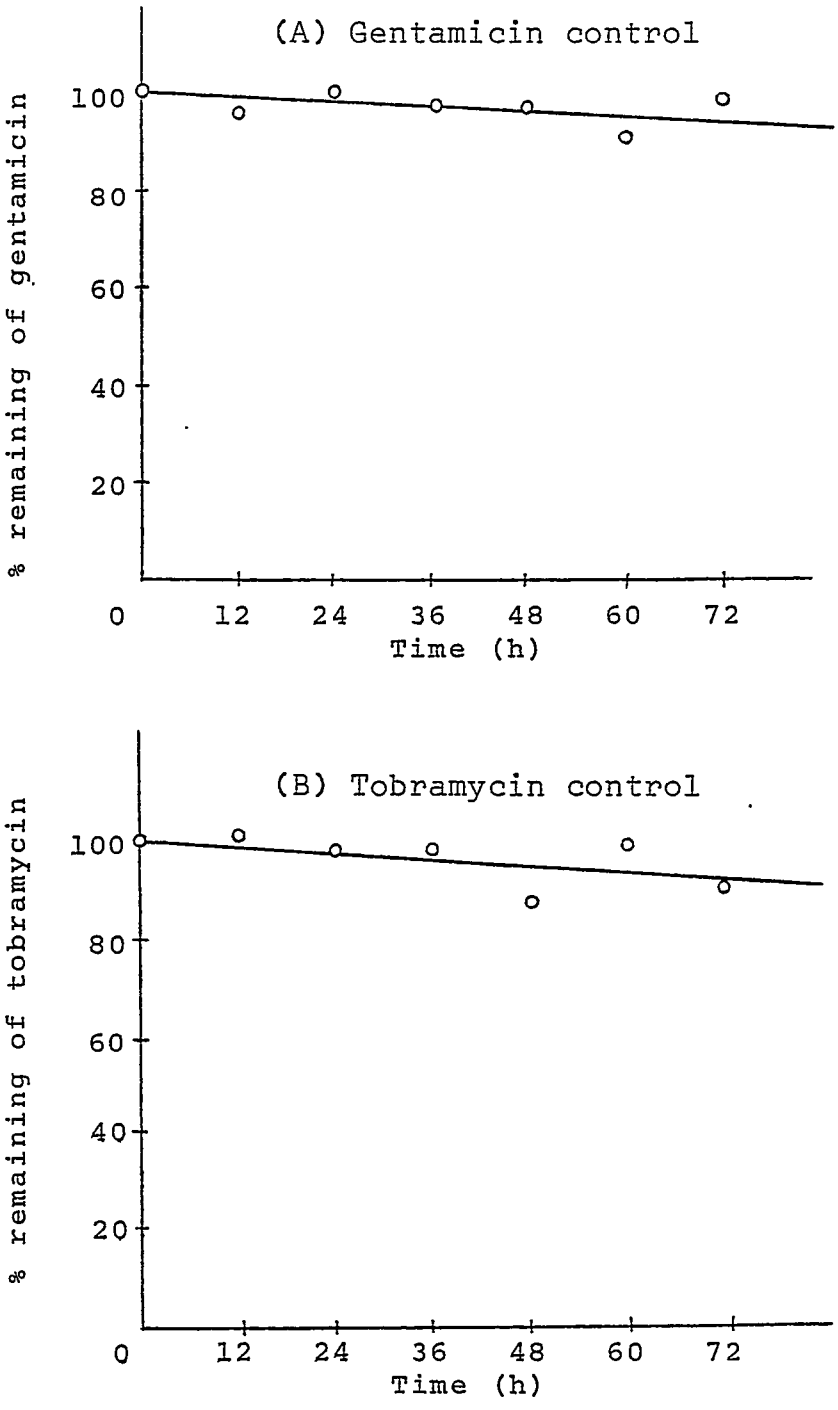


Figure 9: Degradation of gentamicin (A) (10µg/mL) and tobramycin (B) (10µg/mL) in plasma during 3 days' incubation at 37°C (controls) (the above is one of the duplicate results of the study)

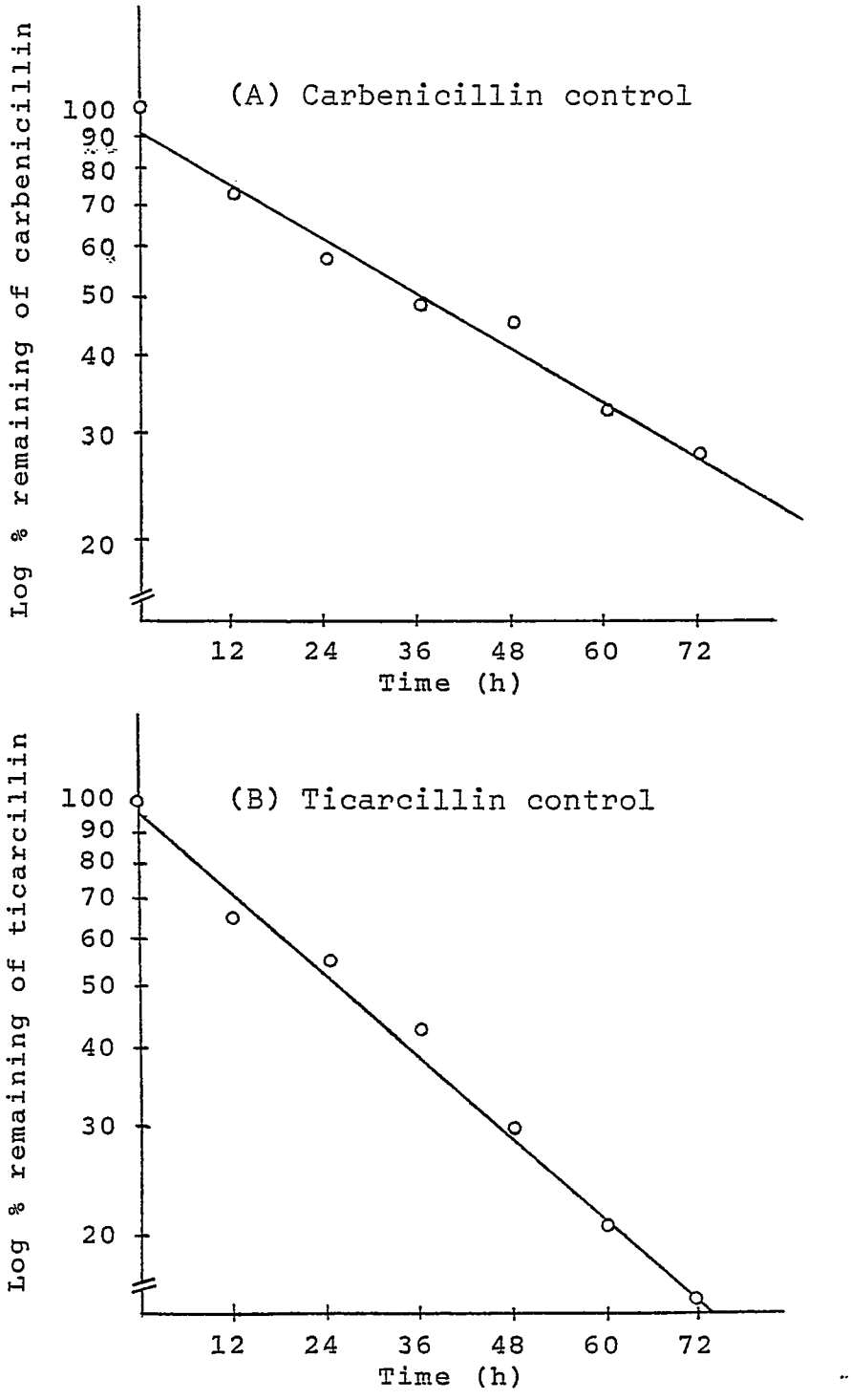


Figure 10: Degradation of carbenicillin (A) (200µg/mL) and ticarcillin (B) (200µg/mL) in plasma during 3 days' incubation at 37°C (controls) (the above is one of the duplicate results of the study)

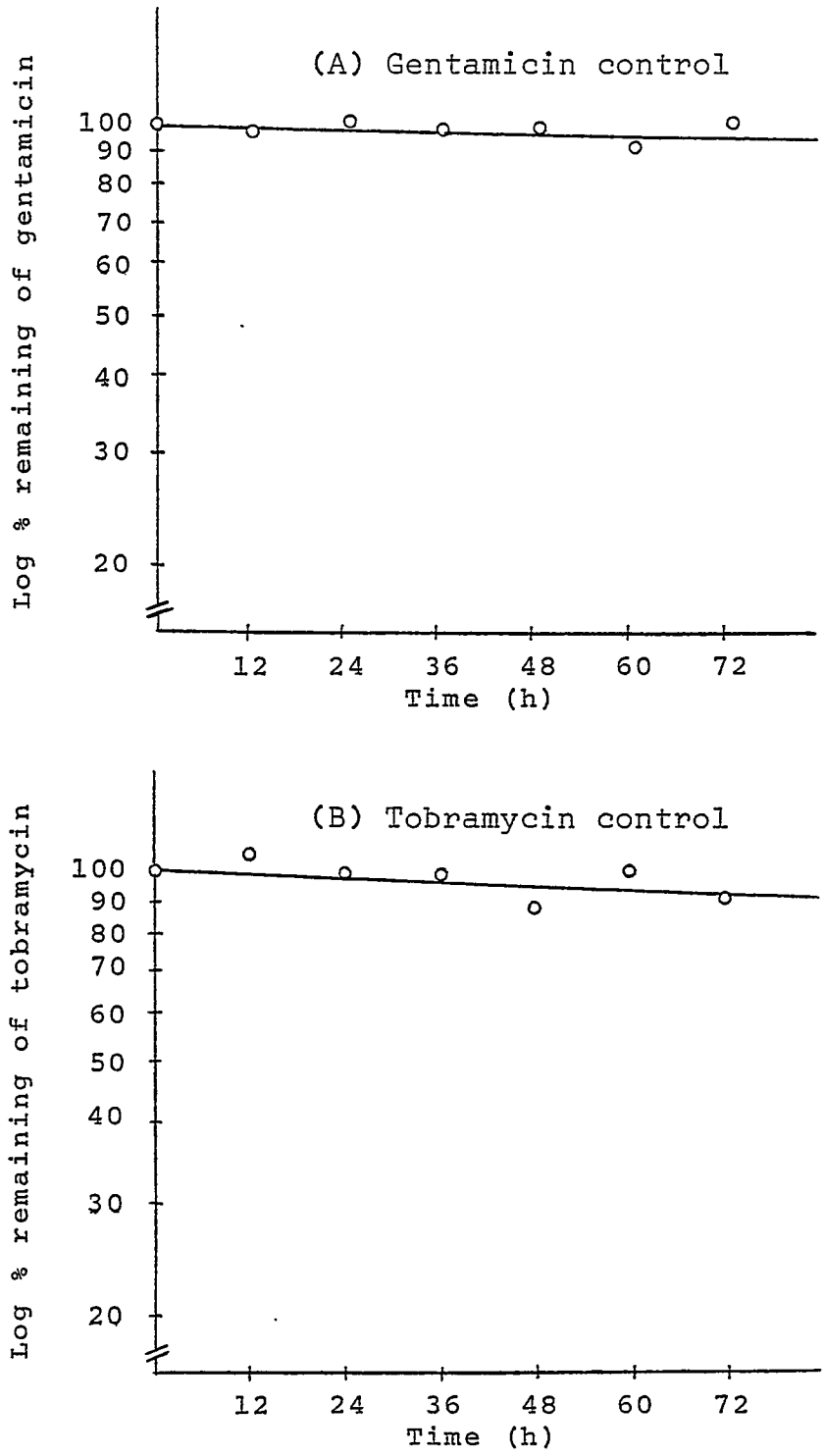


Figure 11: Degradation of gentamicin (A) (10µg/mL) and tobramycin (B) (10µg/mL) in plasma during 3 days' incubation at 37°C (controls) (the above is one of the duplicate results of the study)

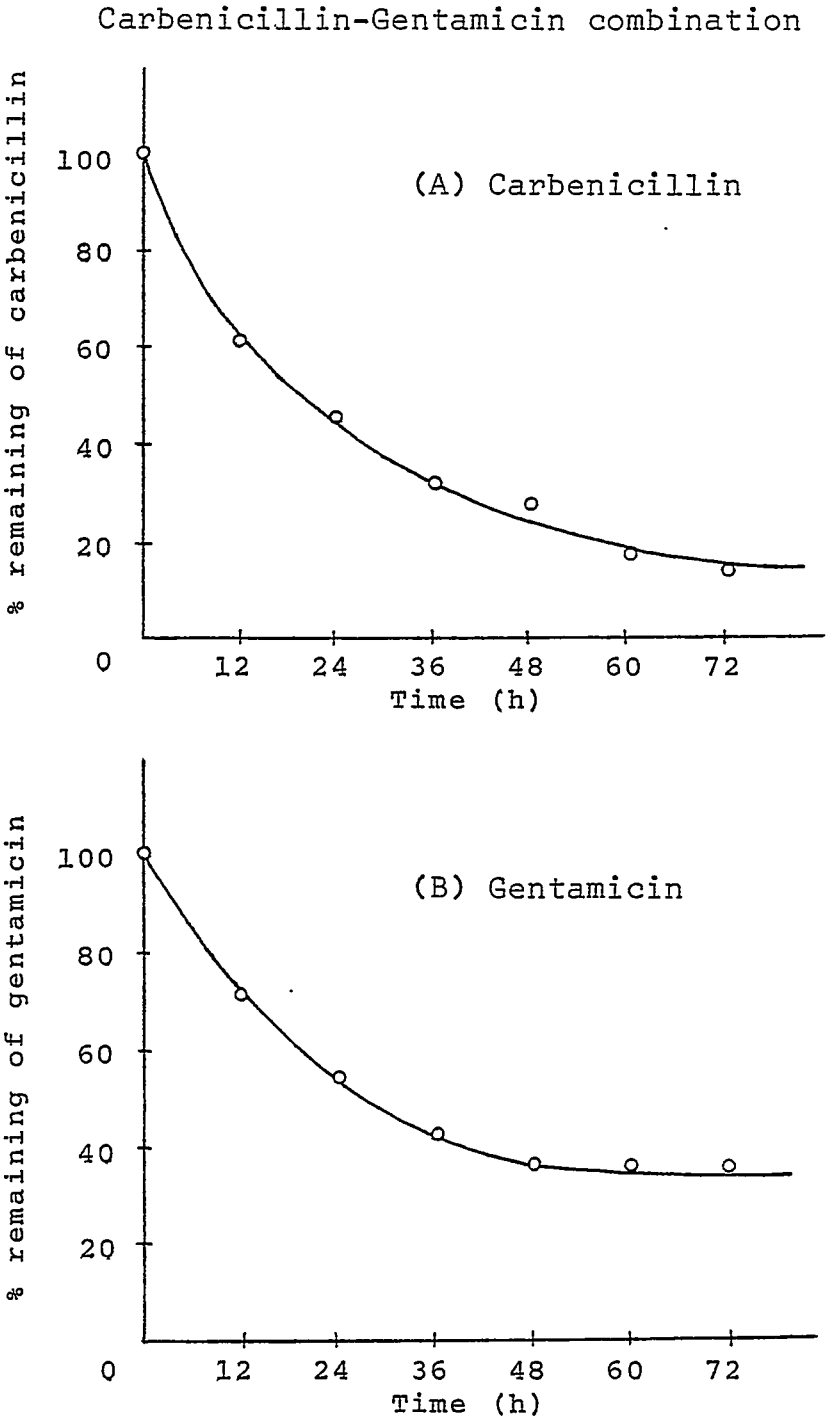


Figure 12: Degradation of carbenicillin (A) (200µg/mL) and gentamicin (B) (10µg/mL) in carbenicillin-gentamicin mixture during 3 days' incubation in plasma at 37°C (the above is one of the duplicate results of the study)

Ticarcillin-Gentamicin combination

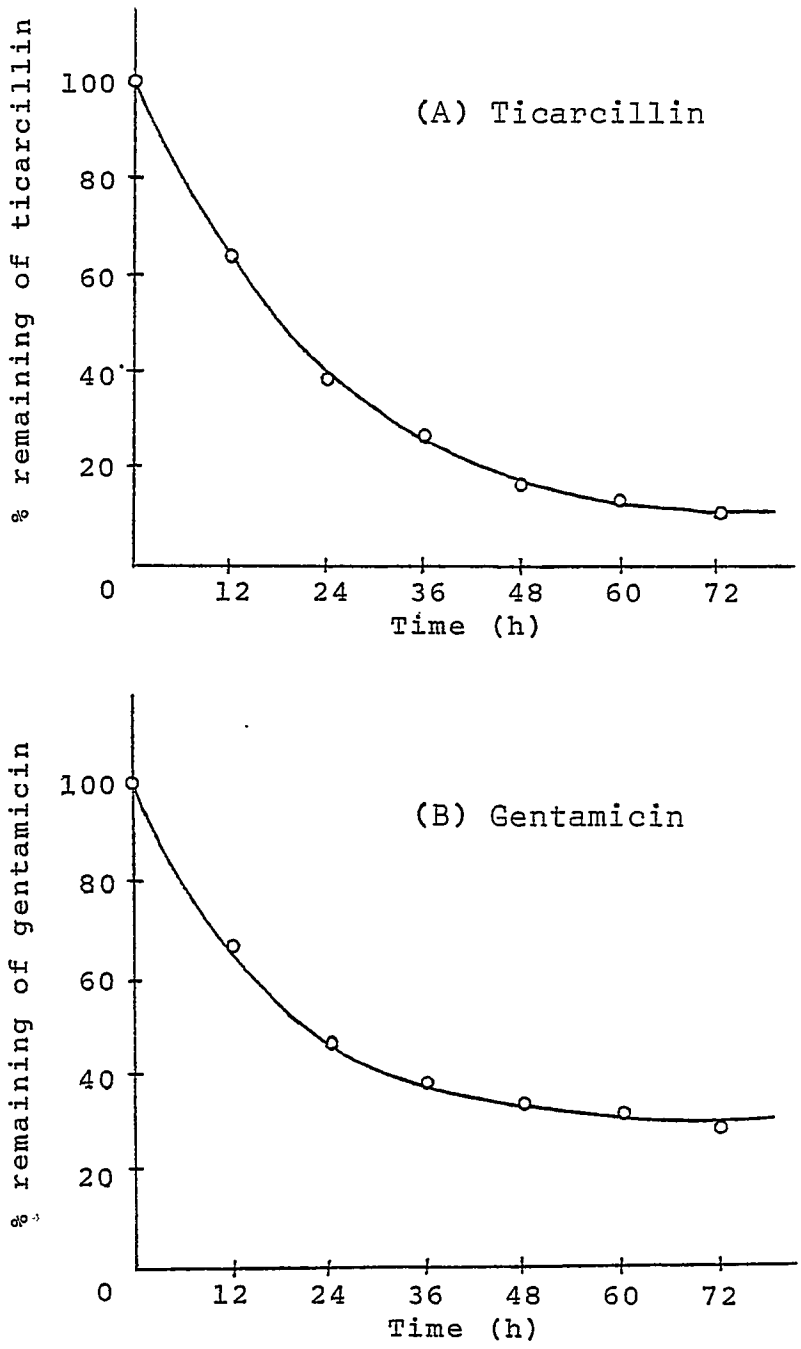


Figure 13: Degradation of ticarcillin (A) (200µg/mL) and gentamicin (B) (10µg/mL) in ticarcillin-gentamicin mixture during 3 days' incubation in plasma at 37°C (the above is one of the duplicate results of the study)

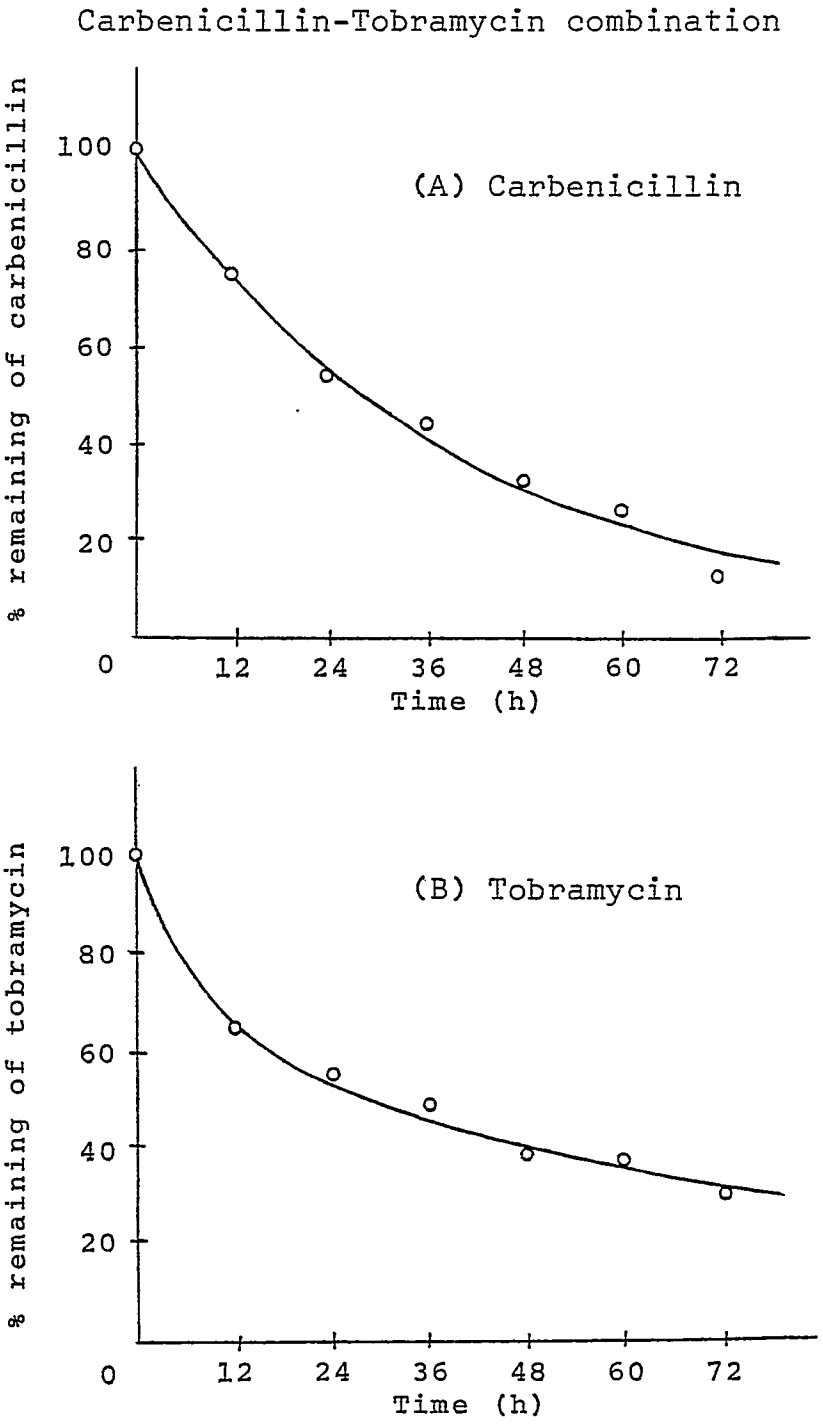


Figure 14: Degradation of carbenicillin (A) (200µg/mL) and tobramycin (B) (10µg/mL) in carbenicillin-tobramycin mixture during 3 days' incubation in plasma at 37°C (the above is one of the duplicate results of the study)

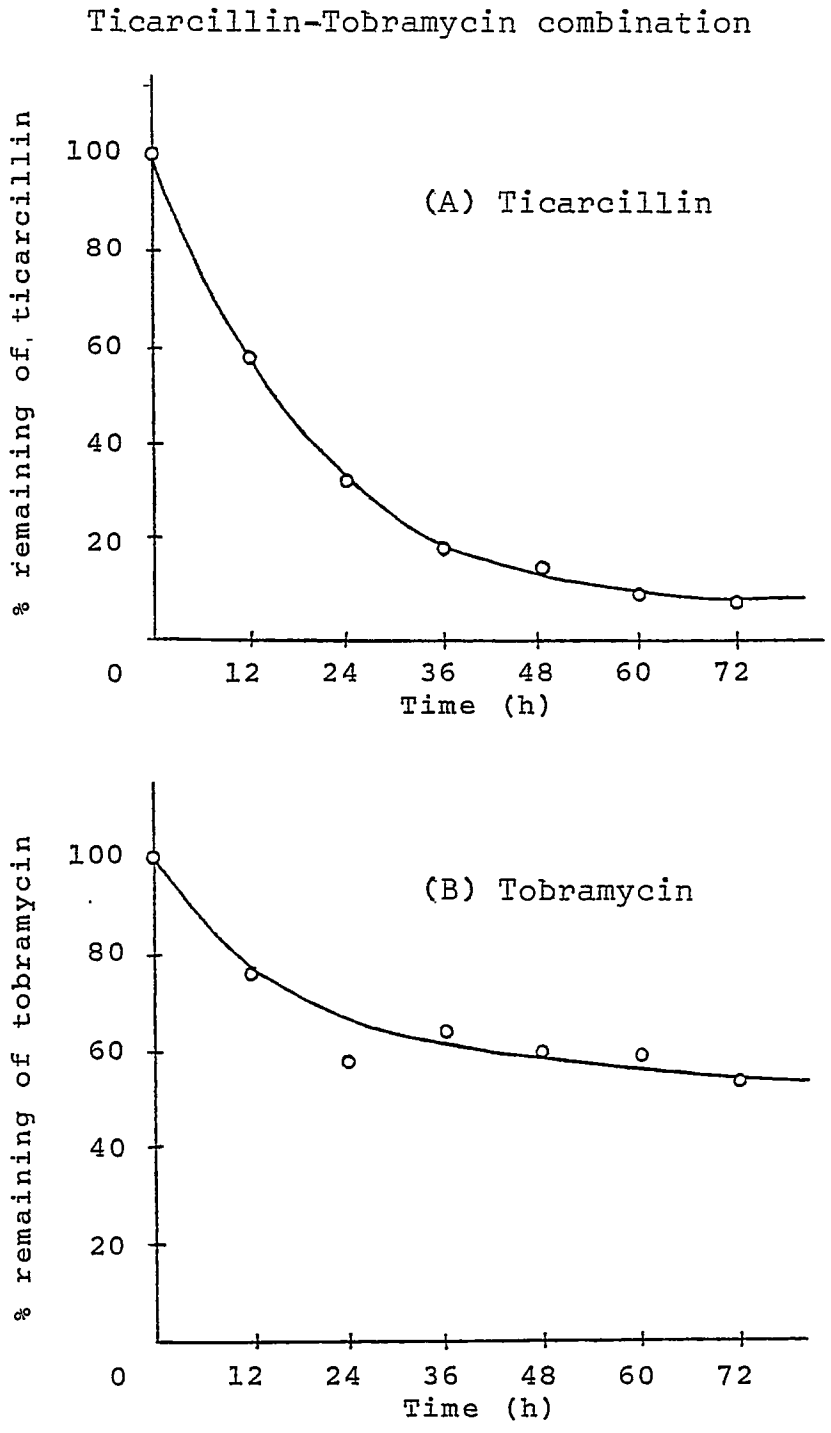


Figure 15: Degradation of ticarcillin (A) (200µg/mL) and tobramycin (B) (10µg/mL) in ticarcillin-tobramycin mixture during 3 days' incubation in plasma at 37°C (the above is one of the duplicate results of the study)

Carbenicillin-Gentamicin combination

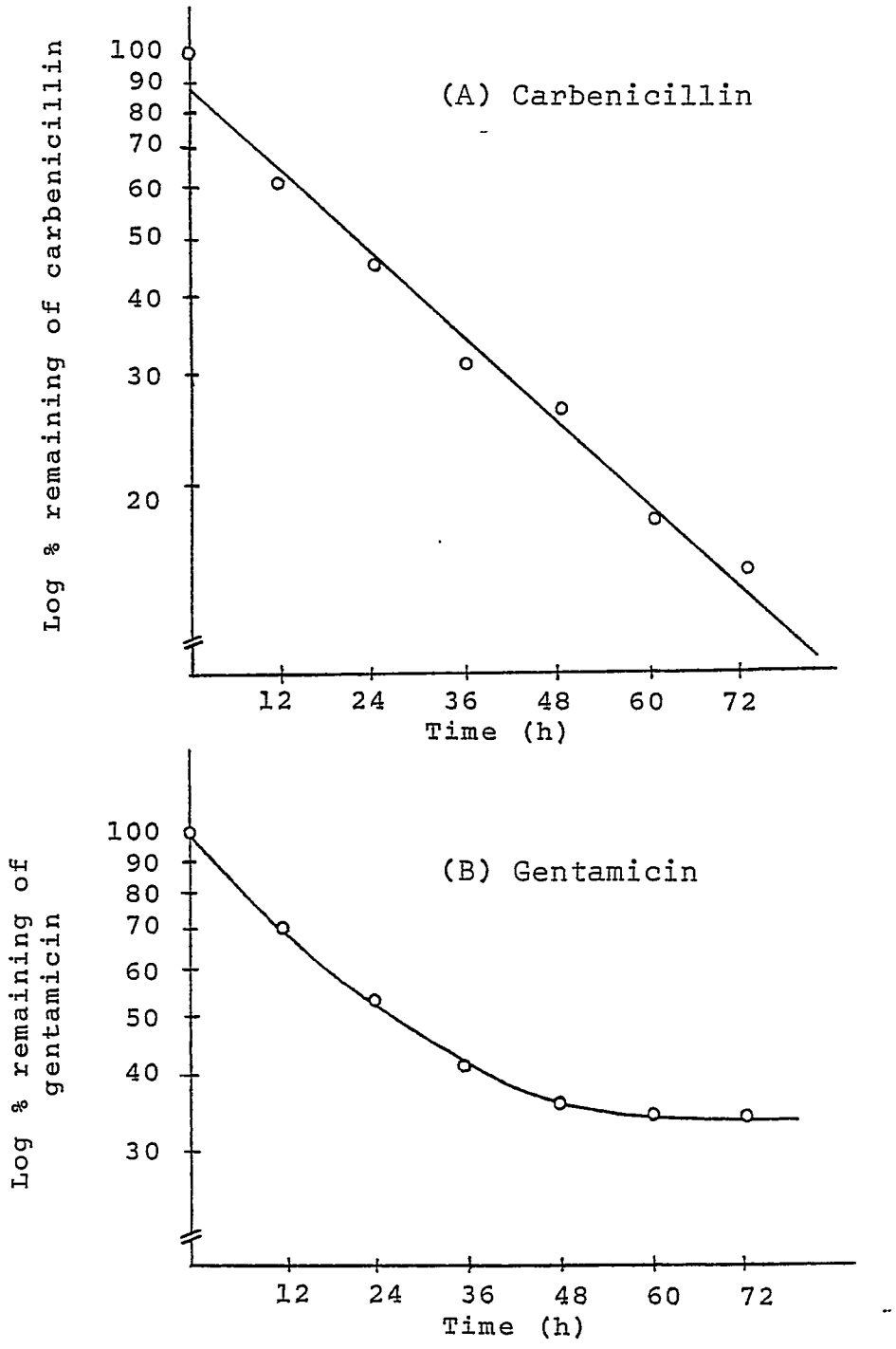


Figure 16: Degradation of carbenicillin (A) (200µg/mL) and gentamicin (B) (10µg/mL) in carbenicillin-gentamicin mixture during 3 days' incubation in plasma at 37°C (the above is one of the duplicate results of the study)

Ticarcillin-Gentamicin combination

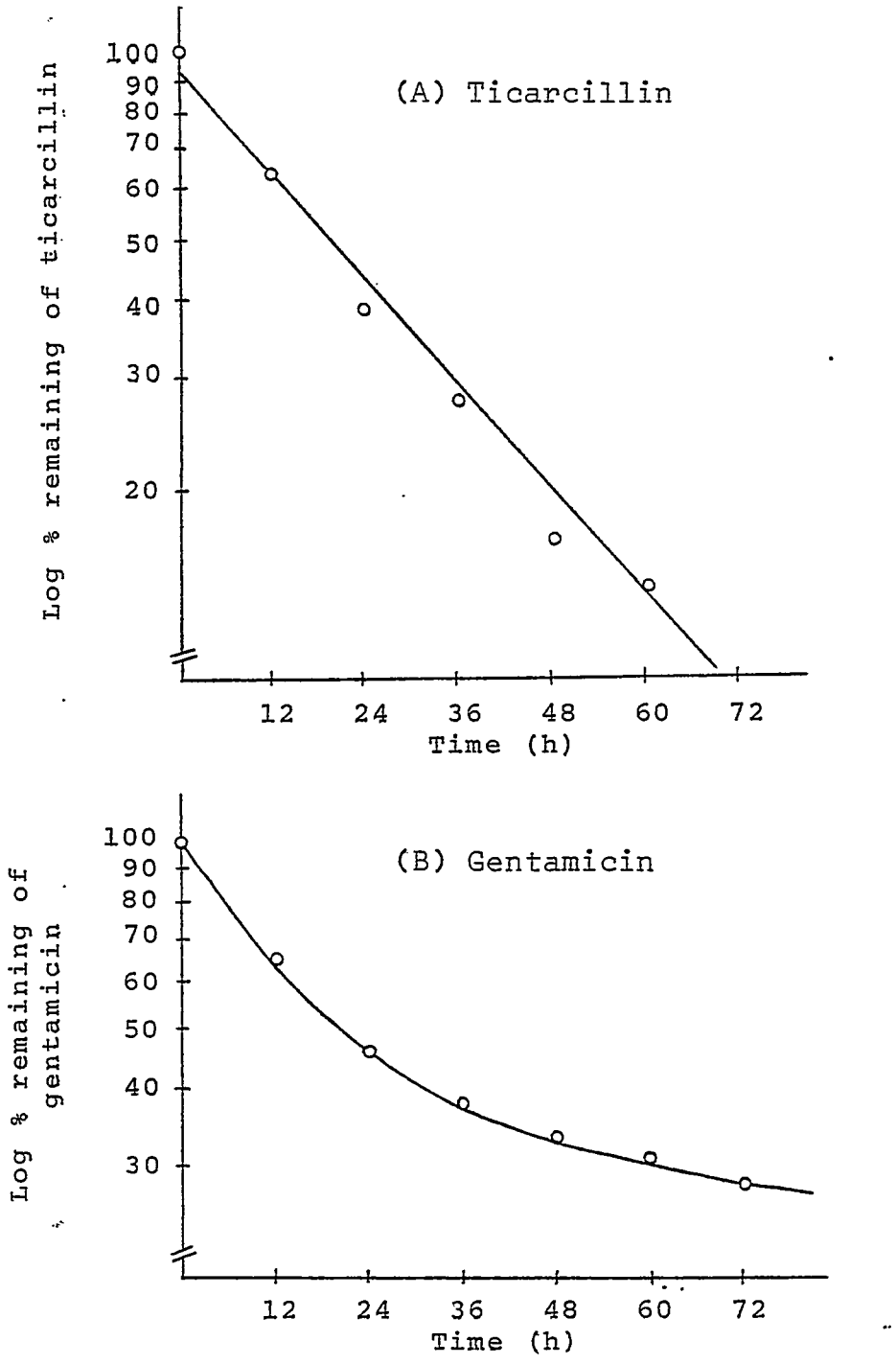


Figure 17: Degradation of ticarcillin (A) (200µg/mL) and gentamicin (B) (10µg/mL) in ticarcillin-gentamicin mixture during 3 days' incubation in plasma at 37°C (the above is one of the duplicate results of the study)

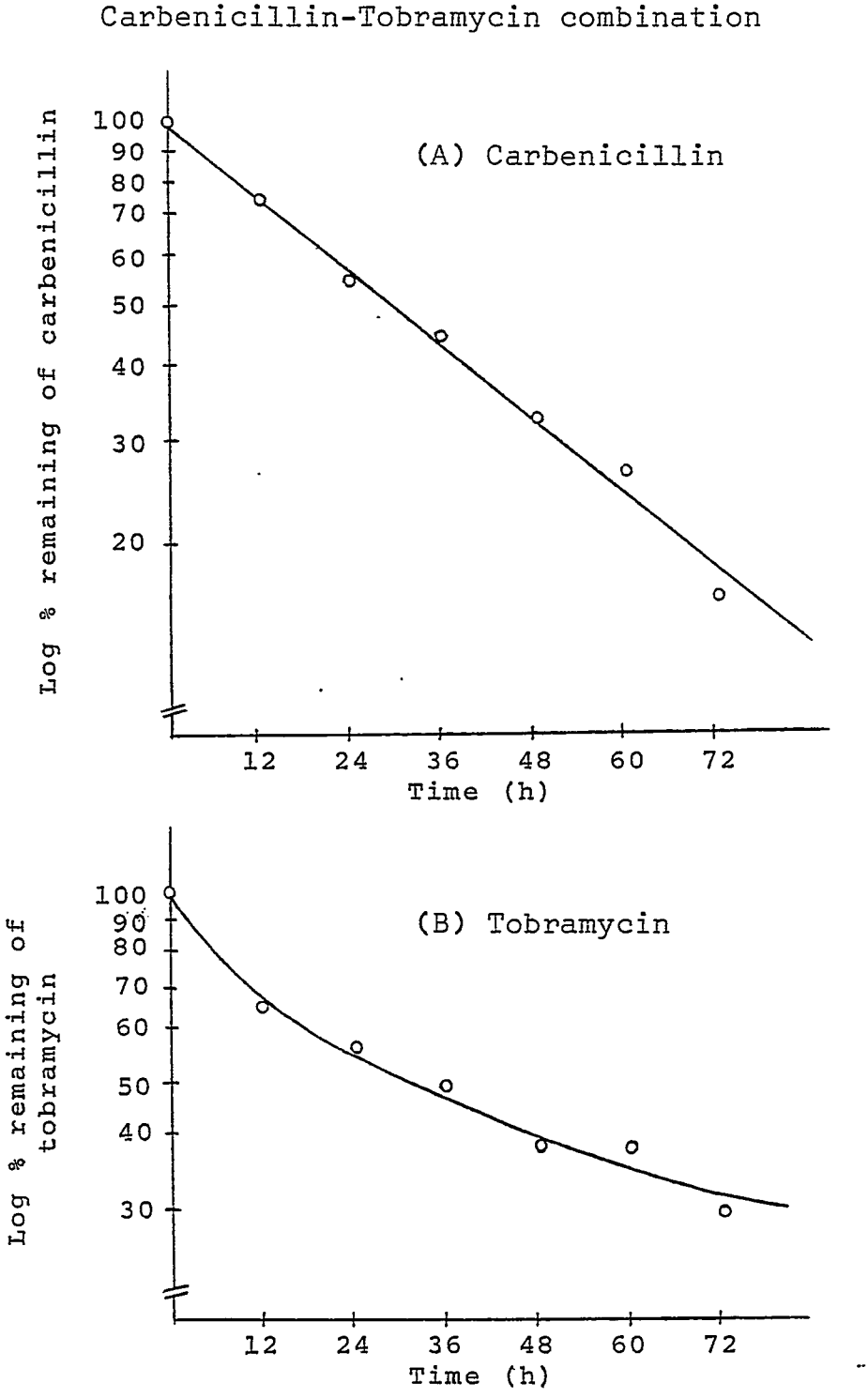


Figure 18: Degradation of carbenicillin (A) (200µg/mL) and tobramycin (B) (10µg/mL) in carbenicillin-tobramycin mixture during 3 days' incubation in plasma at 37°C (the above is one of the duplicate results of the study)

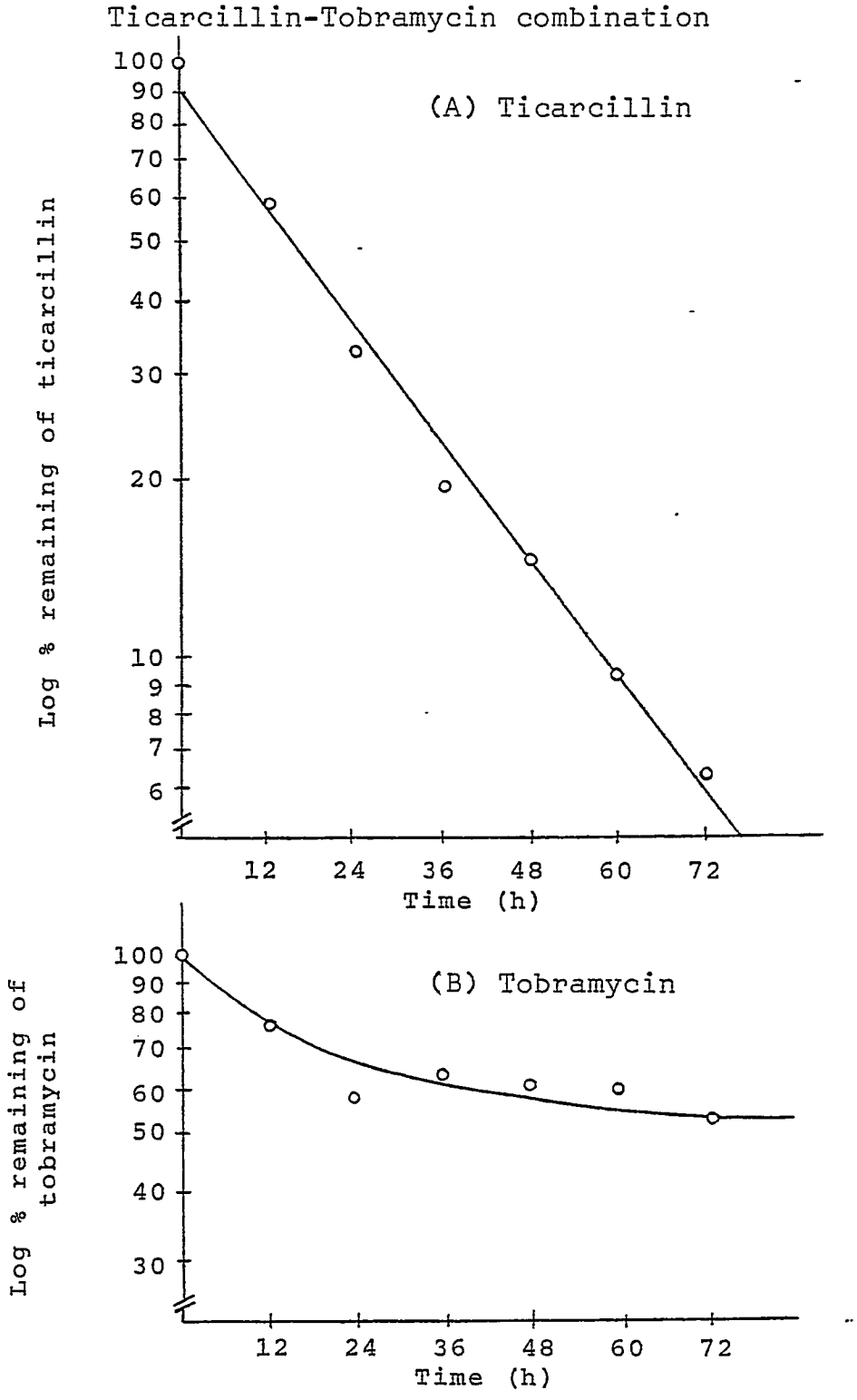


Figure 19: Degradation of ticarcillin (A) (200µg/mL) and tobramycin (B) (10µg/mL) in ticarcillin-tobramycin mixture during 3 days' incubation in plasma at 37°C (the above is one of the duplicate results of the study)

mixtures were still curves on semi-logarithmic graph paper, indicating that the degradation of aminoglycoside by penicillin was a second order or a more complex reaction (Figure 16-19B).

The degradation of the aminoglycosides was dependent on the concentration of the penicillin in incubation mixtures. The higher the concentration of penicillin, the greater the inactivation of the aminoglycoside. For example, while 60% or more of the initial aminoglycoside concentration remained at 24 hours after incubation with 100 µg/mL of penicillin, only 20% or less remained with 600 µg/mL of penicillin (Figure 20-21). The concentration of aminoglycoside did not have as much effect on the inactivation of penicillin as the concentration of penicillin on the inactivation of aminoglycoside. For example, while 60% of the initial carbenicillin concentration remained at 24 hours after incubation with 5 µg/mL of aminoglycoside, 40 to 50% remained with 20 µg/mL of aminoglycoside. The % of ticarcillin remaining at 24 hours after incubation with 20 µg/mL and with 5 µg/mL of aminoglycoside were approximately the same -- 30 to 40% (Figure 22-23).

5. Degradation of Penicillins

Degradation of ticarcillin was more rapid than carbenicillin. The average degradation constants of carbenicillin and ticarcillin in controls were 1.77×10^{-2} h^{-1} ($\pm$ 0.406×10^{-2} S.D.)

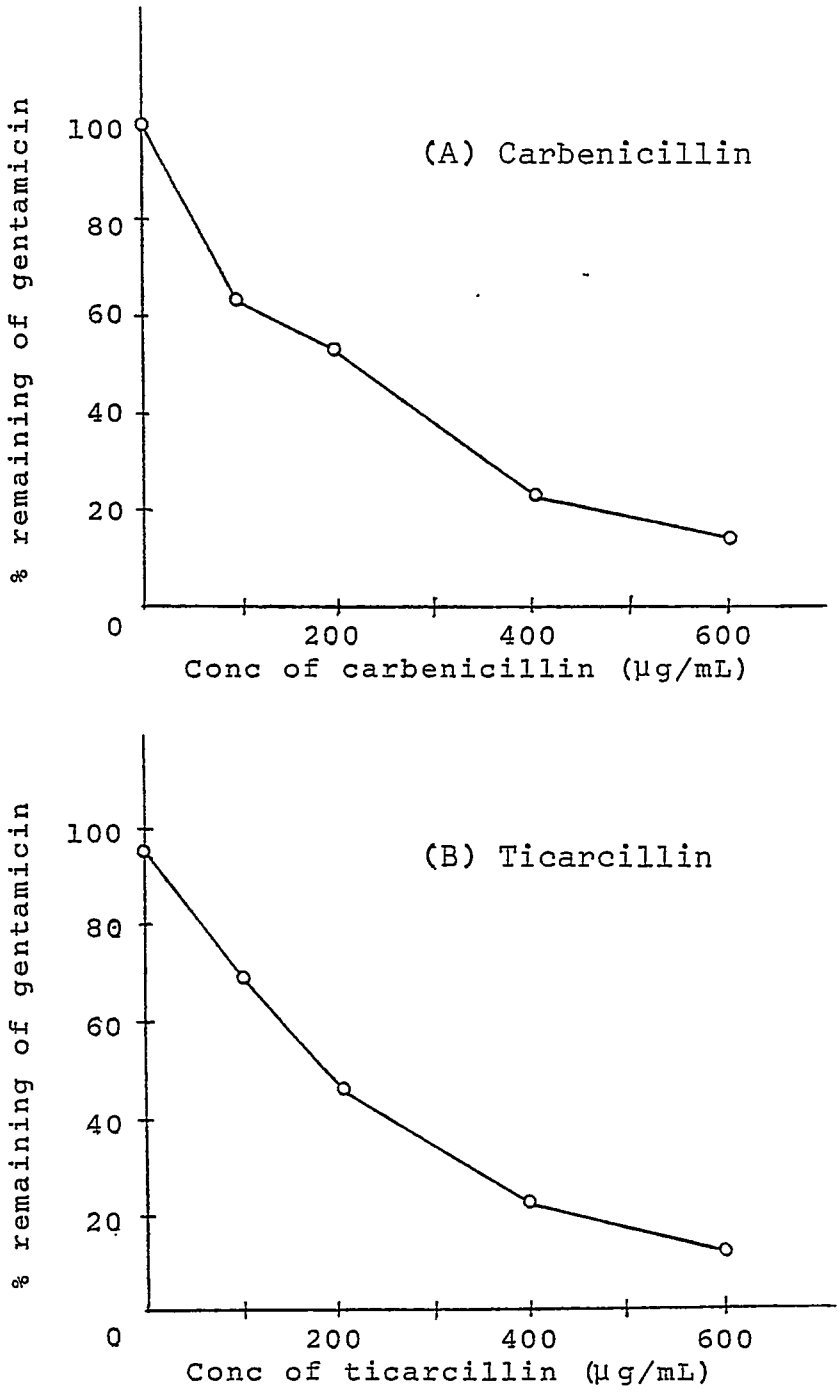


Figure 20: Percentage remaining of gentamicin (10µg/mL) after incubation with various concentrations of carbenicillin (A) and ticarcillin (B) in plasma at 37°C for 24h (the above is one of the duplicate results of the study)

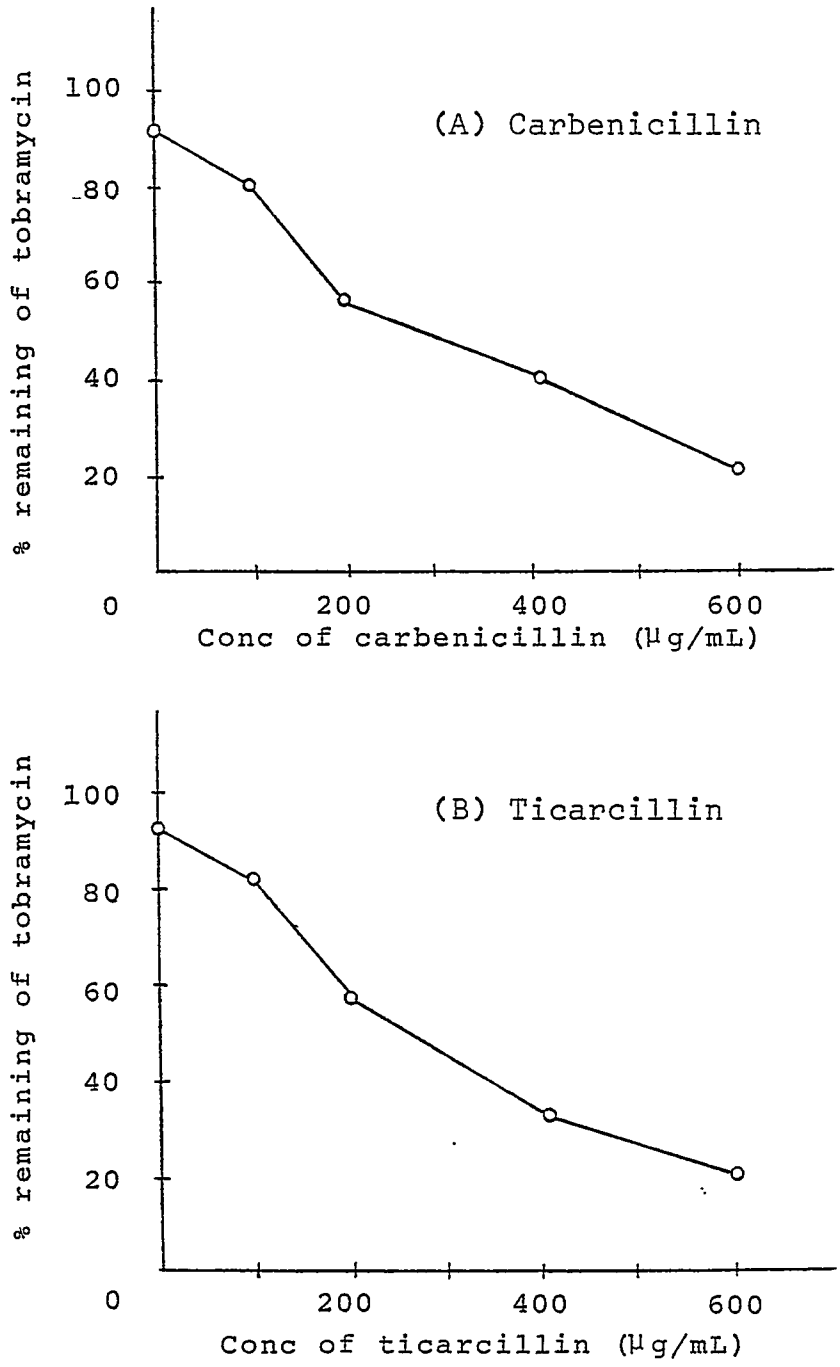


Figure 21: Percentage remaining of tobramycin (10µg/mL) after incubation with various concentrations of carbenicillin (A) and ticarcillin (B) in plasma at 37°C for 24h (the above is one of the duplicate results of the study)

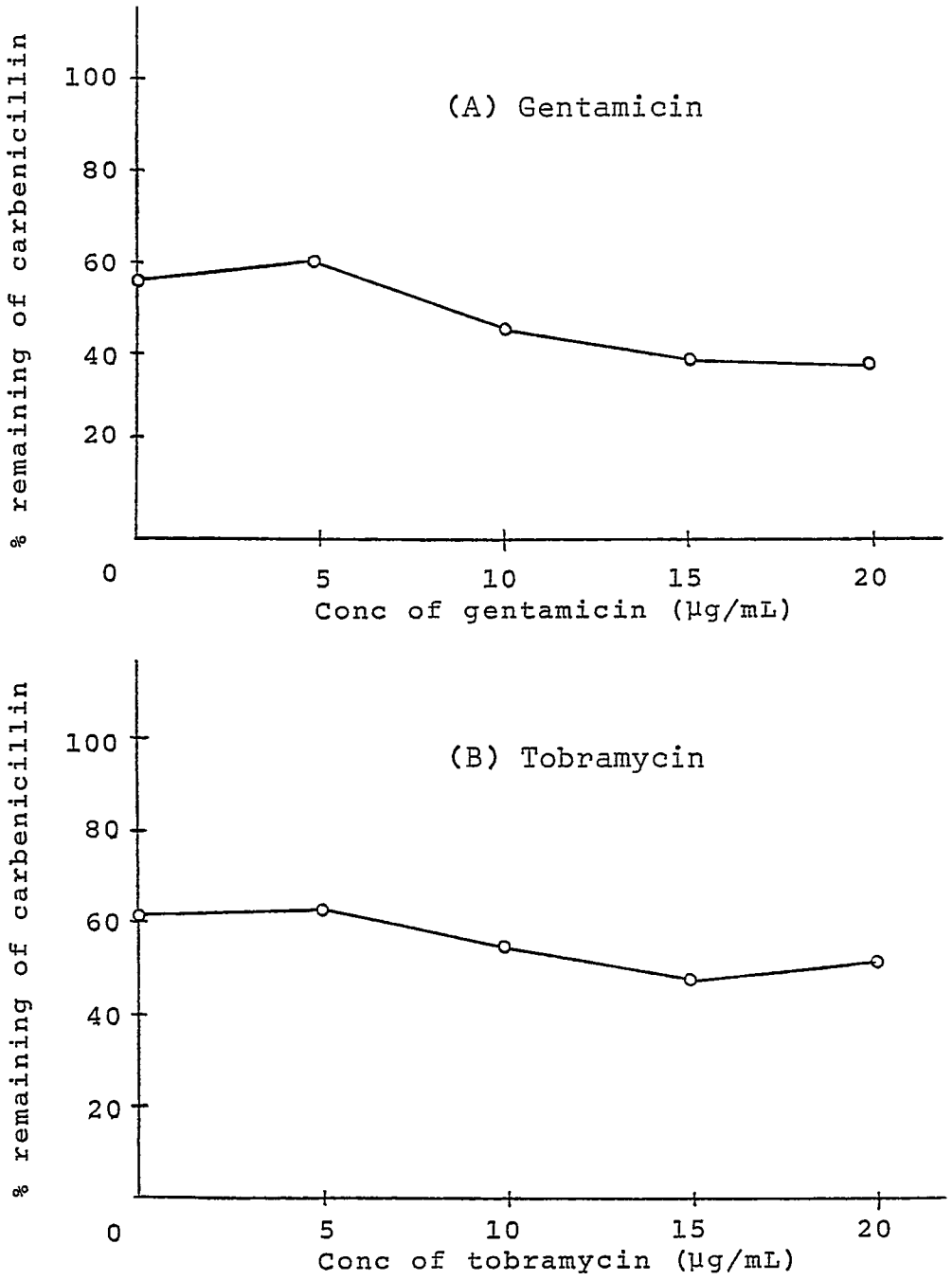


Figure 22: Percentage remaining of carbenicillin (200µg/mL) after incubation with various concentrations of gentamicin (A) and tobramycin (B) in plasma at 37°C for 24h (the above is one of the duplicate results of the study)

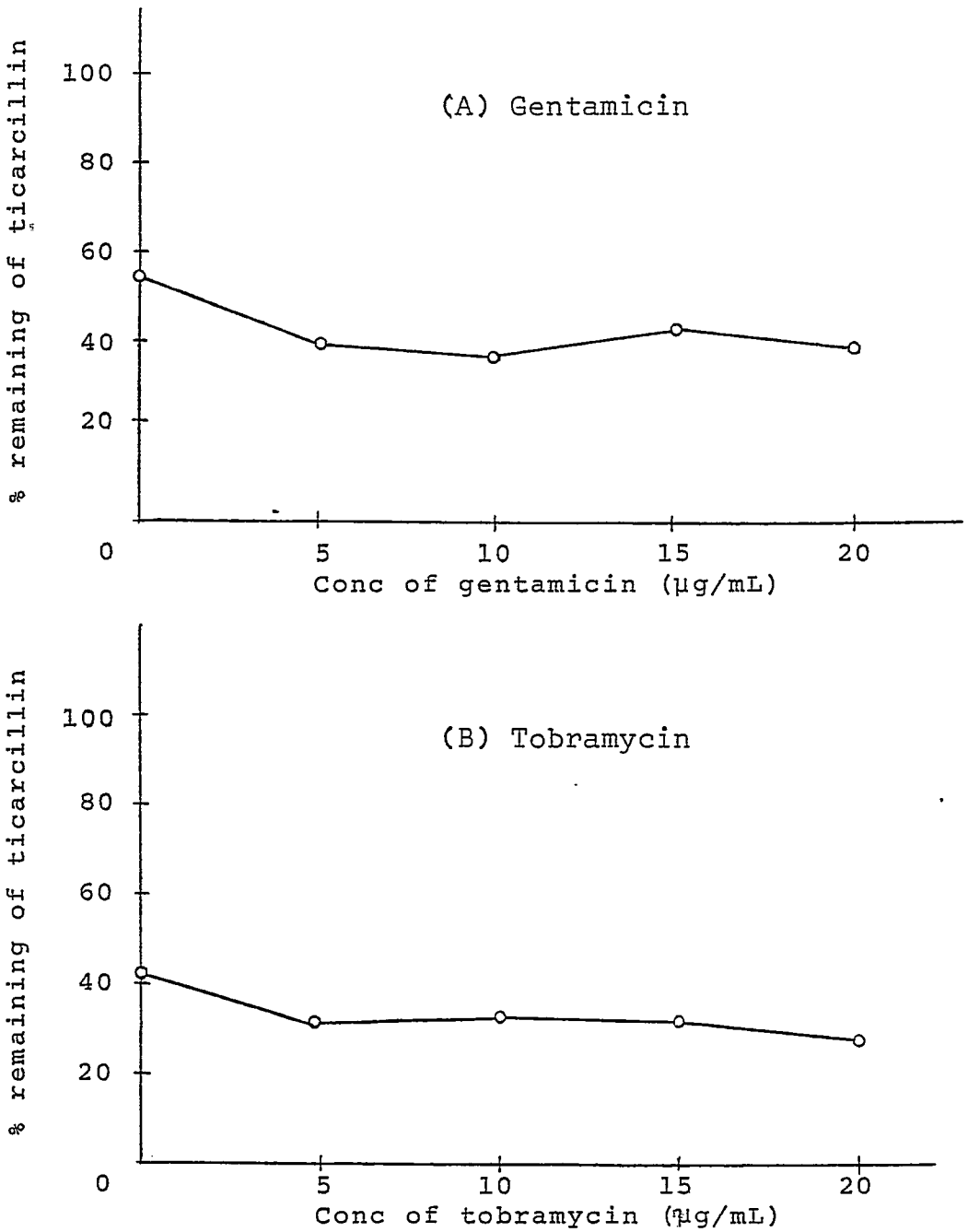


Figure 23: Percentage remaining of ticarcillin (200µg/mL) after incubation with various concentrations of gentamicin (A) and tobramycin (B) in plasma at 37°C for 24h (the above is one of the duplicate results of the study)

and 2.63×10^{-2} h^{-1} ($\pm$ 0.927×10^{-2} S.D.), respectively (Table 6). The degradation constants of both carbenicillin and ticarcillin were statistically related to the concentrations of penicillins (one-way analysis of variance, $p<0.05$). Multiple comparison of means indicated that the degradation constants of carbenicillin at concentrations other than 200 and 400 µg/mL were significantly different. For ticarcillin, there was no significant difference in the degradation constants between the two lower concentrations -- 100 and 200 µg/mL -- or between the two higher concentrations -- 400 and 600 µg/mL (Table 7). The reasons for these differences in the K_p at higher penicillin concentrations were not investigated.

The degradation constants of penicillins in antibiotic mixtures were only slightly larger than their controls, with the averages being 2.34×10^{-2} h^{-1} ($\pm$ 0.486×10^{-2} S.D.) (in carbenicillin-gentamicin combinations) and 2.15×10^{-2} h^{-1} ($\pm$ 0.565×10^{-2} S.D.) (in carbenicillin-tobramycin combinations) for carbenicillin, and 2.71×10^{-2} h^{-1} ($\pm$ 0.629×10^{-2} S.D.) (in ticarcillin-gentamicin combinations) and 3.29×10^{-2} h^{-1} ($\pm$ 1.080×10^{-2} S.D.) (in ticarcillin-tobramycin combinations) for ticarcillin (Table 8-11). The degradation constants of carbenicillin were related to the concentration of carbenicillin, gentamicin and tobramycin. However, the degradation constants of ticarcillin were related to the concentration of ticarcillin and gentamicin

Table 6: The degradation constants of carbenicillin and ticarcillin in controls

Penicillin	Concentration of penicillin (μg/mL)	K_P ($h^{-1} \times 10^{-2}$)	$\bar{K}_P \pm$ S.D. ($h^{-1} \times 10^{-2}$)	$\bar{K}_P \pm$ S.D. ($h^{-1} \times 10^{-2}$)
Carbenicillin	100	2.51 2.09 2.02 2.50	2.28 ± 0.259	
	200	2.10 1.71 1.39 1.72	1.73 ± 0.287	
				1.77 ± 0.406
	400	1.85 1.79 1.40 1.89	1.73 ± 0.222	
	600	1.47 1.43 1.16 1.29	1.34 ± 0.142	
Ticarcillin	100	3.17 3.09 3.17 4.75	3.54 ± 0.804	
	200	2.56 2.16 3.64 4.02	3.08 ± 0.887	
				2.63 ± 0.927
	400	1.97 1.90 2.49 2.17	2.13 ± 0.266	
	600	1.81 1.37 1.90 1.96	1.76 ± 0.267	

Table 7: ANOVA table of one-way analysis of variance and multiple comparison of means on the degradation constants of penicillins in controls

Penicillin	Source of variation	d_f	SS	MS	F	Concentration of antibiotic# (µg/mL)
Carbenicillin	Concentration of carbenicillin	3	1.81×10^{-4}	0.60×10^{-4}	10.00*	100 200 400 600
	Error	12	0.66×10^{-4}	0.06×10^{-4}		
Ticarcillin	Concentration of ticarcillin	3	8.22×10^{-4}	2.74×10^{-4}	7.03*	100 200 400 600
	Error	12	4.73×10^{-4}	0.39×10^{-4}		

d_f: degrees of freedom; SS: sum of squares; MS: mean square
*: significant ($p<0.05$)
#: lines join concentrations which are not significantly different (Newman-Keuls test)

Table 8: The degradation constants of carbenicillin in carbenicillin-gentamicin combinations

	K_p (h^{-1} x 10^{-2})				
Concentration of carbenicillin (µg/mL)	Concentration of gentamicin (µg/mL) 5	10	15	20	$\bar{K}_p \pm$ S.D. (h^{-1} x 10^{-2})
100	2.55 2.58	2.30 2.41	2.88 2.45	3.13 3.04	2.67 ± 0.311
200	2.41 2.56	2.40 2.63	2.75 2.90	2.68 3.33	2.71 ± 0.302
400	2.27 1.78	2.12 2.20	1.88 1.90	2.40 2.20	2.10 ± 0.215
600	1.62 1.33	1.87 1.75	1.58 1.70	2.74 2.43	1.88 ± 0.472
$\bar{K}_p \pm$ S.D. (h^{-1} x 10^{-2})	2.14 ±0.491	2.21 ±0.292	2.26 ±0.550	2.75 ±0.397	2.34 ± 0.486

Table 9: The degradation constants of ticarcillin in ticarcillin-gentamicin combinations

K_P (h^{-1} x 10^{-2})					
Concentration of ticarcillin (µg/mL)	Concentration of gentamicin (µg/mL) 5	10	15	20	$\bar{K}_P \pm$ S.D. (h^{-1} x 10^{-2})
100	3.31 2.91	3.59 3.07	3.78 3.43	4.24 3.55	3.48 ± 0.416
200	2.84 2.56	3.19 2.43	2.91 2.86	3.28 3.24	2.91 ± 0.311
400	1.95 2.45	2.33 2.44	2.26 2.34	2.45 3.07	2.41 ± 0.313
600	2.04 1.74	1.96 1.94	2.41 1.88	2.22 2.20	2.05 ± 0.215
$\bar{K}_P \pm$ S.D. (h^{-1} x 10^{-2})	2.47 ±0.540	2.62 ±0.602	2.73 ±0.635	3.03 ±0.710	2/71 ± 0.629

Table 10: The degradation constants of carbenicillin in carbenicillin-tobramycin combinations

Concentration of carbenicillin (µg/mL)	K_P (h^{-1} x 10^{-2}) Concentration of tobramycin (µg/mL) 5	10	15	20	$\bar{K}_P \pm$ S.D. (h^{-1} x 10^{-2})
100	2.01 2.00	1.90 2.72	2.44 2.74	2.61 2.81	2.40 ± 0.377
200	1.48 1.90	2.37 2.90	2.80 2.37	3.27 3.26	2.54 ± 0.639
400	1.37 1.74	2.02 2.01	1.67 1.45	2.21 2.69	1.89 ± 0.433
600	1.22 1.66	1.35 2.48	1.90 1.57	1.71 2.14	1.75 ± 0.411
$\bar{K}_P \pm$ S.D. (h^{-1} x 10^{-2})	1.67 ±0.295	2.22 ±0.500	2.12 ±0.536	2.59 ±0.548	2.15 ± 0.565

Table 11: The degradation constants of ticarcillin in ticarcillin-tobramycin combinations

	K_P (h^{-1} x 10^{-2})				
Concentration of ticarcillin (µg/mL)	Concentration of tobramycin (µg/mL)				$\bar{K}_P \pm$ S.D. (h^{-1} x 10^{-2})
	5	10	15	20	
100	2.67 4.79	2.79 5.02	2.78 5.06	2.81 4.81	3.84 ± 0.116
200	3.70 4.19	3.82 4.68	3.86 4.17	5.57 4.90	4.36 ± 0.643
400	3.00 2.52	2.66 2.50	2.74 2.83	2.94 2.90	2.76 ± 0.187
600	2.23 1.98	1.92 1.98	2.43 2.33	2.46 2.27	2.20 ± 0.212
$\bar{K}_P \pm$ S.D. (h^{-1} x 10^{-2})	3.13 ±0.993	3.17 ±1.192	3.27 ±0.977	3.58 ±1.291	3.29 ± 1.080

but not tobramycin (two-way analysis of variance, $p<0.05$). Multiple comparison of means showed that there was no significant difference in the degradation constants of carbenicillin between the two lower carbenicillin concentrations -- 100 and 200 µg/mL -- in both carbenicillin-aminoglycoside combinations, between the two higher carbenicillin concentrations -- 400 and 600 µg/mL -- in the carbenicillin-tobramycin combinations, within the gentamicin concentration range of 5 to 15 µg/mL and within the tobramycin concentration range of 10 to 15 µg/mL. There was no significant difference in the degradation constants of ticarcillin between the two lower ticarcillin concentrations -- 100 and 200 µg/mL -- or between the two higher ticarcillin concentrations -- 400 and 600 µg/mL -- in the ticarcillin-tobramycin combinations and within the gentamicin concentration range of 5 to 15 µg/mL (Table 12). The degradation constants of carbenicillin and ticarcillin were about 25% larger for a gentamicin concentration of 20 µg/mL compared to 5 µg/mL. The degradation constant of carbenicillin was about 50% larger for a tobramycin concentration of 20 µg/mL compared to 5 µg/mL, and the degradation constant of ticarcillin was about 15% larger for a tobramycin concentration of 20 µg/mL compared to 5 µg/mL (Table 8-11).

The t_{50} values for penicillins in controls and antibiotic mixtures were calculated from the degradation constants of

Table 12: ANOVA table of two-way analysis of variance and multiple comparison of means on the degradation constants of penicillins in antibiotic mixtures

Antibiotic combination	Source of variation	d_f	SS	MS	F	Concentration of penicillin# (μg/mL)
Carbenicillin -gentamicin	Concentration of carbenicillin	3	4.10×10^{-4}	1.37×10^{-4}	34.25*	100 200 400 600
	Concentration of gentamicin	3	1.80×10^{-4}	0.60×10^{-4}	15.00*	5 10 15 20
	Error	16	0.60×10^{-4}	0.04×10^{-4}		
Ticarcillin -gentamicin	Concentration of ticarcillin	3	9.40×10^{-4}	3.13×10^{-4}	34.78*	100 200 400 600
	Concentration of gentamicin	3	1.37×10^{-4}	0.46×10^{-4}	5.11*	5 10 15 20
	Error	16	1.36×10^{-4}	0.09×10^{-4}		
Carbenicillin -tobramycin	Concentration of carbenicillin	3	3.57×10^{-4}	1.19×10^{-4}	10.82*	100 200 400 600
	Concentration of tobramycin	3	3.44×10^{-4}	1.15×10^{-4}	10.45*	5 10 15 20
	Error	16	1.82×10^{-4}	0.11×10^{-4}		
Ticarcillin -tobramycin	Concentration of ticarcillin	3	23.31×10^{-4}	7.77×10^{-4}	12.14*	100 200 400 600
	Concentration of tobramycin	3	0.95×10^{-4}	0.32×10^{-4}	0.50^{NS}	5 10 15 20
	Error	16	10.27×10^{-4}	0.64×10^{-4}		

d_f: degrees of freedom; SS: sum of squares; MS: mean square
*: significant ($p<0.05$); NS: not significant ($p>0.05$)
#: lines join concentrations which are not significantly different (Newman-Keuls test)

penicillins (K_p) using the formula: $t_{50} = 0.693/K_p$ (Table 13-14). For carbenicillin, the t_{50} values in controls were larger than the t_{50} values in antibiotic mixtures (except for 100 and 400 µg/mL of carbenicillin). For ticarcillin, the t_{50} values in controls were similar to the t_{50} values in antibiotic mixtures (except for 600 µg/mL of ticarcillin). In both controls and mixtures, the t_{50} values were larger at higher concentrations of 400 and 600 µg/mL of penicillin than the t_{50} values at lower concentrations of 100 and 200 µg/mL of penicillin. For example, the t_{50} values of carbenicillin were about 30% larger at the higher concentrations of 400 and 600 µg/mL compared to the lower concentrations of 100 and 200 µg/mL of carbenicillin. The t_{50} values of ticarcillin were about 50 to 60% larger at the higher concentrations of 400 and 600 µg/mL compared to the lower concentrations of 100 and 200 µg/mL of ticarcillin.

6. Degradation of Aminoglycosides

Since gentamicin and tobramycin decayed very slowly in plasma during 3 days' incubation at 37°C, their degradation constants were quite small, with an average value of 0.92×10^{-3} h^{-1} ($\pm$ 0.703×10^{-3} S.D.) for gentamicin and 1.15×10^{-3} h^{-1} ($\pm$ 0.760×10^{-3} S.D.) for tobramycin (Table 15). The large variability of the degradation constants of gentamicin and tobramycin was probably due to the relatively small loss of drug during the incubation

Table 13: The t_{50} of carbenicillin and ticarcillin in controls

Penicillin	Concentration of penicillin (µg/mL)	t_{50} (h)	$\bar{t}_{50} \pm$ S.D. (h)	C.V. (%)
Carbenicillin	100	27.62 33.10 34.27 27.74	30.68 ± 3.50	11.41
	200	33.06 40.48 49.71 40.41	40.92 ± 6.82	16.67
	400	37.56 38.76 49.36 36.71	40.60 ± 5.90	14.53
	600	47.14 48.63 59.90 53.72	52.35 ± 5.77	11.02
Ticarcillin	100	21.84 22.44 21.90 14.60	20.20 ± 3.74	18.51
	200	27.56 32.13 19.03 17.26	24.00 ± 7.05	29.38
	400	35.16 36.47 27.80 31.92	32.84 ± 3.86	11.75
	600	38.31 50.62 36.51 35.36	40.20 ± 7.05	17.54

Table 14: The t_{50} of carbenicillin and ticarcillin in antibiotic mixtures

Antibiotic combination	Concentration of penicillin (µg/mL)	t_{50} (h) Concentration of aminoglycoside (µg/mL) 5	10	15	20	$\bar{t}_{50}$ ±S.D. (h)	C.V. (%)
Carbenicillin -gentamicin	100	27.19 26.82	30.16 28.74	24.05 28.33	22.11 32.87	27.53 ±3.37	12.24
	200	28.72 27.03	28.84 26.32	25.16 23.91	25.90 20.79	25.83 ±2.64	10.22
	400	30.58 38.87	32.63 31.47	36.78 36.45	28.89 31.50	33.40 ±3.52	10.54
	600	42.91 51.99	37.08 39.53	43.81 40.81	25.26 28.47	38.73 ±8.56	22.10
Ticarcillin -gentamicin	100	20.93 23.78	19.28 22.60	18.36 20.23	16.33 19.53	20.13 ±2.35	11.67
	200	24.41 27.12	21.71 28.47	23.81 24.21	21.16 21.42	24.04 ±2.67	11.11
	400	35.56 28.31	29.78 28.39	30.70 29.62	28.32 22.59	29.16 ±3.57	12.24
	600	34.00 39.83	35.39 35.80	28.78 36.78	31.23 31.53	34.17 ±3.54	10.36
Carbenicillin -tobramycin	100	34.51 34.67	36.40 25.45	28.40 25.32	26.60 24.64	29.50 ±4.88	16.54
	200	46.98 36.55	29.27 23.91	24.75 29.24	21.19 21.26	29.14 ±8.82	30.27
	400	50.77 39.83	34.27 34.55	41.40 47.76	31.43 25.74	38.22 ±8.38	21.93
	600	56.71 41.82	51.52 28.00	36.51 44.17	40.57 32.41	41.46 ±9.48	22.87
Ticarcillin -tobramycin	100	26.00 14.50	24.82 13.81	24.94 13.70	24.66 14.41	19.61 ±5.90	30.09
	200	18.73 16.55	18.14 14.80	17.95 16.61	12.45 14.15	16.17 ±2.20	13.61
	400	23.12 27.47	26.01 27.71	25.30 24.51	23.59 23.95	25.21 ±1.74	6.90
	600	31.03 35.09	36.02 34.96	28.51 29.81	28.23 30.53	31.77 ±3.12	9.82

Table 15: The degradation constants of gentamicin and tobramycin in controls

Aminoglycoside	Concentration of aminoglycoside (μg/mL)	K_A (h^{-1} x 10^{-3})	$\bar{K}_A \pm$ S.D. (h^{-1} x 10^{-3})	$\bar{K}_A \pm$ S.D. (h^{-1} x 10^{-3})
Gentamicin	5	0.13 0.64 2.18 0.38	0.83 ± 0.923	
	10	ND 0.73 0.67 0.50	0.63 ± 0.119	
				0.98 ± 0.683
	15	1.14 0.76 2.66 0.83	1.35 ± 0.892	
	20	ND 0.78 1.09 1.22	1.03 ± 0.225	
Tobramycin	5	0.75 1.03 0.24 1.45	0.87 ± 0.507	
	10	1.36 1.47 0.30 1.42	1.14 ± 0.559	
				1.15 ± 0.760
	15	1.65 0.25 2.28 0.46	1.16 ± 0.968	
	20	0.67 0.70 1.35 2.98	1.42 ± 1.083	

ND: not determined, the slope of the curve was not significantly different from zero in these samples

period. More reliable degradation constants would be obtained by incubating the control solutions for a longer period of time and calculating the degradation constants from the logarithmic plot of % aminoglycoside remaining versus time. Unfortunately, loss of solutions by evaporation during the longer incubation period made the assessment impossible. The degradation constants of gentamicin and tobramycin in controls were not statistically related to the concentration of aminoglycoside (one-way analysis of variance, $p > 0.05$) (Table 16).

The t_{50} values of aminoglycosides in controls were calculated from the degradation constants of aminoglycoside in controls (K_A) using the formula: $t_{50} = 0.693/K_A$ (Table 17). The average t_{50} values were greater than 25 days. The large variability was probably due to the small degradation constants.

The t_{50} values of aminoglycosides in antibiotic mixtures were estimated from the logarithmic degradation curves of the aminoglycoside. For a penicillin concentration of 100 µg/mL, the t_{50} values of both gentamicin and tobramycin were longer than 72 hours and as the concentration of penicillin became higher, the t_{50} values of both gentamicin and tobramycin became shorter (Table 18). The t_{50} was more a function of the specific penicillin concentration rather than the penicillin-to-aminoglycoside ratio. For example, although the ticarcillin-to-gentamicin ratio in antibiotic

Table 16: ANOVA table of one-way analysis of variance on the degradation constants of aminoglycosides in controls

Aminoglycoside	Source of variation	d_f	SS	MS	F
Gentamicin	Concentration of gentamicin	3	1.57×10^{-6}	0.52×10^{-6}	1.08^{NS}
	Error	11	5.35×10^{-6}	0.49×10^{-6}	
Tobramycin	Concentration of tobramycin	3	0.62×10^{-6}	0.21×10^{-6}	0.31^{NS}
	Error	12	8.04×10^{-6}	0.67×10^{-6}	

d_f: degrees of freedom; SS: sum of squares; MS: mean square
NS: not significant ($p>0.05$)

Table 17: The t_{50} of gentamicin and tobramycin in controls

Aminoglycoside	Concentration of aminoglycoside (µg/mL)	t_{50} (Day)	$\bar{t}_{50} \pm$ S.D. (Day)	C.V. (%)
Gentamicin	5	218.75 45.40 13.23 75.79	88.29 ± 90.65	102.67
	10	NC 39.61 43.03 58.33	46.99 ± 9.97	21.22
	15	25.33 37.99 10.85 35.00	27.29 ± 12.22	44.78
	20	NC 36.93 26.56 23.63	29.04 ± 6.99	24.07
Tobramycin	5	38.76 28.06 121.32 19.97	52.03 ± 46.83	90.01
	10	21.25 19.62 95.93 20.38	39.30 ± 37.76	96.08
	15	17.52 115.96 12.67 62.91	52.27 ± 48.12	92.06
	20	43.23 41.25 21.47 9.69	28.91 ± 16.15	55.86

NC: not calculated, the K_A was not determined in these samples

Table 18: The t_{50} of gentamicin and tobramycin in antibiotic mixtures

Antibiotic combination	Concentration of penicillin (µg/mL)	t_{50} (h) Concentration of aminoglycoside (µg/mL) 5	10	15	20	$\bar{t}_{50}$ ±S.D. (h)	C.V. (%)
Carbenicillin -gentamicin	100	>72 >72	>72 >72	>72 >72	>72 >72	-	-
	200	21.60 27.72	21.60 26.94	32.28 24.54	30.90 42.60	28.52 ±6.90	24.19
	400	9.00 11.04	7.92 9.36	10.08 12.00	9.36 11.28	10.01 ±1.36	13.59
	600	5.40 7.50	6.00 5.76	6.96 7.56	7.38 6.72	6.66 ±0.84	12.61
Ticarcillin -gentamicin	100	>72 67.80	68.40 >72	>72 >72	>72 >72	-	-
	200	26.04 32.04	21.36 30.60	24.30 25.20	23.52 28.44	26.44 ±3.65	13.81
	400	8.94 7.80	9.00 10.32	11.34 9.90	9.60 8.40	9.41 ±1.12	11.90
	600	7.50 6.36	5.10 4.20	5.76 4.32	6.60 6.18	5.75 ±1.15	20.00
Carbenicillin -tobramycin	100	45.90 >72	74.40 >72	>72 >72	>72 >72	-	-
	200	30.00 29.04	30.00 59.40	43.50 37.44	32.64 >72	-	-
	400	15.24 16.32	15.90 15.24	13.44 11.52	13.32 16.92	14.74 ±1.82	12.35
	600	10.32 11.04	8.28 9.96	6.90 7.50	8.76 8.70	8.93 ±1.42	15.90
Ticarcillin -tobramycin	100	>72 >72	>72 >72	>72 >72	>72 >72	-	-
	200	>72 67.80	>72 68.40	49.20 51.00	70.80 56.70	-	-
	400	22.50 19.92	12.24 11.40	11.64 9.12	10.68 9.00	13.31 ±5.05	37.94
	600	9.36 9.60	7.50 6.48	6.96 6.60	6.18 4.56	7.16 ±1.67	23.32

mixtures containing 200 µg/mL ticarcillin and 5 µg/mL gentamicin, 400 µg/mL ticarcillin and 10 µg/mL gentamicin or 600 µg/mL ticarcillin and 15 µg/mL gentamicin was the same -- 40 : 1 (Table 5), the average t_{50} values were 29.04, 9.66 and 5.04 hours, respectively (Table 18).

The t_{50} values of gentamicin and tobramycin were statistically related to the concentration of penicillin and the concentration of tobramycin but not the concentration of gentamicin (two-way analysis of variance, $p<0.05$). Multiple comparison of means showed that in carbenicillin-tobramycin combinations, only the t_{50} of 15 µg/mL of tobramycin was significantly different from the others. In ticarcillin-tobramycin combinations, there was no significant difference between the t_{50} values of tobramycin within the concentration range of 10 to 20 µg/mL of tobramycin (Table 19).

There was no significant difference in the t_{50} values of gentamicin between carbenicillin-gentamicin and ticarcillin-gentamicin combinations. However, there was a significant difference in the t_{50} of tobramycin between carbenicillin-tobramycin and ticarcillin-tobramycin combinations (three-way analysis of variance, $p<0.05$) (Table 20). At lower concentrations of 100 and 200 µg/mL of penicillins, the t_{50} values of carbenicillin-tobramycin combinations were smaller than those of ticarcillin-tobramycin combinations

Table 19: ANOVA table of two-way analysis of variance and multiple comparison of means on the t_{50} of aminoglycosides in antibiotic mixtures

Antibiotic combination	Source of variation	d_f	SS	MS	F	Concentration of antibiotic# (µg/mL)
Carbenicillin -gentamicin	Concentration[a] of carbenicillin	2	2218.82	1109.41	94.50*	100 200 400 600
	Concentration of gentamicin	3	93.00	31.00	2.64[NS]	5 10 15 20
	Error	12	140.82	11.74		
Ticarcillin -gentamicin	Concentration[a] of ticarcillin	2	1949.64	974.82	148.83*	100 200 400 600
	Concentration of gentamicin	3	7.13	2.38	0.36[NS]	5 10 15 20
	Error	12	78.65	6.55		
Carbenicillin -tobramycin	Concentration[b] of carbenicillin	1	134.79	134.79	98.39*	100 200 400 600
	Concentration of tobramycin	3	24.77	8.26	6.03	5 10 20 15
	Error	8	10.97	1.37		
Ticarcillin -tobramycin	Concentration[b] of ticarcillin	1	151.66	151.66	118.48*	100 200 400 600
	Concentration of tobramycin	3	145.83	48.61	37.98*	5 10 15 20
	Error	8	10.20	1.28		

d_f: degrees of freedom; SS: sum of squares; MS: mean square
*: significant ($p<0.05$); NS: not significant ($p>0.05$)
a: concentration of penicillin tested - 200, 400 & 600µg/mL
b: concentration Of penicillin tested - 400 & 600µg/mL
#: lines join concentrations which are not significantly different (Newman-Keuls test)

Table 20: ANOVA table of three-way analysis of variance on the t_{50} of aminoglycosides in antibiotic mixtures

Antibiotic combinations	Source of variation	d_f	SS	MS	F
Carbenicillin-gentamicin & Ticarcillin-gentamicin	Antibiotic combination	1	17.15	17.15	2.10^{NS}
	Concentration[a] of penicillin	2	4163.51	2081.76	255.43*
	Concentration of gentamicin	3	45.67	15.22	1.87^{NS}
	Error	23	187.38	8.15	
Carbenicillin-tobramycin & Ticarcillin-tobramycin	Antibiotic combination	1	20.51	20.51	15.19*
	Concentration[b] of penicillin	1	286.20	286.20	212.00*
	Concentration of tobramycin	3	124.38	41.46	30.71*
	Error	15	20.28	1.35	
Carbenicillin-gentamicin & Carbenicillin-tobramycin	Antibiotic combination	1	98.14	98.14	104.40*
	Concentration[b] of carbenicillin	1	167.45	167.45	178.14*
	Concentration of aminoglycoside	3	7.17	2.39	2.54^{NS}
	Error	15	14.14	0.94	
Ticarcillin-gentamicin & Ticarcillin-tobramycin	Antibiotic combination	1	56.13	56.13	122.02*
	Concentration[b] of ticarcillin	1	192.97	192.97	419.50*
	Concentration of aminoglycoside	3	73.62	24.54	53.35*
	Error	15	6.90	0.46	

d_f: degrees of freedom; SS: sum of squares; MS: mean square
*: significant ($p<0.05$); NS: not significant ($p>0.05$)
a: concentration of penicillin tested - 200, 400 & 600µg/mL
b: concentration of penicillin tested - 400 & 600µg/mL

However, at higher concentrations of 400 and 600 µg/mL of penicillins, the t_{50} values of carbenicillin-tobramycin combinations were larger than those of ticarcillin-tobramycin combinations (Table 18). There were significant differences in the t_{50} of aminoglycosides between carbenicillin-gentamicin and carbenicillin-tobramycin combinations and between ticarcillin-gentamicin and ticarcillin-tobramycin combinations (three-way analysis of variance, $p<0.05$) (Table 20). The t_{50} values of gentamicin were slightly smaller than those of tobramycin in the antibiotic mixtures (Table 18).

The degradation constants of aminoglycosides in antibiotic mixtures, calculated from computer fitting, were less than 1×10^{-8} h^{-1}. Computer estimates of these values were probably small because of the limited number of data points in the terminal portion of the curve. The loss of aminoglycosides during the incubation period was due mainly to the interaction rather than the degradation. More accurate values of K_A could be obtained by prolonging the incubation to a time when penicillin concentrations were negligible, i.e., $K_i \times P < K_A$.

7. Interaction Rate Constants

The interaction rate constants (K_i) were calculated from computer fitting (Table 21-24). The mean values of the interaction rate constants of the four antibiotic mixtures ranged from approximately 1.5×10^{-4} to 2.3×10^{-4} mL/µgxh.

Table 21: The interaction constants of carbenicillin-gentamicin combinations

K_i (mL/µgxh x 10^{-4})

Concentration of carbenicillin (µg/mL)	Concentration of gentamicin (µg/mL) 5	10	15	20	$\bar{K}_i \pm$ S.D. (mL/µgxh x 10^{-4})
100	1.75	2.84	2.84	2.40	2.38 ± 0.469
	1.62	2.75	2.45	2.37	
200	2.74	2.66	2.23	2.64	2.44 ± 0.357
	1.82	2.49	2.83	2.08	
400	2.56	1.46	2.42	2.48	2.13 ± 0.388
	1.84	2.13	2.30	1.81	
600	1.55	1.42	1.91	1.95	1.64 ± 0.229
	1.28	1.73	1.58	1.68	
$\bar{K}_i \pm$ S.D. (mL/µgxh x 10^{-4})	1.90 ±0.501	2.19 ±0.584	2.32 ±0.428	2.18 ±0.346	2.14 ± 0.476

Table 22: The interaction constants of ticarcillin-gentamicin combinations

K_i (mL/μgxh x 10^{-4})					
Concentration of ticarcillin (μg/mL)	Concentration of gentamicin (μg/mL) 5	10	15	20	$\bar{K}_i \pm$ S.D. (mL/μgxh x 10^{-4})
100	1.36 2.56	2.48 2.33	2.54 3.69	3.10 4.67	2.84 ± 0.992
200	2.37 1.91	2.85 2.20	2.73 2.61	2.58 2.53	2.47 ± 0.303
400	1.46 1.90	2.38 2.06	2.06 3.05	2.58 2.43	2.24 ± 0.481
600	1.04 1.14	1.98 1.86	1.58 2.18	1.93 1.69	1.68 ± 0.405
$\bar{K}_i \pm$ S.D. (mL/μgxh x 10^{-4})	1.72 ±0.560	2.27 ±0.316	2.56 ±0.644	2.69 ±0.909	2.31 ± 0.719

Table 23: The interaction constants of carbenicillin-tobramycin combinations

K_i (mL/µgxh x 10^{-4})					
Concentration of carbenicillin (µg/mL)	Concentration of tobramycin (µg/mL)				$\bar{K}_i \pm$ S.D. (mL/µgxh x 10^{-4})
	5	10	15	20	
100	2.81 1.73	1.89 1.44	1.85 1.93	2.87 1.95	2.06 ± 0.509
200	0.84 1.63	1.80 1.15	1.29 1.50	1.75 1.06	1.38 ± 0.347
400	1.30 1.19	0.99 1.41	0.91 1.58	1.83 1.38	1.32 ± 0.301
600	1.32 1.27	1.35 1.22	1.41 1.55	1.57 1.76	1.43 ± 0.182
$\bar{K}_i \pm$ S.D. (mL/µgxh x 10^{-4})	1.51 ±0.591	1.41 ±0.309	1.50 ±0.320	1.75 ±0.530	1.55 ± 0.453

Table 24: The interaction constants of ticarcillin-tobramycin combinations

	K_i (mL/μgxh x 10^{-4})				
Concentration of ticarcillin (μg/mL)	Concentration of tobramycin (μg/mL)				$\bar{K}_i \pm$ S.D. (mL/μgxh x 10^{-4})
	5	10	15	20	
100	0.56	1.00	0.38	#	2.04 ± 1.510
	2.24	3.47	2.25	4.39	
200	1.10	1.66	1.41	1.69	1.61 ± 0.352
	1.44	1.40	1.95	2.22	
400	1.03	1.58	1.62	1.95	1.57 ± 0.444
	0.77	1.76	1.84	2.01	
600	1.14	1.46	1.58	1.85	1.58 ± 0.324
	1.14	1.64	1.74	2.06	
$\bar{K}_i \pm$ S.D. (mL/μgxh x 10^{-4})	1.18 ±0.504	1.75 ±0.735	1.60 ±0.554	2.31 ±0.932	1.69 ± 0.770

#: the K_i was not significantly different from zero

The interaction rate constants were statistically related to the concentration of penicillin in all antibiotic mixtures and the concentration of aminoglycoside in ticarcillin-aminoglycoside mixtures (two-way analysis of variance, $p<0.05$). Multiple comparison of means showed that only the interaction rate constant of the lower penicillin concentration -- 100 µg/mL -- in penicillin-tobramycin mixtures or that of the higher penicillin concentration -- 600 µg/mL -- in penicillin-gentamicin mixtures was significantly different from the others. There was no significant difference in the interaction rate constants within the gentamicin concentration range of 10 to 20 µg/mL and within the tobramycin concentration range of 10 to 15 µg/mL (Table 25).

Although the interaction constants (K_i) decreased with increasing penicillin concentrations, the "effective" interaction rate constants ($K_i \times P$) were larger for the higher penicillin concentrations. For example, in the carbenicillin-gentamicin combination, the average interaction rate constant at 100 µg/mL of carbenicillin -- 2.38×10^{-4} mL/µgxh -- was larger than the average interaction rate constant at 600 µg/mL -- 1.64×10^{-4} mL/µgxh. However, the "effective" interaction rate constant at 100 µg/mL of carbenicillin -- 2.38×10^{-2} h^{-1} (2.38×10^{-4} mL/µgxh x 100 µg/mL) -- was smaller than the "effective" interaction

Table 25: ANOVA table of two-way analysis of variance and multiple comparison of means on the interaction rate constants

Antibiotic combination	Source of variation	d_f	SS	MS	F	Concentration of antibiotic# (µg/mL)
Carbenicillin -gentamicin	Concentration of carbenicillin	3	3.18×10^{-8}	1.06×10^{-8}	9.64*	100 200 400 600
	Concentration of gentamicin	3	0.77×10^{-8}	0.26×10^{-8}	2.36NS	5 10 15 20
	Error	16	1.75×10^{-8}	0.11×10^{-8}		
Ticarcillin -gentamicin	Concentration of ticarcillin	3	5.51×10^{-8}	1.84×10^{-8}	8.36*	100 200 400 600
	Concentration of gentamicin	3	4.45×10^{-8}	1.48×10^{-8}	6.73*	5 10 15 20
	Error	16	3.47×10^{-8}	0.22×10^{-8}		
Carbenicillin -tobramycin	Concentration of carbenicillin	3	2.83×10^{-8}	0.94×10^{-8}	6.27*	100 200 400 600
	Concentration of tobramycin	3	0.59×10^{-8}	0.20×10^{-8}	1.33NS	5 10 15 20
	Error	16	2.35×10^{-8}	0.15×10^{-8}		
Ticarcillin -tobramycin	Concentration[a] of ticarcillin	2	0.01×10^{-8}	0.01×10^{-8}	0.25NS	100 200 400 600
	Concentration of tobramycin	3	2.32×10^{-8}	0.77×10^{-8}	19.25*	5 10 15 20
	Error	12	0.51×10^{-8}	0.04×10^{-8}		

d_f: degrees of freedom; SS: sum of squares; MS: mean square
*: significant ($p<0.05$); NS: not significant ($p>0.05$)
a: concentration of ticarcillin tested - 200, 400 & 600µg/mL
#: lines join concentrations which are not significantly different (Newman-Keuls test)

rate constant at 600 µg/mL -- 9.84×10^{-2} h^{-1} (1.64×10^{-4} mL/µgxh x 600 µg/mL) (Table 21). In other words, at higher penicillin concentration loss of aminoglycosides due to the interaction was more rapid and the degradation t_{50} smaller.

There was a significant difference between the interaction rate constants of the four penicillin-aminoglycoside combinations (three-way analysis of variance, $p<0.05$). Multiple comparison of means showed that there was no significant difference in the interaction rate constants (or t_{50} values) between carbenicillin-gentamicin and ticarcillin-gentamicin combinations (Table 26). The interaction rate constants of gentamicin-penicillin combinations were slightly larger than those of tobramycin-penicillin combinations.

The assumption that the contribution of the interaction to the decrease in penicillin concentration was negligible was examined by comparing values of $K_p \times P$ and $K_i \times A \times P$ (for $t = 0$) using the carbenicillin-gentamicin combination as an example. The ratio of $K_p \times P$ to $K_i \times A \times P$ for the carbenicillin-gentamicin combination ranged from approximately 6 : 1 to 30 : 1. The lower the concentration of aminoglycoside, the greater was the ratio of $K_p \times P$ to $K_i \times A \times P$ (Table 27). The assumption was also verified by computer simulation. Using the carbenicillin-gentamicin

Table 26: ANOVA table of three-way analysis of variance and multiple comparison of means on the interaction rate constants

Source of variation	d_f	SS	MS	F	Multiple comparison of means#
Antibiotic combination	1	9.70×10^{-8}	9.70×10^{-8}	149.69*	Carb-gen Tica-gen Carb-tob Tica-tob
Concentration[a] of penicillin	2	2.53×10^{-8}	1.27×10^{-8}	20.06*	
Concentration[b] of aminoglycoside	3	3.38×10^{-8}	1.13×10^{-8}	16.98*	
Error	71	4.56×10^{-8}	0.06×10^{-8}		

d_f: degrees of freedom; SS: sum of squares; MS: mean square
*: significant ($p<0.05$)
a: concentration of penicillin tested - 200, 400 & 600µg/mL
b: concentration of aminoglycoside tested - 5, 10, 15 & 20µg/mL
#: line join antibiotic combinations which are not significantly different (Newman-Keuls test) (Abbreviations - Carb: carbenicillin; Tica: ticarcillin; gen: gentamicin; tob: tobramycin)

Table 27: Comparison of initial values of $K_P xP$ and $K_i xAxP$ using the carbenicillin-gentamicin combination as an example

Concentration of gentamicin (µg/mL)	Initial concentration of carbenicillin (µg/mL)	K_P ($h^{-1} x 10^{-2}$)	K_i (mL/µgxhx10^{-4})	$K_P xP$	$K_i xAxP$	$K_P xP / K_i xAxP$
5	100	2.55	1.75	2.55	0.09	28.33
	600	1.33	1.28	7.98	0.38	21.00
20	100	3.13	2.40	3.13	0.48	6.52
	600	2.74	1.95	16.44	2.34	7.03

combination as an example, the degradation of carbenicillin and gentamicin in two different combinations -- 100 µg/mL of carbenicillin & 5 µg/mL of gentamicin and 600 µg/mL of carbenicillin & 5 µg/mL of gentamicin -- were predicted by computer simulation using the differential equations: $dP/dt = -K_P \times P - K_i \times A \times P$ or $dP/dt = -K_P \times P$ and $dA/dt = -K_A \times A - K_i \times A \times P$ and the values of K_P, K_A and K_i in each combination. There were only minor differences in the concentrations of carbenicillin with and without the interaction term (Table 28).

Although many researchers have studied the interaction of penicillin and aminoglycoside, very few have calculated a rate constant for the interaction. Even in those studies calculating an interaction rate constant, major differences in the analysis of data between the present study and previously published reports make it difficult to compare results. Thompson, et al. (1982) studied the interaction of gentamicin and penicillins in vivo. In their study, twelve subjects with end-stage renal disease were given gentamicin alone (2 mg/Kg), in combination with carbenicillin (2 g every 8 hours for 6 doses) or in combination with piperacillin (4 g every 12 hours for 4 doses). Blood samples were drawn and individual's kinetic parameters were calculated. The interaction rate constant of each gentamicin-penicillin combination was calculated by subtracting the elimination rate constant of gentamicin with penicillin (β_{TP})

Table 28: Computer simulation of the degradation of carbenicillin (100 & 600µg/mL) and gentamicin (5µg/mL) in two carbenicillin-gentamicin combinations

Concentration of carbenicillin (µg/mL)	Time[a] (h)	With interaction for carbenicillin $dP/dt = -K_P \times P - K_i \times A \times P$ $dA/dt = -K_A \times A - K_i \times A \times P$		Without interaction for carbenicillin $dP/dt = -K_P \times P$ $dA/dt = -K_A \times A - K_i \times A \times P$	
		Concentration of carbenicillin (µg/mL)	Concentration of gentamicin (µg/mL)	Concentration of carbenicillin (µg/mL)	Concentration of gentamicin (µg/mL)
100	0	100.00	5.00	100.00	5.00
	12	72.94	4.18	73.64	4.17
	24	53.27	3.66	54.23	3.65
	36	38.94	3.33	39.93	3.31
	48	28.48	3.10	29.41	3.08
	60	20.84	2.95	21.61	2.92
	72	15.26	2.84	15.95	2.81
600	0	600.00	5.00	600.00	5.00
	12	508.88	2.14	511.49	2.13
	24	432.81	1.04	436.04	1.03
	36	368.53	0.56	371.72	0.56
	48	313.95	0.33	316.88	0.33
	60	267.53	0.21	270.14	0.21
	72	228.00	0.15	230.29	0.14

a: A 6-hour interval was used for computer simulation but only the data for 12-hour intervals are presented here for comparison

from the elimination rate constant of gentamicin alone (β), i.e., $K_i = \beta - \beta_{TP}$. This method of calculating a K_i value, however, assumes K_i is a first order rate constant (with the same unit as β, h^{-1}) and does not change as penicillin concentration changes with time. The *in vivo* interaction rate constants of gentamicin-carbenicillin combination reported by Thompson, *et al*. ranged from 0.0128 to 0.0363 h^{-1} with the average 0.0253 h^{-1} ($\pm$ 0.0114 S.D.). In order to compare these first order, *in vivo* interaction rate constants with our second order, *in vitro* interaction rate constants, these *in vivo* constants were divided by a serum carbenicillin level estimated as: $C_{p(0)} = D_o/V_d$. With an initial carbenicillin concentration of about 230 µg/mL, the *in vivo* interaction rate constant would therefore be converted to 1.1×10^{-4} mL/µgxh. The average of our *in vitro* interaction rate constants for the carbenicillin-gentamicin combination was 2.14×10^{-4} mL/µgxh ($\pm$ 0.476 S.D.) which was the same order of magnitude as the values calculated from the data of Thompson, *et al*.

Konishi, *et al*. (1983) also studied the interaction of tobramycin and penicillins *in vivo*. In their study, solutions containing 5 g of penicillin and 80 mg of tobramycin were administered intravenously to six adult male volunteers with normal renal function. Blood samples were drawn and individual's kinetic parameters were estimated. The

interaction rate constants of tobramycin-penicillin combinations were calculated by the same method as Thompson, et al. The reported in vivo interaction rate constants were 0.047 h^{-1} (± 0.039 S.D.) and 0.042 h^{-1} (± 0.026 S.D.) for tobramycin-carbenicillin and tobramycin-ticarcillin combinations, respectively. At the end of 1 hour infusion of 5 g of penicillins, serum penicillin levels would be about 400 µg/mL and 430 µg/mL for carbenicillin and ticarcillin, respectively (estimated as: $C_p = K_o \times (1-e^{-K_d t})/K_d \times V_d$). The second-order rate constants for the interaction estimated from the reported interaction rate constants and the initial penicillin concentrations would, therefore, be 1.18×10^{-4} mL/µgxh and 0.98×10^{-4} mL/µgxh for tobramycin-carbenicillin and tobramycin-ticarcillin combinations, respectively. The average of our in vitro interaction rate constants were 1.55×10^{-4} mL/µgxh (± 0.453 S.D.) and 1.69×10^{-4} mL/µgxh (± 0.770 S.D.) for tobramycin-carbenicillin and tobramycin-ticarcillin, respectively, i.e., in the same order of magnitude as the values calculated from the data of Konishi, et al. Interpretation of the data of Konishi, et al. is complicated since the drugs were mixed prior to administration. The authors fail to note that changes in the peak concentration in the presence of the penicillin are larger than would be anticipated by the relatively small difference in rate constants.

8. Clinical Significance of the Interaction

In vivo, the loss of aminoglycoside would be the sum of elimination of aminoglycoside by the kidney and interaction with beta-lactam penicillin to form an inactive amide. The overall rate of loss could be described by the following formula:

$$dA/dt = -k_d \times A - K_i \times A \times P = -(k_d + K_i \times P) \times A$$

where: A = Concentration of aminoglycoside (µg/mL)
P = Concentration of penicillin (µg/mL)
k_d = Elimination rate constant of aminoglycoside (h^{-1})
K_i = Interaction rate constant (mL/µgxh)
t = Time after drug administration (h)

In patients with normal renal function, elimination of both aminoglycoside and penicillin is rapid. The elimination rate constant of gentamicin or tobramycin is approximately 0.35 h^{-1} (i.e., $t_{\frac{1}{2}}$ = 2h) in patients with normal renal function. Since K_i is very small, k_d would be much larger than $K_i \times P$, i.e., loss due to interaction ($K_i \times A \times P$) would be significantly less than loss due to renal excretion ($k_d \times A$). In other words, the interaction of penicillin and aminoglycoside would not affect the elimination rate of aminoglycoside from the body. Therefore, in patients with normal renal function, the interaction of penicillin and aminoglycoside should not be clinically significant unless the concentration of penicillin is very high (Table 29). Although k_d is much larger than $K_i \times P$ in patients with

Table 29: Comparison of k_d and $K_i xP$ using carbenicillin-gentamicin as an example

Patient's renal function	k_d^a (h^{-1})	Concentration of carbenicillin (μg/mL)	K_i^b (mL/μgxhx10^{-4})	$K_i xP$ (h^{-1}x10^{-2})	$k_d/K_i xP$
Normal	0.35 ($t_{1/2}$ = 2h)	100	2.42	2.42	14.46
		200	2.44	4.88	7.17
		400	2.13	8.52	4.11
		600	1.64	9.84	3.56
Impaired	0.069 ($t_{1/2}$ = 10h)	100	2.42	2.42	2.85
		200	2.44	4.88	1.41
		400	2.13	8.52	0.81
		600	1.64	9.84	0.70
	0.035 ($t_{1/2}$ = 20h)	100	2.42	2.42	1.45
		200	2.44	4.88	0.72
		400	2.13	8.52	0.41
		600	1.64	9.84	0.36
	0.023 ($t_{1/2}$ = 30h)	100	2.42	2.42	0.95
		200	2.44	4.88	0.47
		400	2.13	8.52	0.27
		600	1.64	9.84	0.23

a: k_d ia a literature value for the elimination rate constant of aminoglycoside from the body with varying degrees of renal impairment

b: K_i is the average value for each concentration of carbenicillin (Table 21)

normal renal function, the ratio of k_d to K_i x P at a higher initial penicillin concentration is smaller than the ratio of k_d to K_i x P at a lower initial penicillin concentration. For example, at the lower initial carbenicillin concentration of 100 µg/mL, k_d is about 14 times larger than K_i x P but at the higher initial carbenicillin concentration of 600 µg/mL, k_d is only 4 times K_i x P. Therefore, the interaction of penicillin and aminoglycoside might become significant in patients with normal renal function when the concentration of penicillin is high.

In patients with impaired renal function, the elimination rate constant of aminoglycoside decreases substantially to about 0.023 to 0.012 h^{-1} for gentamicin and 0.014 to 0.010 h^{-1} for tobramycin. The k_d is only slightly larger or even smaller than K_i x P. The ratio of k_d to K_i x P decreases with increasing concentration of penicillin and/or with decreasing elimination rate constant of aminoglycoside (k_d). For example, when k_d is 0.069 h^{-1} ($t_{\frac{1}{2}}$ = 10h or renal function approximately 20% of normal), k_d is 2.9 times larger than K_i x P at an initial carbenicillin concentration of 100 µg/mL but is 1.4 times smaller than K_i x P at an initial carbenicillin concentration of 600 µg/mL. When k_d decreases to 0.023 h^{-1} ($t_{\frac{1}{2}}$ = 30h or renal function approximately 3% of normal), k_d is 1.1 times smaller than K_i x P at an initial carbenicillin concentration of 100 µg/mL and is

4.3 times smaller than K_i x P at an initial carbenicillin concentration of 600 µg/mL. Therefore, the contribution of K_i x P to the elimination rate of aminoglycoside from the body would become larger and the interaction of penicillin and aminoglycoside, clinically significant. In addition, the decreased elimination rate of penicillin as a result of renal failure would produce a longer contact time for the interaction of penicillin and aminoglycoside to occur in the body. Since K_i x P is larger at higher concentrations of penicillin compared to lower concentrations of penicillin, the contribution of the interaction to the elimination rate of aminoglycoside from the body would, therefore, be larger for higher concentrations of penicillin. In addition, the interaction would be especially significant in patients with severe renal failure when the elimination rate constant of aminoglycoside is extremely small (Table 29).

The effect of the interaction of penicillin and aminoglycoside in patients with normal and impaired renal function was also examined by computer simulation. For the purposes of the simulation, patients were assumed to receive both antibiotics simultaneously. For patients with normal renal function, receiving both gentamicin and carbenicillin, the loss of gentamicin with or without carbenicillin would be similar. At 8 h, the concentrations of gentamicin in the presence of initial carbenicillin concentrations of 100 and

600 µg/mL are 0.29 and 0.25 µg/mL, respectively and 0.31 µg/mL without carbenicillin (Table 30). Thus the interaction would not be significant in patients with normal renal function. In patients with renal dysfunction, the concentration of gentamicin is significantly reduced by co-administration of carbenicillin, especially at higher concentrations of carbenicillin and/or with greater impairment of renal function. For example, when the elimination rate constant of gentamicin is 0.069 h^{-1} ($t_{\frac{1}{2}}$ = 10h or renal function approximately 20% of normal), the concentrations of gentamicin at 24 h in the presence of initial carbenicillin concentrations of 100 and 600 µg/mL are 0.78 and 0.39 µg/mL, respectively and 0.95 µg/mL without carbenicillin. When the elimination rate constant of gentamicin decreases to 0.023 h^{-1} ($t_{\frac{1}{2}}$ = 30h or renal function approximately 3% of normal), the concentrations of gentamicin at 24 h in the presence of initial carbenicillin concentrations of 100 and 600 µg/mL are 2.09 and 0.70 µg/mL, respectively and 2.88 µg/mL without carbenicillin. Since aminoglycosides have a very narrow therapeutic window, the interaction, therefore, would be significant in patients with impaired renal function, especially if the patient is given a very high dose of penicillin or if the impairment of renal function is severe. Unless adjustments in the aminoglycoside dose or the time of administering the penicillin are made, patients may have sub-therapeutic

Table 30: Computer simulation of the degradation of carbenicillin (100 & 600µg/mL) and gentamicin (5µg/mL) in two carbenicillin-gentamicin combinations with and without interaction in patients with normal renal function (k_d & k'_d = 0.35 h^{-1} or $t_{½}$ = 2 h)

		With interaction $dP/dt = -k'_d \times P - K_i \times A \times P$ $dA/dt = -k_d \times A - K_i \times A \times P$		Without interaction $dP/dt = -k'_d \times P$ $dA/dt = -k_d \times A$	
Concentration of carbenicillin (µg/mL)	Time[a] (h)	Concentration of carbenicillin (µg/mL)	Concentration of gentamicin (µg/mL)	Concentration of carbenicillin (µg/mL)	Concentration of gentamicin (µg/mL)
100	0	100.00	5.00	100.00	5.00
	4	24.64	1.19	24.71	1.24
	8	6.09	0.29	6.11	0.31
	12	1.50	0.07	1.51	0.08
600	0	600.00	5.00	600.00	5.00
	4	147.84	1.05	148.24	1.24
	8	36.49	0.25	36.65	0.31
	12	9.01	0.06	9.06	0.08

a: A 2-hour interval was used for computer simulation but only the data for 4-hour intervals are presented here for comparison

serum levels of the aminoglycoside (Table 31).

Table 31: Computer simulation of the degradation of carbenicillin (100 & 600µg/mL) and gentamicin (5µg/mL) in two carbenicillin-gentamicin combinations with and without interaction in patients with impaired renal function

			With interaction $dP/dt = -k'_d \times P - K_i \times A \times P$ $dA/dt = -k_d \times A - K_i \times A \times P$		Without interaction $dP/dt = -k'_d \times P$ $dA/dt = -k_d \times P$	
k_d & k'_d (h^{-1})	Concentration of carbenicillin (µg/mL)	Time[a] (h)	Concentration of carbenicillin (µg/mL)	Concentration of gentamicin (µg/mL)	Concentration of carbenicillin (µg/mL)	Concentration of gentamicin (µg/mL)
0.069 ($t_{1/2}$=10h)	100	0	100.00	5.00	100.00	5.00
		8	57.29	2.59	57.58	2.88
		12	43.41	1.90	43.69	2.18
		24	18.92	0.78	19.09	0.95
	600	0	600.00	5.00	600.00	5.00
		8	344.40	1.80	345.49	2.88
		12	261.15	1.17	262.17	2.18
		24	113.98	0.39	114.55	0.95
0.035 ($t_{1/2}$=20h)	100	0	100.00	5.00	100.00	5.00
		8	75.15	3.35	75.58	3.78
		12	65.19	2.77	65.70	3.29
		24	42.64	1.63	43.17	2.16
	600	0	600.00	5.00	600.00	5.00
		8	451.90	2.21	453.47	3.78
		12	392.49	1.55	394.23	3.29
		24	257.49	0.62	259.03	2.16
0.023 ($t_{1/2}$=30h)	100	0	100.00	5.00	100.00	5.00
		8	82.70	3.66	83.19	4.16
		12	75.25	3.16	75.88	3.79
		24	56.79	2.09	57.58	2.88
	600	0	600.00	5.00	600.00	5.00
		8	497.38	2.38	499.16	4.16
		12	453.19	1.70	455.29	3.79
		24	343.30	0.70	345.48	2.88

a: A 2-hour interval was used for computer simulation but only the data for 0, 8, 12 & 24 hour (the usual dosage intervals for aminoglycoside administration) are presented here for comparison

SUMMARY AND CONCLUSIONS

1. Degradation and Interaction Curves

Carbenicillin and ticarcillin decayed rapidly in human plasma at 37°C even when present as a single component. Only 50% of the original concentration remained at 24 hours. Gentamicin and tobramycin decayed slowly in human plasma. More than 85% of the original concentration remained after 3 days. The degradation of both penicillin (carbenicillin and ticarcillin) and aminoglycoside (gentamicin and tobramycin) in control samples were first order reactions. When carbenicillin or ticarcillin was incubated with gentamicin or tobramycin in human plasma at 37°C, there was a substantial loss of gentamicin and tobramycin. The degradation of aminoglycoside was faster before 24 hours and thereafter much slower and less extensive, probably a result of progressively diminishing concentration of penicillin. The loss of aminoglycoside in penicillin-aminoglycoside mixtures appeared to be a second order reaction dependent on the concentration of both penicillin and aminoglycoside. The decrease in the concentration of aminoglycoside was due to both its degradation in plasma ($-K_A \times A$) and its interaction with penicillin ($-K_i \times A \times P$), i.e., $dA/dt = -K_A \times A - K_i \times A \times P$. On the other hand, the loss of penicillin in penicillin-aminoglycoside mixtures

appeared to be a first order reaction. The interaction did not contribute significantly to the loss of penicillin, i.e., $dP/dt = -K_P \times P$.

2. Degradation of Penicillins

The degradation constants of penicillins in controls and antibiotic mixtures were calculated (Equation 3 & 6) and analyzed statistically (one-way ANOVA by concentration of penicillin for controls, two-way ANOVA by concentration of penicillin and concentration of aminoglycoside, followed by multiple comparison of means). The degradation constants of penicillin in control samples averaged 1.8×10^{-2} h^{-1} for carbenicillin and 2.6×10^{-2} h^{-1} for ticarcillin. They were statistically related to the concentration of penicillin (although there was no significant difference between the two penicillin concentrations of 200 and 400 µg/mL or between the two lower ticarcillin concentrations -- 100 and 200 µg/mL). The degradation constants of penicillins in antibiotic mixtures averaged 2.2×10^{-2} for carbenicillin and 3.0×10^{-2} for ticarcillin. They were statistically related to the concentration of penicillin (although there was no significant difference between the two lower penicillin concentrations -- 100 and 200 µg/mL -- in all antibiotic mixtures except ticarcillin-gentamicin mixtures or between the two higher penicillin concentrations -- 400 and 600 µg/mL

-- in all penicillin-tobramycin mixtures). Except for the ticarcillin-tobramycin combination, the degradation constants of penicillins were also statistically related to the concentration of aminoglycoside (although there was no significant difference within the gentamicin concentration range of 5 to 15 µg/mL and within the tobramycin concentration range of 10 to 15 µg/mL).

In both controls and mixtures, the t_{50} values of penicillins were 30 and 55% larger, for carbenicillin and ticarcillin respectively, at higher penicillin concentrations of 400 and 600 µg/mL compared to lower penicillin concentrations of 100 and 200 µg/mL.

3. Degradation of Aminoglycosides

The degradation constants of aminoglycosides in controls were calculated (Equation 4) and analyzed statistically (one-way ANOVA by concentration of aminoglycoside, followed by multiple comparison of means). The degradation constants of aminoglycosides in controls averaged 0.9×10^{-3} h^{-1} for gentamicin and 1.2×10^{-3} h^{-1} for tobramycin. They were not statistically related to the concentration of aminoglycoside. The degradation constants of aminoglycosides in antibiotic mixtures, calculated from computer fitting, were less than 1×10^{-8} h^{-1}.

The t_{50} values of aminoglycosides in antibiotic mixtures were shorter than in controls and were statistically

related to the concentration of penicillin. The t_{50} values of aminoglycoside were longer than 72 hours at a penicillin concentration of 100 µg/mL. As the concentration of penicillin became higher, the t_{50} values became shorter and were less than 10 hours for a penicillin conentration of 600 µg/mL. The t_{50} values of tobramycin in carbenicillin-tobramycin and ticarcillin-tobramycin combinations were also related to the concentration of tobramycin. The statistical difference, however, was only associated with one of the four aminoglycoside concentrations -- 15 µg/mL for the carbenicillin-tobramycin combinations and 5 µg/mL for the ticarcillin-tobramycin combinations.

Examination of the t_{50} values of aminoglycosides in antibiotic mixtures indicated that there was no difference in the ability of carbenicillin and ticarcillin to inactivate gentamicin but a significant difference in the ability of carbenicillin and ticarcillin to inactivate tobramycin. Of the two aminoglycosides, tobramycin was inactivated less by carbenicillin or ticarcillin than gentamicin.

4. Interaction of Penicillins and Aminoglycosides

The interaction rate constants were determined by computer fitting and analyzed statistically (two-way ANOVA by concentration of penicillin and concentration of aminoglycoside, followed by multiple comparison of means).

The interaction rate constants averaged 2.2×10^{-4} mL/μgxh for both carbenicillin and ticarcillin interactions with gentamicin and 1.6×10^{-4} mL/μgxh for both carbenicillin and ticarcillin interactions with tobramycin. Except for ticarcillin-tobramycin mixtures, the interaction rate constants were statistically related to the concentration of penicillin (although only the interaction rate constants of the lower penicillin concentration -- 100 μg/mL -- in penicillin-tobramycin mixtures and only that of the higher penicillin concentration -- 600 μg/mL -- in penicillin-gentamicin mixtures was significantly different from the others). The interaction rate constants were also statistically related to the concentration of aminoglycoside in ticarcillin-aminoglycoside mixtures (although there was no significant difference within the gentamicin concentration range of 10 to 20 μg/mL and within the tobramycin concentration range of 10 to 15 μg/mL).

The interaction was significantly faster in penicillin-gentamicin mixtures than in penicillin-tobramycin mixtures (three-way ANOVA by antibiotic combination, concentration of penicillin and concentration of aminoglycoside, followed by multiple comparison of means). There was no significant difference between the interaction rate produced by carbenicillin and ticarcillin.

5. Clinical Significance of the Interaction

Based on the t_{50} values of aminoglycosides in antibiotic mixtures and the interaction rate constants determined in vitro, the combination of tobramycin and carbenicillin or ticarcillin is a better choice than the combination of gentamicin and carbenicillin or ticarcillin if the interaction of penicillin and aminoglycoside is to be minimized.

Based on computer simulations using the interaction rate constants measured in this study, the interaction of penicillin and aminoglycoside would be significant in patients with impaired renal function and might be significant in patients with normal renal function when the concentration of penicillin is very high. Additional simulations are necessary to determine the optimal dosage regimen for combination therapy.

RECOMMENDATIONS FOR FUTURE RESEARCH

The purpose of our study was to investigate the kinetics of interaction of penicillin and aminoglycoside *in vitro*. Further research could then be performed *in vivo*, to confirm our *in vitro* results. The interaction of penicillin and aminoglycoside appeared to be a second order reaction and yielded values for the interaction rate constant. With these values, computer simulations could be used to predict the effect of various dosage schedules on serum concentration of the antibiotics. Similar studies could also be performed on other penicillin-aminoglycoside combinations.

APPENDIX I

MATERIALS, REAGENTS AND SOLVENTS

MATERIALS

1. Sodium biphosphate (monobasic, mono-hydrate) (J. T. Baker Chemical Company, Phillipsburg, N.J.)
2. Sodium phosphate (dibasic, 7-hydrate) (J. T. Baker Chemical Company, Phillipsburg, N.J.)
3. Gentamicin sulfate (potency: 590 µg/mg) (Sigma Chemical Company)
4. Tobramycin sulfate (potency: 942 µg/mg) (Sigma Chemical Company)
5. Carbenicillin disodium (Sigma Chemical Company)
6. Ticarcillin disodium (Sigma Chemical Company)
7. Penicillin-G potassium (benzylpenicillin) (potency: 1595 units/mg) (Sigma Chemical Company)

REAGENTS AND SOLVENTS

1. Sulfuric acid (98%, reagent grade) (Fisher Scientific Company, Fair Lawn, N.J.)
2. Acetonitrile (HPLC grade, UV cutoff 190nm) (Fisher Scientific Company, Fair Lawn, N.J.)
3. Ethyl acetate (reagent grade) (Fisher Scientific Company, Fair Lawn, N.J.)
4. Methylene chloride (reagent grade) (Caledon Laboratories Ltd., Georgetown, Ontario)
5. Phosphoric acid (85%, reagent grade) (Fisher Scientific Company, Fair Lawn, N.J.)
6. Gentamicin (^{125} I) Radioimmunoassay Kit (New England Nuclear, North Billerica, M.A.)
7. Tobramycin (^{125} I) Radioimmunoassay Kit (New England Nuclear, North Billerica, M.A.)
8. Human plasma (University Hospital)

APPENDIX II

APPARATUS

APPARATUS

1. HIGH-PRESSURE LIQUID CHROMATOGRAPHY PUMP, Model 8500 (Varian)
2. μ-BONDAPAK C_{18} COLUMN (Waters Scientific Ltd., Milford, Mass.)
3. SOLVECON PRE-COLUMN KIT, includes a Whatman silica pre-column (L-type) and Whatman pre-column gel (Terochem Laboratories Ltd, Toronto) (the pre-column was placed between the pump and the injector to protect the column from low pH mobile phase)
4. SYRINGE LOADING SAMPLE INJECTOR, 37-53 microns, model 7125 (Rheodyne, Inc.)
5. UV-VIS DIGITAL SPECTROPHOTOMETER AND VARIABLE WAVELENGTH DETECTOR, Model 55, with deuterium lamp and wavelength set at 210 nm during the experiment (Perkin-Elmer Coleman)
6. GAMMA SCINTILLATION COUNTER, Gamma 7000 Counting System (Beckman Instruments, Inc.), connected to a silent 700 Electronic Data Terminal (Texas Instruments Incorporated, California)
7. RECORDER, with speed set at 20 mL/h during the experiment (Canlab)
8. HAMILTON MICROLITER SYRINGE, 100 μL (Hamilton Company, Reno, Nevada)

9. LABORATORY VORTEX MIXER, hand switch (Canadian Laboratory Supplies, Division of McGaw Supply Ltd.)
10. RADIOMETER pH METER, type PHM 62 (Bach-Simpson Ltd., Laboratory Equipment Division, London, Ontario)
11. REFRIGERATED CENTRIFUGE, Model TJ-6 Centrifuge, with Model TJ-R Refrigeration unit (Beckman)
12. IEC HN-S II CENTRIFUGE (Damon/IEC Division, International Equipment Company, Needham Heights, Mass.)
13. POLYPROPYLENE TUBES, 12 x 75 mm, for HPLC use
14. POLYSTYRENE TUBES, 12 x 75 mm, for RIA use
15. FREEZER -20°C
16. EPPENDORF PIPETTE TIPS, 1-100µL and 100µL-1mL (Fisher Scientific Ltd.)
17. EPPENDORF DIGITAL PIPETTE, 100µL-1mL (Brinkmann Instruments, Inc., Westbury, N.Y.)
18. INCUBATOR, with the temperature set at 37°C during the experiment (Lab-Line Instruments, Inc., Melrose Park, Illinois)
19. MLAB PROGRAMME AND DEC-20 COMPUTER, Division of Computer Research and Technology (National Institutes of Health, Bethesda, Maryland)

APPENDIX III

DATA USED IN CONSTRUCTING STANDARD CURVES AND DETERMINING EXTRACTION EFFICIENCY FOR HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

STANDARD CURVES OF CARBENICILLIN IN WATER AND PLASMA

Concentration of carbenicillin (µg/mL)	Peak height ratio		Calculated carbenicillin concentration (µg/mL)	% Extraction efficiency
	Water standards	Serum standards		
0	0.00	0.00	-	-
100	0.40	0.48	86.0	86.0
200	0.80	0.91	172.0	86.0
400	1.56	1.75	337.0	84.3
600	2.24	2.78	501.5	83.6

Concentration of carbenicillin (µg/mL)	Peak height ratio		Calculated carbenicillin concentration (µg/mL)	% Extraction efficiency
	Water standards	Serum standards		
0	0.00	0.00	-	-
100	0.35	0.46	78.5	78.5
200	0.66	0.84	160.0	80.0
400	1.41	1.78	313.0	78.0
600	2.30	2.65	472.0	78.7

STANDARD CURVES OF TICARCILLIN IN WATER AND PLASMA

Concentration of ticarcillin (µg/mL)	Peak height ratio		Calculated ticarcillin concentration (µg/mL)	% Extraction efficiency
	Water standards	Serum standards		
0	0.00	0.00	-	-
100	0.40	0.46	96.0	96.0
200	0.78	0.81	183.0	91.0
400	1.57	1.67	358.0	89.5
600	2.43	2.80	532.5	88.8

Concentration of ticarcillin (µg/mL)	Peak height ratio		Calculated ticarcillin concentration (µg/mL)	% Extraction efficiency
	Water standards	Serum standards		
0	0.00	0.00	-	-
100	0.28	0.36	83.0	83.0
200	0.55	0.67	170.0	85.0
400	1.12	1.26	345.0	86.3
600	1.72	2.00	521.5	86.9

APPENDIX IV

DATA USED IN CONSTRUCTING STANDARD CURVES FOR RADIOIMMUNOASSAY (RIA)

STANDARD CURVE OF GENTAMICIN

	Tube #	CPM Bound	Mean CPM	Net CPM Bound	Normalized % Bound	µg/mL From Curve
Total	1	28962	29455	-	-	-
	2	29948				
Blank	3	1821	1728	-	-	-
	4	1634				
"0" Standard	5	15768	15794	14066	100.0	-
	6	15820				
1 µg/mL	7	14385	14382	12654	90.0	-
	8	14378				
2 µg/mL	9	12908	12809	11081	78.8	-
	10	12709				
4 µg/mL	11	10081	9891	8163	58.0	-
	12	9701				
8 µg/mL	13	6888	6899	5171	36.8	-
	14	6909				
16 µg/mL	15	4834	4668	2940	20.9	-
	16	4502				
Control serum I	17	11056	10937	9209	65.5	3.30
	18	10817				
Control serum II	19	5278	5392	3664	26.0	12.30
	20	5506				

STANDARD CURVE OF TOBRAMYCIN

	Tube #	CPM Bound	Mean CPM	Net CPM Bound	Normalized % Bound	µg/mL From Curve
Total	1	43376	42840	-	-	-
	2	42304				
Blank	3	2523	2495	-	-	-
	4	2466				
"0" Standard	5	30388	30406	27911	100.0	-
	6	30423				
1 µg/mL	7	26000	26180	23685	84.9	-
	8	26360				
2 µg/mL	9	22911	22658	20163	72.2	-
	10	22404				
4 µg/mL	11	17539	17602	15107	54.1	-
	12	17664				
8 µg/mL	13	12571	12508	10013	35.9	-
	14	12445				
16 µg/mL	15	8375	8393	5898	21.1	-
	16	8410				
Control serum I	17	20058	19982	17487	62.7	2.98
	18	19906				
Control serum II	19	9828	8827	7332	26.3	12.30
	20	9826				

APPENDIX V

AVERAGE pH VALUES OF THE ANTIBIOTIC MIXTURES

Antibiotic combinations	$\overline{pH} \pm$ S.D.
Carbenicillin-gentamicin	8.39 ± 0.13
Ticarcillin-gentamicin	8.37 ± 0.13
Carbenicillin-tobramycin	8.25 ± 0.39
Ticarcillin-tobramycin	7.70 ± 0.28

APPENDIX VI

DATA OF CONCENTRATIONS OF PENICILLIN (CARBENICILLIN OR TICARCILLIN) AND AMINOGLYCOSIDE (GENTAMICIN OR TOBRAMYCIN) IN ANTIBIOTIC MIXTURES DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

CONCENTRATIONS OF CARBENICILLIN AND GENTAMICIN IN CARBENICILLIN-GENTAMICIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

Conc of Carb (μg/mL)	Time (h)	Conc of Gen (μg/mL) 0		5		10		15		20	
		Conc of Carb	Conc of Gen	Conc of Carb	Conc of Gen	Conc of Carb	Conc of Gen	Conc of Carb	Conc of Gen	Conc of Carb	Conc of Gen
0	0				4.83		9.45		16.00		21.20
	12				4.35		9.10		15.40		20.10
	24				4.46		9.45		15.20		19.85
	36				4.34		9.20		14.10		19.80
	48				4.50		9.25		15.60		18.45
	60				4.30		8.60		15.50		19.70
	72				4.65		9.35		14.40		19.20
100	0	80.00		83.00	4.47	88.00	9.95	73.00	13.40	75.00	17.30
	12	52.50		55.50	3.52	50.00	7.15	47.00	9.80	44.50	13.75
	24	38.00		43.50	3.48	36.00	6.25	41.50	9.70	30.00	11.55
	36	28.00		23.50	3.11	27.50	5.85	28.00	7.95	20.50	11.55
	48	25.00		22.00	3.24	19.50	5.55	20.50	7.85	15.00	12.15
	60	18.00		15.50	3.15	16.00	5.83	15.50	7.95	12.00	12.15
	72	18.00		13.50	3.30	15.50	5.87	12.50	10.40	7.50	11.70
200	0	163.00		163.00	4.10	160.00	9.05	161.00	12.60	152.00	14.75
	12	120.00		132.50	2.82	97.50	6.45	82.50	7.85	82.50	11.50
	24	92.00		97.50	2.15	71.50	4.88	62.50	6.50	58.00	8.95
	36	77.50		57.50	1.85	50.00	3.77	45.50	4.97	45.50	7.65
	48	72.50		50.00	1.71	42.00	3.26	41.00	4.73	27.50	7.50
	60	52.50		35.00	1.75	28.00	3.18	25.00	3.97	20.00	7.25
	72	45.00		28.00	1.68	23.00	3.15	16.00	4.40	12.00	6.70
400	0	300.00		283.50	4.13	318.00	7.50	290.50	10.40	285.00	14.20
	12	230.50		252.50	2.11	222.50	3.51	202.00	5.67	200.00	6.80
	24	170.50		152.00	1.16	172.50	1.77	143.00	2.93	161.00	4.50
	36	152.00		130.00	1.09	137.50	1.24	126.00	1.92	126.00	3.87
	48	140.00		121.00	0.92	108.50	1.26	97.50	1.62	90.50	3.45
	60	112.00		99.00	0.75	71.00	1.04	80.50	1.35	68.50	3.30
	72	70.00		77.50	0.56	67.50	1.02	72.50	1.27	60.00	3.00
600	0	520.00		507.50	3.41	475.00	7.25	455.00	9.30	441.00	15.60
	12	378.00		349.00	1.27	347.00	2.27	407.50	3.50	358.00	5.50
	24	307.00		337.00	0.66	301.50	1.07	295.50	1.51	296.00	2.91
	36	273.00		298.00	0.59	256.00	0.68	252.00	1.17	165.50	2.32
	48	235.50		220.00	0.50	214.00	0.80	183.00	0.90	125.00	1.97
	60	197.50		205.00	0.56	165.50	0.81	170.50	0.86	103.50	1.77
	72	177.50		187.50	0.39	122.50	0.92	142.50	0.70	88.00	1.58

CONCENTRATIONS OF CARBENICILLIN AND GENTAMICIN IN CARBENICILLIN-GENTAMICIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

Conc of Carb (μg/mL)	Time (h)	Conc of Gen (μg/mL) 0		5		10		15		20	
		Conc of Carb	Conc of Gen	Conc of Carb	Conc of Gen	Conc of Carb	Conc of Gen	Conc of Carb	Conc of Gen	Conc of Carb	Conc of Gen
0	0				5.35		9.60		14.00		21.90
	12				4.25		8.00		13.30		21.40
	24				4.50		8.70		12.70		20.20
	36				4.40		7.70		11.50		19.20
	48				4.32		7.80		11.40		19.30
	60				4.40		8.75		11.60		20.90
	72				4.15		8.70		11.80		20.00
100	0	72.00		75.00	4.85	70.00	10.20	72.00	12.90	72.00	18.50
	12	46.00		48.00	3.60	47.00	7.20	43.00	10.50	43.00	15.30
	24	40.00		33.00	3.65	36.00	6.60	33.00	8.85	27.50	14.60
	36	27.50		26.00	3.65	27.50	5.50	25.00	8.65	21.00	13.40
	48	18.00		16.00	3.75	22.50	6.30	13.50	7.70	12.00	12.40
	60	14.00		14.00	3.55	14.00	6.10	12.00	7.95	10.00	12.30
	72	12.50		12.50	3.48	14.00	6.70	9.00	7.40	7.50	12.00
200	0	162.50		145.00	5.50	152.00	9.00	152.00	12.00	152.50	17.60
	12	103.00		97.50	3.40	103.00	5.80	103.00	8.40	87.50	13.20
	24	75.00		71.00	2.42	63.00	4.30	70.50	6.73	54.00	10.25
	36	62.00		52.50	2.30	54.00	3.62	52.00	5.80	42.50	7.60
	48	47.00		37.50	1.96	42.50	3.10	35.00	5.47	31.00	7.58
	60	43.00		32.50	1.82	28.00	3.17	26.00	4.60	23.50	6.30
	72	32.50		25.00	1.85	28.00	2.97	22.00	4.35	22.00	6.28
400	0	336.00		280.00	4.73	287.50	8.05	280.50	11.55	297.00	17.20
	12	236.00		212.00	2.01	190.00	3.25	177.50	5.30	177.50	7.50
	24	200.00		170.00	1.27	152.50	2.09	166.00	2.82	124.00	4.86
	36	138.00		120.00	0.78	97.50	1.49	122.50	2.05	107.50	3.56
	48	128.00		97.50	0.97	86.00	1.16	106.00	1.70	90.00	2.88
	60	100.00		66.00	0.34	83.00	1.12	86.00	1.41	57.00	2.33
	72	87.50		58.00	0.90	56.00	1.01	64.00	1.39	48.00	2.27
600	0	458.50		440.00	5.45	450.00	9.30	427.50	9.90	420.00	15.30
	12	322.00		298.00	1.36	419.00	3.17	345.00	3.37	257.00	5.75
	24	263.00		273.50	1.27	303.00	2.00	255.00	1.57	173.00	3.44
	36	243.00		216.00	1.17	200.00	1.45	181.50	1.05	145.00	2.55
	48	210.00		177.00	1.35	176.50	1.35	160.50	0.99	100.00	2.25
	60	162.50		157.00	0.68	153.00	1.17	150.50	0.97	71.00	2.14
	72	150.20		127.50	0.81	130.00	1.03	147.50	0.70	55.00	2.14

CONCENTRATIONS OF TICARCILLIN AND GENTAMICIN IN TICARCILLIN-GENTAMICIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

Conc of Tica (μg/mL)	Time (h)	Conc of Gen (μg/mL) 0 Conc of Tica	0 Conc of Gen	5 Conc of Tica	5 Conc of Gen	10 Conc of Tica	10 Conc of Gen	15 Conc of Tica	15 Conc of Gen	20 Conc of Tica	20 Conc of Gen
0	0				4.60		9.70		14.00		17.30
	12				4.45		8.45		12.30		15.90
	24				4.43		9.10		13.20		15.50
	36				4.40		8.90		12.70		16.45
	48				4.25		8.35		12.60		16.10
	60				4.22		8.45		12.75		15.90
	72				4.50		9.20		12.75		15.65
100	0	80.00		77.00	4.18	72.00	8.90	75.00	13.00	70.00	16.20
	12	54.00		45.50	3.65	45.00	6.85	42.50	9.90	40.00	13.10
	24	40.00		36.50	3.43	25.50	6.15	27.00	9.65	25.00	11.70
	36	27.00		24.00	2.86	20.00	5.70	16.50	9.00	15.00	11.00
	48	17.50		14.80	2.70	12.50	5.10	12.50	8.65	-	10.20
	60	12.50		10.00	2.52	-	4.37	-	9.10	-	11.00
	72	8.00		7.00	2.51	-	4.37	-	8.65	-	9.70
200	0	178.00		177.00	4.65	174.00	8.85	162.00	12.60	170.00	16.50
	12	115.00		115.00	3.25	111.50	5.80	93.50	7.95	100.00	10.60
	24	99.00		71.00	2.42	66.50	4.03	71.00	6.35	67.00	7.90
	36	76.00		48.00	1.99	48.00	3.40	52.50	5.65	40.50	7.30
	48	52.50		42.00	1.71	28.50	2.97	35.00	4.45	32.00	6.55
	60	37.00		28.00	1.64	24.00	2.72	24.00	4.43	22.50	6.10
	72	28.00		22.50	1.49	18.00	2.47	19.50	4.25	15.00	5.65
400	0	347.50		332.50	4.40	357.50	8.45	346.00	11.00	308.00	14.40
	12	227.50		248.00	1.92	232.50	3.52	222.50	5.30	242.00	6.30
	24	195.00		218.00	1.55	196.50	1.77	175.00	2.95	152.50	3.52
	36	146.00		142.00	1.25	138.00	1.20	126.50	2.10	100.50	2.23
	48	114.00		119.00	1.05	103.00	0.96	94.50	1.64	86.00	1.74
	60	102.00		114.00	0.92	87.00	0.80	75.00	1.60	75.00	1.45
	72	78.00		77.00	0.86	63.00	0.48	70.00	1.48	52.50	1.12
600	0	534.00		536.00	3.90	467.50	7.75	569.00	11.10	512.00	12.70
	12	402.50		421.50	1.53	381.00	1.95	354.00	3.27	336.00	4.15
	24	347.50		332.00	1.08	292.50	0.88	270.00	1.91	248.00	2.00
	36	292.00		265.00	0.82	230.50	0.69	222.00	1.28	182.50	1.23
	48	212.50		162.50	0.84	170.50	0.56	157.50	1.20	152.50	1.07
	60	196.00		157.00	0.84	140.50	0.42	118.00	1.16	112.50	0.96
	72	134.00		134.00	0.67	121.50	0.52	95.50	1.11	104.00	0.74

CONCENTRATIONS OF TICARCILLIN AND GENTAMICIN IN TICARCILLIN-GENTAMICIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

Conc of Tica (μg/mL)	Time (h)	Conc of Gen (μg/mL) 0 Conc of Tica	0 Conc of Gen	5 Conc of Tica	5 Conc of Gen	10 Conc of Tica	10 Conc of Gen	15 Conc of Tica	15 Conc of Gen	20 Conc of Tica	20 Conc of Gen
0	0				4.50		8.55		12.60		16.30
	12				4.47		8.50		12.00		16.70
	24				4.15		9.75		12.70		17.95
	36				4.65		8.20		13.40		17.50
	48				4.65		8.55		12.75		19.00
	60				4.50		8.80		13.20		19.20
	72				4.25		9.20		11.95		20.00
100	0	75.00		77.50	4.30	75.00	8.25	76.00	14.20	74.00	19.30
	12	47.00		40.00	3.20	42.50	7.00	42.50	10.55	32.50	15.60
	24	38.00		23.00	3.08	30.00	5.93	27.50	9.20	22.50	12.00
	36	26.00		21.50	3.02	20.20	5.72	18.00	8.05	12.50	10.40
	48	15.00		17.00	2.40	15.00	5.88	10.10	8.90	8.00	10.75
	60	12.00		10.50	2.36	10.00	5.40	8.00	7.90	8.00	10.40
	72	8.00		8.00	2.11	8.00	5.54	7.00	7.55	5.00	10.60
200	0	147.50		162.50	4.05	140.00	7.30	147.50	12.50	162.50	15.55
	12	112.00		108.00	2.74	97.50	5.20	112.50	8.45	88.00	11.25
	24	77.50		72.50	2.27	78.00	4.10	74.00	6.65	64.00	7.90
	36	62.00		56.50	1.93	57.50	3.20	49.00	4.80	49.00	7.35
	48	50.50		42.50	1.76	47.50	3.00	40.00	4.35	30.00	6.15
	60	37.50		31.00	1.75	32.50	3.57	25.00	4.10	20.00	6.07
	72	31.50		25.50	2.05	22.50	2.49	20.00	3.63	15.00	5.50
400	0	302.00		322.50	4.20	312.00	7.15	302.50	11.65	318.00	15.35
	12	207.50		252.50	1.65	266.00	3.28	217.50	5.33	200.50	6.85
	24	178.00		160.00	1.00	188.00	1.83	166.50	2.45	115.50	4.00
	36	157.50		155.00	0.75	130.00	1.31	123.00	1.69	87.00	3.32
	48	119.00		93.00	1.13	108.00	0.92	103.00	1.23	65.00	2.79
	60	86.00		72.00	1.05	77.50	0.89	75.00	0.96	40.50	2.70
	72	74.00		57.50	1.08	55.50	0.96	52.50	0.64	36.00	2.50
600	0	546.50		496.00	3.58	425.00	6.90	427.50	11.95	517.50	14.00
	12	441.50		436.00	1.12	382.50	1.64	306.00	2.88	390.50	4.23
	24	384.00		347.50	1.00	299.00	1.00	267.00	1.62	272.00	1.96
	36	302.00		296.00	0.74	227.50	0.81	212.00	0.89	204.00	1.68
	48	260.00		230.50	0.51	174.00	0.59	174.00	1.08	165.00	1.60
	60	218.00		182.00	0.46	132.50	0.48	140.00	1.05	130.00	1.41
	72	215.00		145.00	0.44	118.00	0.55	100.70	0.97	108.50	1.42

CONCENTRATIONS OF CARBENICILLIN AND TOBRAMYCIN IN CARBENICILLIN-TOBRAMYCIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

Conc of Carb (µg/mL)	Time (h)	Conc of Tob (µg/mL) 0		5		10		15		20	
		Conc of Carb	Conc of Tob	Conc of Carb	Conc of Tob	Conc of Carb	Conc of Tob	Conc of Carb	Conc of Tob	Conc of Carb	Conc of Tob
0	0				5.10		10.80		15.50		20.10
	12				4.90		9.40		15.00		18.50
	24				4.30		9.80		14.30		19.90
	36				4.90		9.70		14.50		19.80
	48				4.27		9.50		14.90		19.60
	60				5.70		10.50		12.75		18.15
	72				4.50		9.80		13.20		17.60
100	0	86.50		84.50	5.00	72.00	10.45	81.50	16.40	84.50	21.00
	12	64.50		66.00	4.70	57.50	8.95	62.50	13.25	52.00	17.10
	24	60.50		50.00	2.86	45.50	8.30	52.50	11.50	25.50	14.50
	36	50.00		46.00	2.74	38.50	6.63	42.50	12.00	21.00	12.70
	48	36.00		27.50	2.25	34.50	6.28	32.00	10.10	16.00	14.50
	60	29.50		37.00	2.06	22.50	5.60	16.00	9.25	14.50	10.45
	72	18.00		16.00	2.30	17.50	5.70	15.50	10.25	12.50	13.80
200	0	198.00		172.50	5.28	167.50	10.40	194.50	15.20	174.00	23.10
	12	133.00		128.50	4.10	125.00	6.70	126.00	11.50	140.00	16.80
	24	123.00		108.50	2.95	91.00	5.70	92.50	9.15	88.50	12.00
	36	127.00		95.00	3.01	74.00	5.08	74.00	8.27	77.00	9.00
	48	100.00		77.50	1.83	55.00	4.00	52.00	7.33	42.00	10.65
	60	79.00		76.00	1.57	44.00	3.93	40.00	5.67	30.00	9.40
	72	63.00		52.50	1.42	28.00	3.07	22.00	6.35	16.00	11.35
400	0	398.00		402.00	5.30	395.00	9.60	330.00	16.00	333.50	19.50
	12	318.00		292.50	3.05	315.00	5.37	248.00	8.45	256.50	10.25
	24	242.50		227.50	1.83	222.00	3.83	215.50	5.63	194.50	6.60
	36	213.50		223.50	1.85	192.50	3.16	166.00	4.07	147.00	3.61
	48	181.50		204.00	0.85	128.50	2.25	141.50	3.41	111.00	4.20
	60	158.00		183.00	0.72	117.50	1.84	119.00	1.13	82.50	2.22
	72	145.00		123.50	0.71	95.00	1.36	95.00	2.04	72.50	3.15
600	0	507.00		491.00	5.10	513.50	9.70	500.50	15.40	583.50	20.90
	12	402.50		455.50	2.32	426.50	3.80	437.50	5.50	435.00	8.45
	24	397.50		352.00	1.01	382.00	2.01	394.00	3.33	412.00	4.14
	36	321.50		302.50	0.94	322.50	1.47	323.50	1.69	262.00	1.34
	48	272.50		283.00	0.41	285.00	0.78	262.50	1.75	258.00	1.91
	60	242.00		251.00	0.62	225.50	0.62	171.00	0.49	205.50	0.69
	72	221.00		200.00	0.22	192.00	0.39	128.00	1.34	166.00	1.13

CONCENTRATIONS OF CARBENICILLIN AND TOBRAMYCIN IN CARBENICILLIN-TOBRAMYCIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

Conc of Carb (µg/mL)	Time (h)	Conc of Tob (µg/mL) 0		5		10		15		20	
		Conc of Carb	Conc of Tob	Conc of Carb	Conc of Tob	Conc of Carb	Conc of Tob	Conc of Carb	Conc of Tob	Conc of Carb	Conc of Tob
0	0				4.87		9.90		14.40		19.25
	12				5.20		9.95		14.40		20.10
	24				4.80		9.70		14.30		20.30
	36				5.10		9.70		14.50		17.70
	48				4.10		8.55		13.20		16.30
	60				4.73		9.80		13.25		16.10
	72				4.65		8.90		14.85		17.20
100	0	83.50		97.00	5.15	90.00	10.30	96.00	15.25	81.00	21.55
	12	70.00		72.50	4.50	72.50	9.30	48.50	12.35	53.50	17.90
	24	55.50		52.50	3.34	55.00	8.00	37.50	10.60	45.50	15.50
	36	38.00		50.50	3.48	40.00	6.80	27.50	11.85	26.50	17.15
	48	27.00		36.50	2.76	26.00	6.65	18.00	10.80	17.00	14.80
	60	22.00		28.00	3.37	21.00	7.75	15.00	9.97	15.50	11.80
	72	14.00		22.00	2.97	12.50	6.60	12.50	9.40	11.00	16.40
200	0	163.50		211.00	4.75	167.00	9.93	163.00	15.50	178.50	19.60
	12	138.00		142.50	3.92	113.00	7.60	142.50	12.20	106.00	14.75
	24	120.00		114.00	2.30	72.50	7.00	102.00	9.40	67.50	14.40
	36	105.00		93.00	2.06	54.00	6.20	81.00	7.80	44.50	14.55
	48	82.50		77.50	1.55	30.50	5.55	62.50	7.42	32.00	13.60
	60	60.50		68.00	1.96	28.00	7.71	49.00	5.53	24.00	11.00
	72	47.00		47.00	1.47	22.00	6.40	27.50	7.63	16.00	15.00
400	0	354.00		412.00	4.43	367.00	9.20	332.00	15.50	390.50	20.00
	12	300.00		316.50	2.65	246.00	5.20	227.50	7.65	277.00	12.20
	24	228.50		243.50	1.34	205.50	3.55	189.50	4.80	205.00	7.70
	36	167.50		226.50	1.26	142.50	2.43	187.50	3.45	128.50	6.00
	48	122.00		186.50	0.89	137.50	2.12	161.00	2.60	92.50	4.76
	60	108.00		142.00	1.19	97.00	2.28	123.00	0.96	72.50	3.16
	72	104.00		109.50	0.63	82.50	1.54	104.00	2.32	61.00	4.25
600	0	517.50		549.00	4.65	545.00	8.60	501.00	14.75	495.50	20.00
	12	408.50		403.50	2.25	448.00	4.27	414.00	6.17	346.00	8.20
	24	288.50		352.50	0.88	343.00	1.90	312.50	2.82	287.50	4.10
	36	272.50		293.00	0.82	235.00	1.34	281.00	1.62	191.00	2.90
	48	250.00		238.00	0.62	177.00	1.26	215.00	0.90	165.50	2.52
	60	229.50		218.00	0.82	128.00	1.34	202.00	0.80	133.50	0.89
	72	188.00		147.50	0.25	98.00	0.87	158.00	1.20	102.50	1.64

CONCENTRATIONS OF TICARCILLIN AND TOBRAMYCIN IN TICARCILLIN-TOBRAMYCIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

Conc of Tica (μg/mL)	Time (h)	Conc of Tob (μg/mL) 0		5		10		15		20	
		Conc of Tica	Conc of Tob	Conc of Tica	Conc of Tob	Conc of Tica	Conc of Tob	Conc of Tica	Conc of Tob	Conc of Tica	Conc of Tob
0	0				4.95		9.85		15.75		19.80
	12				4.83		10.02		15.70		19.10
	24				4.53		9.20		14.70		18.01
	36				4.80		9.85		13.80		20.50
	48				4.85		9.70		15.05		18.80
	60				5.00		8.20		14.50		17.50
	72				4.35		9.50		13.70		19.20
100	0	100.20		108.00	4.40	84.50	9.55	95.00	14.35	84.50	19.50
	12	56.00		52.00	3.94	53.00	8.80	57.50	13.45	57.00	18.05
	24	36.00		42.50	3.95	31.00	7.70	32.00	12.75	33.50	16.80
	36	26.00		24.00	3.87	22.50	8.00	22.00	12.10	22.50	15.15
	48	17.50		20.00	3.95	16.00	8.10	20.50	13.05	17.50	13.50
	60	12.50		17.50	4.30	13.50	8.80	16.00	9.90	14.00	11.25
	72	10.00		14.50	3.64	11.50	7.40	11.50	11.55	11.50	11.65
200	0	192.00		192.00	4.40	175.00	9.55	208.00	15.70	191.50	21.05
	12	114.50		102.00	3.40	102.00	7.25	90.00	11.30	98.50	15.30
	24	82.50		60.50	2.96	57.50	5.43	64.50	9.40	52.00	13.70
	36	42.00		36.50	3.06	34.00	6.07	34.50	9.60	26.00	10.65
	48	34.00		25.00	3.17	25.00	5.65	23.50	9.50	17.50	13.70
	60	18.00		17.00	3.75	16.00	5.60	17.50	5.87	7.50	11.45
	72	15.00		13.50	2.61	11.00	5.00	11.50	7.40	3.00	10.25
400	0	387.50		377.50	4.07	360.00	9.55	320.00	15.70	347.50	20.95
	12	248.50		245.50	2.61	215.00	4.82	236.00	7.78	228.00	10.02
	24	184.50		197.00	1.73	159.50	3.11	152.50	4.85	137.50	6.82
	36	128.00		113.50	1.74	123.00	2.81	116.50	4.20	102.50	4.56
	48	100.00		83.00	1.84	90.00	2.51	82.00	4.43	73.00	5.50
	60	80.00		65.00	2.01	68.00	2.41	56.50	1.40	53.50	3.82
	72	62.00		42.50	1.21	47.50	2.21	47.50	2.72	42.00	3.73
600	0	494.00		554.50	4.13	516.00	9.40	560.00	15.60	517.50	19.50
	12	366.50		353.50	1.85	356.00	3.33	393.00	5.37	346.00	6.48
	24	272.00		285.00	1.05	299.00	1.86	277.50	2.76	252.00	3.57
	36	254.50		204.50	0.99	203.00	1.55	212.50	2.17	187.00	1.81
	48	184.00		154.00	0.94	166.50	1.23	157.50	2.47	125.50	2.39
	60	142.50		139.00	1.15	148.00	1.07	121.00	1.00	102.50	1.79
	72	126.00		104.00	0.58	130.50	0.91	97.50	1.25	94.00	1.21

CONCENTRATIONS OF TICARCILLIN AND TOBRAMYCIN IN TICARCILLIN-TOBRAMYCIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

Conc of Tica (μg/mL)	Time (h)	Conc of Tob (μg/mL) 0		5		10		15		20	
		Conc of Tica	Conc of Tob	Conc of Tica	Conc of Tob	Conc of Tica	Conc of Tob	Conc of Tica	Conc of Tob	Conc of Tica	Conc of Tob
0	0				4.85		9.60		15.60		20.40
	12				4.90		10.10		13.60		18.60
	24				4.80		8.50		14.45		19.60
	36				4.73		9.25		13.20		16.30
	48				4.57		9.45		14.45		20.00
	60				4.50		8.90		15.00		20.00
	72				4.65		8.55		15.35		17.85
100	0	100.00		98.50	4.67	94.00	8.43	93.00	13.90	98.00	22.50
	12	47.00		47.00	4.00	42.00	5.80	40.00	10.40	38.00	14.70
	24	22.50		22.50	3.17	21.50	5.03	22.50	10.60	18.00	15.70
	36	15.00		13.50	3.72	12.50	4.40	10.00	9.00	7.50	10.50
	48	15.00		7.50	3.20	7.50	5.50	6.00	10.70	6.00	14.90
	60	5.00		5.00	2.73	5.00	5.65	5.00	11.55	5.00	14.15
	72	2.50		3.00	3.08	2.00	6.45	2.00	11.00	2.50	14.80
200	0	182.00		207.00	4.30	187.00	8.50	190.00	14.60	176.50	21.70
	12	103.00		108.00	3.79	99.00	7.33	102.50	9.40	107.00	12.20
	24	75.00		60.00	2.58	66.50	5.27	53.50	8.40	48.00	12.90
	36	30.00		38.00	2.70	31.50	4.65	38.00	6.20	32.00	8.80
	48	26.50		24.50	2.39	25.50	5.40	24.00	8.80	21.50	11.50
	60	18.50		14.00	2.37	14.00	4.20	16.00	7.75	12.50	10.60
	72	9.00		10.00	2.64	5.00	4.40	8.00	8.20	4.00	12.50
400	0	337.00		350.00	4.58	337.00	9.55	372.50	15.25	400.00	21.00
	12	228.00		250.00	3.00	230.00	4.67	242.50	6.13	202.50	7.80
	24	174.00		160.00	1.75	170.00	3.22	179.00	4.90	167.50	6.64
	36	120.00		122.50	2.05	127.50	2.14	135.00	2.71	97.00	3.70
	48	108.00		98.00	1.42	103.00	2.37	90.00	3.20	87.50	4.70
	60	84.00		74.00	0.70	72.00	1.64	63.00	2.37	61.50	3.38
	72	67.60		55.00	0.80	52.50	1.62	48.50	3.01	43.00	4.10
600	0	596.00		552.00	4.40	543.00	8.90	580.00	13.80	581.00	20.00
	12	416.50		403.50	1.97	389.50	2.89	367.50	4.25	342.00	4.88
	24	292.00		307.00	1.03	294.50	1.87	272.50	2.69	241.00	3.62
	36	235.00		246.50	1.37	241.50	0.67	208.00	1.04	195.50	1.21
	48	198.00		193.50	0.86	211.00	1.22	176.00	1.57	148.50	2.03
	60	162.50		157.50	0.48	162.50	0.66	132.50	0.99	127.00	1.14
	72	141.50		132.00	0.71	118.00	0.91	98.00	1.30	104.00	1.73

APPENDIX VII

AN EXAMPLE SHOWING PERCENTAGE REMAINING OF CARBENICILLIN AND GENTAMICIN IN CARBENICILLIN-GENTAMICIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

PERCENTAGE REMAINING OF CARBENICILLIN AND GENTAMICIN IN CARBENICILLIN-GENTAMICIN MIXTURE DURING 3 DAYS' INCUBATION IN PLASMA AT 37°C

Conc of Carb (μg/mL)	Time (h)	Conc of Gen (μg/mL) 0 % remaining of Carb	0 % remaining of Gen	5 % remaining of Carb	5 % remaining of Gen	10 % remaining of Carb	10 % remaining of Gen	15 % remaining of Carb	15 % remaining of Gen	20 % remaining of Carb	20 % remaining of Gen
0	0				100.00		100.00		100.00		100.00
	12				79.44		83.33		95.00		97.72
	24				84.11		90.63		90.71		92.24
	36				82.24		80.21		82.14		87.67
	48				80.75		81.25		81.43		88.13
	60				82.24		91.15		82.86		95.43
	72				77.57		90.63		84.29		91.32
100	0	100.00		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	12	63.89		64.00	74,23	67.14	70.59	59.72	81.40	59.72	82.70
	24	55.56		44.00	75.26	57.43	64.71	45.83	68.60	38.19	78.92
	36	38.19		34.67	75.26	39.29	53.92	34.72	67.05	29.17	72.43
	48	25.00		21.33	77.32	32.14	61.76	18.75	59.69	16.67	67.03
	60	19.44		18.67	73.20	20.00	59.80	16.67	61.63	13.89	66.49
	72	17.36		16.67	71.75	20.00	65.69	12.50	57.36	10.42	64.86
200	0	100.00		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	12	63.34		67.24	61.82	67.76	64.44	67.76	70.00	57.38	75.00
	24	46.15		49.00	44.00	41.45	47.78	46.38	56.08	35.41	58.24
	36	38.15		36.21	41.82	35.53	40.22	34.21	48.33	27.87	43.18
	48	28.92		25.86	35.63	27.96	34.44	23.03	45.58	20.33	43.07
	60	26.46		22.41	33.09	18.42	35.22	17.11	38.33	15.41	35.80
	72	20.00		17.24	33.64	18.42	33.00	14.47	36.25	14.43	35.68
400	0	100.00		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	12	70.24		75.71	42.49	66.09	40.37	63.28	45.89	59.76	43.60
	24	59.52		60.71	26.85	53.04	25.96	59.36	24.42	41.75	28.26
	36	41.11		42.86	16.49	33.91	18.51	43.67	17.75	36.20	20.70
	48	38.10		34.82	20.51	29.99	14.41	37.79	14.72	30.30	16.74
	60	29.76		23.58	7.19	28.92	13.91	30.66	12.21	19.19	13.55
	72	26.04		20.71	19.03	19.48	12.55	22.82	12.03	16.16	13.20
600	0	100.00		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	12	70.22		67.73	24.95	93.11	34.09	80.70	34.04	61.19	37.58
	24	57.36		62.16	23.30	67.33	21.51	59.65	15.86	41.19	22.48
	36	53.00		49.09	21.47	44.44	15.59	42.46	10.61	34.52	16.67
	48	45.80		40.23	24.77	39.22	14.52	37.54	10.00	23.81	14.71
	60	35.44		35.68	12.48	34.00	12.58	35.20	9.80	16.90	13.99
	72	32.76		28.98	14.86	28.89	11.06	34.50	7.07	13.10	13.99

APPENDIX VIII

ESTIMATED DEGRADATION CONSTANTS OF GENTAMICIN AND TOBRAMYCIN IN ANTIBIOTIC MIXTURES

(FROM EQUATION 8, p.73)

Antibiotic combination	Concentration of penicillin (μg/mL)	K_A (h^{-1} x 10^{-2}) Concentration of aminoglycoside (μg/mL) 5	10	15	20
Carbenicillin -gentamicin	100	0.17	ND	0.36	0.28
		ND	0.01	ND	0.16
	200	0.61	0.48	0.86	0.63
		0.22	0.47	0.45	0.36
	400	0.52	1.90	1.13	1.30
		1.84	0.65	1.19	0.67
	600	1.49	0.97	1.03	0.48
		0.94	0.84	1.32	1.05
Ticarcillin -gentamicin	100	0.38	0.79	0.06	0.25
		0.91	0.15	0.26	0.06
	200	0.76	0.87	0.72	0.70
		0.41	0.48	0.75	0.74
	400	1.05	2.44	0.90	1.87
		0.19	0.81	2.63	0.74
	600	0.51	0.95	0.39	1.36
		1.39	1.14	0.45	0.53
Carbenicillin -tobramycin	100	0.51	0.47	0.47	0.07
		0.23	0.08	0.65	0.30
	200	1.06	1.27	0.87	ND
		0.65	ND	0.30	0.10
	400	2.53	2.28	2.65	0.87
		1.49	1.08	1.82	1.20
	600	3.29	3.51	1.64	1.28
		2.74	1.03	0.85	2.29
Ticarcillin -tobramycin	100	0.08	0.13	0.09	0.63
		0.60	ND	ND	0.03
	200	0.26	0.49	1.05	0.25
		0.06	0.35	0.29	0.54
	400	0.84	0.63	2.05	0.81
		2.39	1.00	0.56	0.02
	600	1.17	1.45	2.13	1.25
		2.13	1.22	0.79	0.67

ND: not determined, the slope of the terminal portion of the curve was not significantly different from zero in these samples. The mean values of control samples with the same concentration of aminoglycoside were used as the initial estimates of K_A in these samples for computer fitting.

APPENDIX IX

ESTIMATED INTERACTION RATE CONSTANTS

(FROM EQUATION 8, p.73)

K_i (mL/μgxh x 10^{-4})

Antibiotic combination	Concentration of penicillin (μg/mL)	Concentration of aminoglycoside (μg/mL) 5	10	15	20
Carbenicillin -gentamicin	100	0.70	2.50	1.17	1.04
		1.25	1.49	2.77	1.10
	200	1.16	1.23	0.73	1.04
		1.15	1.23	1.42	1.13
	400	1.33	1.02	0.91	0.92
		0.41	1.07	0.84	0.83
	600	0.34	0.62	0.68	1.08
		0.37	0.64	0.61	0.85
Ticarcillin -gentamicin	100	1.08	0.89	1.78	1.83
		0.26	1.23	1.90	2.71
	200	0.96	1.20	1.09	1.11
		0.96	1.07	1.33	1.03
	400	0.53	0.66	0.91	0.95
		0.94	1.19	0.75	1.25
	600	0.50	0.90	0.86	0.79
		0.41	0.84	0.96	0.82
Carbenicillin -tobramycin	100	0.77	1.19	0.08	0.72
		1.16	0.78	0.62	1.43
	200	0.59	1.09	0.91	0.59
		0.48	0.39	0.47	2.05
	400	0.31	0.50	0.44	0.59
		0.12	0.16	0.24	0.88
	600	0.19	0.65	0.67	0.50
		0.15	0.18	0.64	0.64
Ticarcillin -tobramycin	100	0.17	0.34	0.49	0.34
		0.31	5.15	3.18	2.04
	200	0.39	0.60	0.21	1.39
		1.01	1.05	0.87	1.08
	400	0.35	0.75	0.50	0.96
		0.12	0.79	1.10	1.20
	600	0.37	0.49	0.49	0.82
		0.20	0.57	0.77	0.83

APPENDIX X

COMPARISON OF THE DIFFERENTIAL EQUATION (EQUATION 5, p.72) AND THE INTEGRATED EQUATION (EQUATION 7, p.73) BY COMPUTER SIMULATION

COMPARISON OF COMPUTER SIMULATIONS OF THE DEGRADATION OF GENTAMICIN IN A CARBENICILLIN (100µg/mL)-GENTAMICIN (5µg/mL) COMBINATION USING DIFFERENTIAL EQUATIONS AND AN INTEGRATED EQUATION

	Concentration of gentamicin (µg/mL)		
	Differential equation[b]		
Time[a] (h)	With interaction for penicillin[c]	Without interaction for penicillin[d]	Integrated equation[e]
0	5.000	5.000	5.000
12	4.176	4.173	4.173
24	3.662	3.652	3.652
36	3.326	3.311	3.311
48	3.101	3.080	3.080
60	2.946	2.921	2.921
72	2.837	2.808	2.808

a: A 6-hour interval was used for computer simulation but only the data for 12-hour intervals are presented here for comparison

b: $dA/dt = -K_A \times A - K_i \times A \times P$

c: $dP/dt = -K_P \times P - K_i \times A \times P$

d: $dP/dt = -K_P \times P$

e: $A = A_o \times \exp(-K_A \times t + K_i \times P_o \times e^{-K_P t}/K_P - K_i \times P_o/K_P)$

b-e: $K_P = 2.55 \times 10^{-2}\ h^{-1}$

$K_A = 0.00\ h^{-1}$

$K_i = 1.75 \times 10^{-4}$ mL/µgxh

COMPARISON OF COMPUTER SIMULATIONS OF THE DEGRADATION OF GENTAMICIN IN A CARBENICILLIN (600µg/mL)-GENTAMICIN (5µg/mL) COMBINATION USING DIFFERENTIAL EQUATIONS AND AN INTEGRATED EQUATION

	Concentration of gentamicin (µg/mL)		
	Differential equation[b]		
Time[a] (h)	With interaction for penicillin[c]	Without interaction for penicillin[d]	Integrated equation[e]
0	5.000	5.000	5.000
12	2.139	2.133	2.133
24	1.039	1.032	1.032
36	0.563	0.556	0.556
48	0.333	0.328	0.328
60	0.214	0.209	0.209
72	0.146	0.142	0.142

a: A 6-hour interval was used for computer simulation but only the data for 12-hour intervals are presented here for comparison

b: $dA/dt = -K_A \times A - K_i \times A \times P$

c: $dP/dt = -K_P \times P - K_i \times A \times P$

d: $dP/dt = -K_P \times P$

e: $A = A_o \times \exp(-K_A \times t + K_i \times P_p \times e^{-K_P t}/K_P - K_i \times P_o/K_P)$

b-e: $K_P = 1.33 \times 10^{-2}\ h^{-1}$

$K_A = 0.00\ h^{-1}$

$K_i = 1.28 \times 10^{-4}$ mL/µgxh

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