CHAPTER II

REVIEW OF LITERATURE

Since the discovery and clinical use of streptomycin by Waksman, Bugie, and Schatz (1944, quoted in Siegenthaler, Bonetti, and Luthy, 1986), a new chemical class of antibiotics, the aminoglycosidic aminocyclitols, i.e., the aminoglycosides, has emerged on the war against bacteria. More than 40 years ago, various aminoglycosides have been developed and introduced in clinical medicine (Table 1).

Table 1. Discovery of the Aminoglycosides

Year	Antibiotic	Species
1944	Streptomycin	From *Streptomyces griseus*
1949	Neomycin	From *Streptomyces fradiae*
1957	Kanamycin	From *Streptomyces kanamyceticus*
1963	Gentamicin	From *Micromonospora purpurea*
1967	Tobramycin	From *Streptomyces tenebrarius*
1970	Sisomicin	From *Micromonospora inyoensis*
1972	Amikacin	Semisynthetic derivative of kanamycin A
1975	Netilmicin	Semisynthetic derivative of sisomicin

from Siegenthaler et al., 1986.

The aminoglycosides are quite similar in physical, chemical, pharmacokinetic, pharmacologic, and toxicologic properties. Some of the advantages and disadvantages of these antibotics are listed in table 2. The advantages include chemical stability without metabolic changes, broad antibacterial spectrum, rapid bactericidal action, and comprehensive experience over many years. This experience has shown that the efficacy and success rate can be improved by the use of controlled, optimal serum concentrations. Two other advantages are the very rare occurrences of allergic side effects, and the synergism demonstrated when used along with beta-lactam antibiotics

Table 2. The Advantages and Disadvantages of the Aminoglycosides

Advantages	Disadvantages
Chemical stability	Nephrotoxicity and ototoxicity
Broad antibacterial spectrum	Lack of activity against anaerobic organisms
Rapid bactericidal action	Low concentration in cerebrospinal fluid and bile
Experience over many years	Variable pharmacokinetics
Rare allergic side effects	Lack of correlation between administered dose and measured serum concentration
Synergism with beta-lactam antibiotics	Inactivation by various factors

from Siegenthaler, et al. 1986. Am. J. Med. 80 (S6B): 2-14.

(penicillins and cephalosporins). For this reason the combination of aminoglycosides and beta-lactam antibiotics is one of the most frequently used regimens in acutely ill patients.

Some disadvantages include the potential for nephrotoxicity and ototoxicity and the associated narrow therapeutic range between suboptimal serum concentrations and toxic values. Other disadvantages are the lack of activity against anaerobic organisms; the very low concentrations in cerebrospinal fluid and bile; the markedly variable pharmacokinetics influenced by factors such as age, renal function, fever, ascites, and obesity; the lack of correlation between the administered dose and the measured serum concentrations; and, finally, the inactivation of aminoglycosides by reversible binding to lysed granulocytes, low pH, anaerobic environment, high concentrations of calcium and magnesium ions, and beta-lactam antibiotics. The last factor, however, probably plays a role only *in vitro*, and is rarely of clinical importance except during concomitant administrations and when renal function is severely impaired (McLaughlin and Reeves, 1971; Riff and Jackson, 1972).

Aminoglycoside antibiotics are among the most useful group of antimicrobial agents in the treatment of life-threatening Gram-negative bacterial infections such as endocarditis, pneumonia, or a bacteremia. Clinically, these agents are used in infections caused by pathogens resistant to other less toxic antibiotics. Some infections in which aminoglycosides are frequently used include malignant otitis externa, hospital-acquired pneumonias, urogenital

infections, endocarditis, intra-abdominal infections, Gram-negative meningitis, infectious arthritis or osteomyelitis, nosocomial septicemia, and infections in immunocompromised patients (Siegenthaler et al., 1986).

Of the several thousand aminoglycoside molecules synthesized in laboratories and by microorganisms, only three are commonly used in the treatment of human disease in the present. These include gentamicin, tobramycin, and amikacin (Schentag, 1980).

Gentamicin

Gentamicin was first approved by the US Food and Drug Administration for intramuscular use in 1969 and for intravenous use in 1971. It rapidly became a major part of the clinician's therapeutic armamentarium because of its effectiveness against many Gram-negative organisms and its presumed low toxicity. Later, three factors have blunted the initial enthusiasm over this effective drug: the emergence of gentamicin resistance, reports of increased toxicity, and the development of newer antibiotics (Appel and Neu, 1978). However, careful attention to achieving appropriate serum levels, avoidance of unnecessary and potentially toxic concomitant drug therapy, and close following of renal and eighth cranial nerve function allow this drug to be used most efficaciously.

1. Chemistry

Gentamicin is an aminoglycoside antibiotic obtained from cultures of *Micromonospora purpurea.* The commercially available drug is a mixture of the sulfate salts of three fractions, gentamicin C_1, gentamicin C_{1A}, and gentamicin C_2; all three components appear to have similar antimicrobial activity. Some commercial samples may contain significant quantities of the minor components gentamicin C_{2A} and gentamicin C_{2B}. Gentamicin sulfate occurs as a white to buff powder that is odorless, and is soluble in water and insoluble in alcohol. It contained when dried not less than 590 units of gentamicin per mg. Gentamicin sulfate injection for IM or IV administration is a clear, colorless to slightly yellow solution with a pH of 3.0-5.5. Gentamicin sulfate intrathecal injection is a clear, colorless solution with a pH of 3.5-5.5 (McEvoy, 1989; Reynolds, 1989).

2. Stability

Gentamicin sulfate injection should generally be stored at a temperature less than 40°C, preferably between 15-30°C, unless otherwise specified by the manufacturer; freezing should be avoided. Gentamicin sulfate is stable for 24 hours at room temperature in most IV infusion fluids including 0.9% sodium chloride or 5% dextrose injection. The manufacturers state that gentamicin sulfate injection for IM or IV administration should not be mixed with other drugs. The intrathecal injection contains no preservatives and should be used immediately after opening; unused portions should be discarded (McEvoy, 1989).

3. Incompatibility

3.1 Beta-Lactam Antibiotics

The aminoglycosides are inactivated if mixed *in vitro* with beta-lactam antibiotics especially the antipseudomonal penicillins, e.g. ticarcillin or carbenicillin, by interaction with the beta-lactam ring. The extent of inactivation is dependent upon temperature, duration of contact, and the concentration of beta-lactam antibiotic and also varies according to the aminoglycoside and beta-lactam combination (Noone and Pattison, 1971; Pickering and Gearhart, 1979; Henderson, Polk, and Kline, 1981; Konishi et al., 1983; Wright et. al, 1986). Tindula, Ambrose, and Harralson (1983) found that ampicillin, benzylpencillin, carbenicillin and ticarcillin produced marked inactivation while cephazolin and cephamandole produced little inactivation; nafcillin, cephapirin, and cefoxitin produced moderate inactivation.

Although there have been occasional reports of reduced concentrations of aminoglycoside when co-administered with beta-lactam antibiotic (McLaughlin and Reeves, 1971; Murillo et al., 1979) in patients with normal renal function, this effect does not usually appear to be of clinical significance (Eykyn, Phillips, and Ridley, 1971) if these agents are administered at separate sites because the relative time of contact *in vivo* is minimal. However, in patients with renal failure contact time is longer and the degree of inactivation may be sufficient to reduce antimicrobial efficacy.

Therefore, it is generally recommended that when these antibiotics need to be used together they should be administered at different sites and not mixed in infusion solutions or given through the same intravenous lines.

3.2 Other Agents

Other antimicrobial agents reported to be incompatible *in vitro* with gentamicin include erythromycin, chloramphenical, and sulphadiazine sodium. Gentamicin is also incompatible with heparin and sodium bicarbonate and there have been reports of incompatibility with furosemide (Thompson et al., 1985).

4. Mechanism of Action

Gentamicin and other aminoglycosides are bactericidal in action and rapidly induce their lethal effects to the bacterial cells. Although the mechanism of action has not known exactly, the drugs appear to act by interfering with bacterial protein synthesis, possibly by binding irreversibly to the 30S portion of the bacterial (not mammalian) ribosome (Gennaro, 1985; Siegenthaler et al., 1986; McEvoy, 1989; Reynolds, 1989).

After binding to the surface and transporting through the cell wall of the bacteria, the drugs bind to the 30S ribosomal subunits and then form the initiation complexes which cannot pass into subsequent stages of protein synthesis. The binding is quite

firm, so that inhibition is severe enough that a bactericidal effect can result. The drugs also appear to interfere with the binding of aminoacetyl-t-RNA, which prevents chain elongation. They further appear to cause misreading of the messenger RNA (RNA codons) during the translation process, such that inappropriate proteins can be formed when protein synthesis is not completely prevented (Gennaro, 1985). The sum of the events from drug entry into the cell to interference with protein synthesis disturbs membrane function and causes potassium, sodium, amino acids, and other essential constituents to leak out, resulting in bacterial death (Siegenthaler et al., 1986).

5. Spectrum of Activity

Gentamicin has a broad antimicrobial spectrum, extending from Gram-positive cocci to Gram-negative bacilli. However, it is inactive against all anaerobic bacteria, viruses, fungi, and yeasts. Some mycoplasmas have been reported to be sensitive to gentamicin (Reynolds, 1989).

The Gram-negative spectrum of gentamicin includes most members of the Enterobacteriaceae and most *Pseudomonas aeruginosa* strains (Table 3), but precise sensitivity patterns are variable and depend on local usage (Appel and Neu, 1978). In general, gentamicin is active against many species of *Campylobacter*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Providencia*, *Pseudomonas*, and *Serratia*. Minimum inhibitory concentrations have been reported to range from 0.06 to 8 mcg/ml (Reynolds, 1989).

Pseudomonas species, other than *P. aeruginosa*, many isolates of *Serratia marcescens* and *Providencia* are resistant.

Among the Gram-positive organisms, most strains of *Staphylococcus aureus* are highly sensitive to gentamicin with minimum inhibitory concentrations being reported within 0.12 to 1 mcg/ml. *Listeria monocytogenes* and some strains of *Staph. epidermidis* may also be sensitive to gentamicin. The activity against enterococci and streptococci is inadequate when used as monotherapy.

Apart from the three main components, gentamicin C_1, gentamicin C_{1A}, and gentamicin C_2, the minor component gentamicin C_{2A} (the 6'-*C* epimer of gentamicin C_2) has been found recently to be present in commercial samples of gentamicin in higher proportions than previously thought, but as it has a similar activity *in vitro* to the C_2 component this increase will not change the activity of gentamicin.

6. Resistance

Almost all pathogens that are sensitive to one or more of the aminoglycosides can develop resistance. Widespread use of aminoglycoside antibiotics has led to an increase in the incidence of resistance found in staphylococci, enterococci, *Pseudomonas aeruginosa*, the Enterobacteriaceae, and other Gram-negative organisms. The incidence of resistance to aminoglycosides appears generally to be higher in the US than in Europe. In addition,

Table 3. In Vitro Activity of Gentamicin*

Organism	Minimal Inhibitory Concentration (mcg/ml)	% Inhibited by 4 mcg/ml
Escherichia coli	0.25	95
Klebsiella pneumoniae	0.25	95
Enterobacter species	0.5	95
Serratia marcescens	1.0	60
Proteus mirabilis	0.5	100
Proteus, indole positive	2.0	70
Providencia	2.0	50
Acinetobacter	1.6	85
Citrobacter	0.5	95
Pseudomonas aeruginosa	1.6	90
Pseudomonas, other	25	25
Staphylococcus aureus	0.2	100
Streptococcus pneumoniae	25	0
Streptococcus pyogenes	12.5	0
Streptococcus faecalis	25	0
Bacteroides	> 100	0

from Appel and Neu, 1978.

* Major differences occur depending on the institution. In some institutions 30% to 60% of *Klebsiella* and *Serratia* organisms are resistant.

individual differences between agents are reported from hospital to hospital due to time- and indication-related induction of resistance (Reynolds, 1989).

Resistance to the aminoglycoside antibiotics occurs by three main mechanisms; alterations in the ribosomal binding site, reduction of aminoglycoside penetration into bacterial cells, or the presence of inactivating enzymes. The first is by mutation leading to reduced affinity for binding to the ribosomal target sites; this type of resistance is importance only for streptomycin (Davies and Courvalin, 1977). Secondly, penetration of aminoglycosides into bacterial cells is by an oxygen- or energy-dependent active transport process and resistance may also occur because of elimination or reduction of this uptake. *Pseudomonas aeruginosa*, *Serratia* species, and *Streptococcus faecalis* can become resistant to aminoglycosides in this way, whereas the group resistance of the anaerobes results from the absence of an oxygen-dependent transport system across the cytoplasmic membrane (Verklin and Mandell, 1977). The third which is the most important cause of resistance is inactivation of aminoglycoside molecule by enzymatic modification. These enzymes can modify the drug molecule in three different ways; by acetylation at an amino group, by phosphorylation, or by adenylation at a hydroxyl group. The drug molecule becomes changed in such a way that it can no longer bind to the ribosome subunits (Siegenthaler et al., 1986).

Since gentamicin has been used worldwide, and during its years of clinical application, resistance and sensitivity changes have appeared repeatedly in *Staphylococcus aureus* (Mayhall, Medeff, and Marr, 1976; Speller et al., 1976; Dowding, 1977), *P. aeruginosa*, and other Enterobacteriaceae (Keys and Washington, 1977; Kauffmann et al., 1978). The most important cause of resistance lies in the bacterial production of modifying enzymes coded by plasmids, transposons, episomes, and phages. Gentamicin can be modified primarily by four enzymes: two acetyltransferases, one adenyltransferase, and one phosphotransferase (Davies, 1983). Cross-resistance with other aminoglycosides is common and, with netilmicin, almost complete (Briedis and Robson, 1976). In contrast, amikacin is still effective against gentamicin-resistant Enterobacteriaceae and *P. aeruginosa* strains, because amikacin is rarely inactivated by gentamicin-modifying enzymes.

7. Pharmacokinetics

7.1 Absorption

Patients must receive gentamicin by parenteral route, because the drug is poorly absorbed from the gastrointestinal tract. Adequate serum levels are obtained with either intramuscular or intravenous administration (Table 4). Average peak serum concentrations of about 4 mcg/ml have been obtained 30 to 60 minutes after intramuscular administration of a dose equivalent to 1 mg of gentamicin per kg body weight although there may be considerable individual variation and higher concentrations in patients with

Table 4. Pharmacokinetic Properties of Gentamicin

Variable	Route	Dose (*mg/kg BW*)	Result
Peak serum level, *mcg/ml*	IM	1	4
	IM	2	6 to 8
	IV (2 min)	1	12 to 20
	IV (30-min infusion)	1.5	4 to 6
Serum half-life, *h*			
Initial phase			2
Second phase			27 to 700
Volume of distribution, *% (body weight)*			20 to 30
Protein binding			0
Clearance on heomodialysis, 6 h, %			50
Dose excreted 8 h, %			85 to 90

from Appel and Neu. 1978. Ann. Intern. Med. 89: 528-538.

Completeness of intramuscular absorption is seldom a problem in ambulatory patients, but in critically ill patients the reliability of intramuscular absorption is never complete (Hull and Sarubbi, 1976); hence, intramuscular administration is best avoided in these patients. In general, the prefered route for most aminoglycosides including gentamicin is intravenous administration. However, there is still controversy over the optimum method of administration.

Systemic absorption of gentamicin and other aminoglycosides has been reported after topical use on denuded skin and burns following instillation into and irrigation of wounds, body-cavities, and joints (Ericsson, Duke, and Pickering, 1978; Reynolds, 1989). Crosby et al. (1987) found that systemic absorption ranged from 1.5 to 34% in patients who were given gentamicin (1 patient) or tobramycin (9 patients) by endotracheal instillation; all had a creatinine clearance of 40 or more ml/min. Although some patients absorbed significant amounts of aminoglycosides, serum concentrations above 1 mcg/ml did not occur.

Gentamicin was rapidly absorbed from the peritoneal cavity in patients undergoing continuous ambulatory peritoneal dialysis with about 49% of the dose added to the dialysate being absorbed over a 6-hour period. However, it was estimated that it may take as long as 48 hours for equilibrium to be achieved between serum and peritoneal fluid. Dialysis clearance was low and 48 hours of dialysis would be necessary to remove 50% of the amount of gentamicin absorbed (Pancorbo and Comty, 1981).

7.2 Distribution

7.2.1 Volume of Distribution.

Following parenteral administration, gentamicin and other aminoglycosides diffuse mainly into extracellular fluids and factors which affect the volume of distribution will also affect serum concentrations (Riff and Jackson, 1971; Christopher et al., 1974; Siber et al., 1975). In normal volunteers, the extracellular fluid compartment approximates 20 to 25% of body weight. This physiological space is quite susceptible to several changes that may occur during Gram-negative sepsis such as dehydration, congestive heart failure, etc. Frequently, patients in initial phases of Gram-negative sepsis are febrile, nauseated, and vomiting, resulting in dehydration. Consequently, the extracellular fluid compartment and drug distribution volume are decreased. In these patients, volume of distribution of the drug is markedly lower than 20% of body weight. Additionally, several subgroups of patients have been identified which are likely to have changes in drug distribution volume. These include patients with congestive heart failure, patients with peritonitis, patients immediately postpartum, or patients receiving intravenous hyperalimentation (Zaske, 1980). In addition, the volume of distribution of gentamicin in neonates and children is greater than that in adults.

Initially, the distribution volume of the aminoglycosides was thought to be consistent from patient to patient. However, the distribution volume demonstrates considerable variability between patients. This interpatient variation appears to have a substantial effect on serum concentrations and dosage

Initially, the distribution volume of the aminoglycosides was thought to be consistent from patient to patient. However, the distribution volume demonstrates considerable variability between patients. This interpatient variation appears to have a substantial effect on serum concentrations and dosage requirements. In addition to the interpatient variation, the drug's distribution volume may change during the course of antibiotic therapy. This is especially true for patients who are markedly dehydrated in the initial phases of sepsis or patients who have a large volume initially (Zaske, 1980).

7.2.2 Diffusion into Body Tissues and Fluids.

Penetration into the bile, synovial space, and pleural and pericardial fluid can also provide therapeutic levels of gentamicin (Riff and Jackson, 1971; Pitt, Roberts, and Johnson, 1973). Although biliary concentrations of gentamicin are usually 30 to 40% of those in serum (Pitt et al., 1973; Mendelson, Portnoy, and Sigman, 1973), concentrations 2 to 4 times higher than those in serum have been obtained in gall-bladder tissue in patients with acute cholecystitis which may explain the effectiveness of gentamicin in hepatobiliary infections. Therapeutic levels have been obtained in synovial fluid after intramuscular injection of gentamicin (Marsh, Matthew, and Persellin, 1974), and some consider that intra-articular injections may not be necessary in the treatment of infectious arthritis (Dee and Kozin, 1977).

Richey and Schleupner (1981) considered diffusion of gentamicin into peritoneal fluid to be therapeutically adequate in cirrhotic patients with spontaneous peritonitis when serum concentrations were also maintained in the therapeutic range. Aminoglycosides have been reported to penetrate into burn eschar in therapeutic concentrations (Polk, et al., 1983).

The drug distributes readily into the perilymph of the inner ear (Reynolds,1989) and is also measurable in renal lymph fluid (Chisholm, Calnan, and Waterworth, 1968), and in sputum and bronchial secretions (Pines, Raafat, and Plucinski, 1967; Pennington and Reynolds, 1973, 1975; Wong, et al., 1975).

In contrast to most body fluid barriers, gentamicin does not pass the blood brain barrier into the cerebrospinal fluid adequately to achieve therapeutic levels even when the meninges are inflamed (Goitein, Michel, and Sacks, 1975; Kaiser and McGee, 1975). Thus, intrathecal and intraventricular administration has been recommended to treat meningitis (Rahal, et al., 1974; Kaiser and McGee, 1975).

Diffusion into the eye is also poor (Reynolds, 1989). Antibacterial concentrations are not achieved in the vitreous humour and are variable in the aqueous humour following parenteral administration or topical application of gentamicin, so intravitreal and subconjunctival injection respectively are required to treat infections at these sites (Mathalone and Harden, 1972).

Gentamicin crosses the placenta (Weinstein, Gibbs, and Gallagher, 1976), but only small amounts have been reported in breast milk (McEvoy, 1989).

The drug is detectable in all body tissues, and the tissue concentrations of all patients slowly rise with multiple dosing (Schentag, Jusko, Plaut et al., 1977a; Schentag, Jusko, Vance et al., 1977b; Reiner, Bloxham, and Thompson, 1978; Bergeron and Trottier, 1979). Highly perfused organs such as liver, lung, and kidney usually have concentrations above those in serum, while muscle, fat, and hard bone usually have concentrations lower than serum (Edwards, et al., 1976; Schentag, Jusko, Vance et al., 1977b; Schentag, et al., 1978). The highest concentrations in the body are found in the kidneys with most drug being concentrated in the renal cortex (Luft and Kleit, 1974; Edwards, et al., 1976; Barza and Scheife, 1977; Schentag and Jusko, 1977a, 1977b), except in severe chronic renal failure, where liver or lung will often be higher (Schentag, 1980). Schentag and Jusko (1977b) reported that 40% of the total amount of gentamicin in the body was present in the kidney. Accumulation appears to be due to tubular reabsorption followed by active transport of gentamicin into the renal cortex (Hsu, Kurtz, and Wellen, 1977).

7.2.3 Protein Binding.

Results of studies on the protein binding of gentamicin have been conflicting and reported values have ranged from 0 to 30% (Gordon, Regamey, and Kirby, 1972; Ramirez-Ronda, Holmes, and Sanford, 1975; Myers, et al., 1978). While Ramirez-Ronda

et al. (1975) found no significance binding to serum proteins under physiological conditions, in studies by Myers et al. (1978) gentamicin was about 20 % protein bound under the same conditions. However, both groups of workers agreed that gentamicin binding was enhanced in the absence of calcium and magnesium ions. Ramirez-Ronda suggested that although under normal conditions binding did not appear to be of clinical significance it might be in certain pathologic states. Myers et al. were unable to demonstrate a significant difference between the binding capacity of serum proteins from healthy subjects and that from uremic patients receiving chronic hemodialysis but did not find that protein binding appeared to be increased in the presence of heparin due to direct binding of gentamicin to heparin.

7.3 Elimination

Gentamicin and other aminoglycosides do not appear to be metabolized and are excreted unchanged solely or almost entirely in the urine by glomerular filtration (Gyselynck, Forrey, and Cutler, 1971; Riff and Jackson, 1971; Barza and Scheife, 1977). After a single dose, 40% to 60% is recovered in the urine during the first 24 hours, and ultimately almost 90% is excreted (Wilson et al., 1973), and after repeated administration the amount of gentamicin recovered in the urine approaches the dose administered (Gingell et al., 1969).

There is no evidence that gentamicin is metabolized, although metabolism was postulated when early investigators (Chan et al., 1972) noted intercepts in the relation between creatinine

clearance (Cl_{cr}) and the elimination rate constant (k_{el}), or were unable to recover the entire administered dose in a 24-hour urine (Gyselynck et al., 1971; Simon, Mosinger, and Malerczy, 1973; Wilson et al., 1973). Commplete urine recovery can be accomplished when the urine is collected for 20 to 30 days after a week's therapy (Schentag and Jusko, 1977a), and a two compartment model explains the intercept (Schentag, Jusko, Vance, et al., 1977b).

A wide interpatient variation in elimination of the aminoglycosides has been recognized and is now a clinical problem of concern. This interpatient variation occurs in patients with normal serum creatinine or with normal creatinine clearance. The magnitude of this variation seems greater in patients being treated for Gram-negative sepsis than in normal volunteers. This variation may also be greater in the initial phase of treatment, rather than later in the treatment course when patients have stabilized (Zaske, 1980).

In volunteers with normal renal function, the half-life of gentamicin was initially reported to vary between 2.5 and 4 hours (Gyselynck et al., 1971). Zaske (1980) studied a large group of patients in the early course of sepsis and found that the half-life of gentamicin ranged from 0.4 to 32.7 hours in 855 patients with normal serum creatinine ($\leq$ 1.5 mg/dl) and 0.4 to 7.6 hours in 331 patients with normal creatinine clearance ($\geq$ 100 ml/min/1.73m^2). The total body clearance of the aminoglycosides also demonstrated considerable patient to patient variability. For gentamicin, the total body clearance varied from 7.0 to 249 ml/hr/kg in 855 patients

with a normal serum creatinine and from 8.4 to 242 ml/hr/kg in 331 patients with a normal creatinine clearance. Thus, considerable patient to patient variability occured in the elimination and clearance of gentamicin, even in patients with normal serum creatinine or creatinine clearance.

8. Uses

Clinically, aminoglycosides are used in infections caused by pathogens resistant to other less toxic antibiotics. When used empirically or as specific therapy in severe Gram-negative, hospital-acquired infections, especially in neutropenic patients, aminoglycosides are often combined with beta-lactam antibiotics because of the possible synergy.

The major therapeutic role of gentamicin is in the treatment of infections caused by the Gram-negative Enterobacteriaceae and *P. aeruginosa*. It is used IM or IV in the short-term treatment of serious infections such as septicemia (including neotatal sepsis), bone and joint infections, skin and soft tissue infections (including those resulting from burns), respiratory tract infections, and postoperative and inra-abdominal infections (including peritonitis). The drug is also effctive in serious, complicated, recurrent urinary tract infections caused by susceptible Gram-negative bacteria; however, it is not indicated for the initial treatment of uncomplicated urinary tract infections unless the causative organisms are resistant to other less toxic anti-infectives. Gentamicin may also be used IM or IV in the treatment of serious infections caused by susceptible Gram-positive

bacteria, but only when other less toxic anti-infectives are ineffective or contraindicated.

8.1 Monotherapy

A review of the many studies in which aminoglycosides have been used as monotherapy in septicemia reveals success rates of 24 to 100 percent (Siegenthaler et al., 1986). In neutropenic patients with septicemia, failures often occur. However, consistently positive results can be attained in patients with normal host defenses (Jackson and Riff, 1971). Pathogens for which an aminoglycoside is the drug of choice are discussed later, as are those compounds that are equally active (Sanford, 1985, quoted in Siegenthaler et al., 1986). In infections with *Acinetobacter anitratus* or *lwoffi*, an aminoglycoside, an antipseudomonal penicillin, or one of the new 5-quinolone preparations can all probably be considered equally effective as alternative therapy. Cefotaxime, ceftizoxime, ceftazidime, or imipenem (plus cilastatin) are the alternatives to an aminoglycoside in infections with *Enterobacter* species. In infections with *Hafnia alvei*, an aminoglycoside is the drug of first choice, and chloramphenicol is only the drug of second choice. Against *Morganella* species, imipenem (plus cilastatin) will most likely be as effective as an aminoglycoside. *Yersinia enterocolitica* is most successfully treated with an aminoglycoside, but ceftizoxime, ceftriaxone, and moxalactam are also effective. Only prolonged clinical experience and controlled comparative studies will establish the relative reliability and value of a given drug against a specific pathogen.

8.2 Combination Therapy

The need to broaden the spectrum of antibiotic therapy has led to the administration of antibiotic combinations. The rationale for combination therapy also includes the enhancement of antibacterial activity due to synergistic or additive interactions. Increased activity reduces the risk of therapeutic failure that might result when bacterial subpopulations develop resistance to one or both antibiotics. In addition, these synergistic or additive effects often allow a reduction in dosage and in consequent toxic side effects. The value of combining aminoglycosides with beta-lactam antibiotics is recognized worldwide. However, drug combinations may also have antagonistic interactions, increased side effects caused by both drugs, possible provocation of multiply resistant organisms, misinterpretation of therapeutic safety, and finally, higher costs.

Regardless of the potential disadvantages, in many clinical situations, the combination of an aminoglycoside with a beta-lactam antibiotic continues to be the optimal therapy. This is true in life-threatening infections with unknown pathogens, in mixed aerobic/anaerobic infections, in infections in neutropenic or immunodeficient patients, in bacterial endocarditis, and in systemic *Pseudomonas* infections. Above all, clinical trials have shown that patients with granulocytopenia appear to benefit from combined antibiotic therapy. Love and co-workers (1980) and Young et al. (1981) reported a success rate of 80 percent in granulocytopenic patients with septicemia when both antibiotics used in the combination, a beta-lactam plus an aminoglycoside, were individually

active against the causative organisms. The success rate declined to about 60 percent when one of the antibiotics in the combination proved inactive when tested alone. If both antibiotics were inactive *in vitro*, the success rate dropped below 20 percent. There are now numerous animal and clinical trials that confirm the superiority of combined antibiotic therapy in appropriate indications (Scott and Robson, 1976; Klastersky, Meunier-Carpentier, and Prevost, 1977; Levin, 1981; Young, 1984; Baltch and Smith, 1985), a fact that had been recognized as early as 1971 by Schimpff and co-workers. Today, however, in various infections, new antibiotic groups alone or in combination show similar results, a fact that has to be considered when evaluating a therapeutic regimen.

Among the important infections of the upper respiratory tract is malignant otitis externa caused by *P. aeruginosa*. It can lead to severe complications, such as osteomyelitis, basal meningtitis with cranial nerve involvement, cerebritis, and venous sinus thromboses, and therefore requires combined therapy with an antipseudomonal penicillin or a third-generation cephalosporin with an aminoglycoside.

Hospital-acquired bronchopneumonias are generally caused by *Pseudomonas*, *E. coli*, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Providencia*, and *Acinetobacter* species, and, rarely *S. aureus*. Depending on the bacterial resistance pattern of the particular hospital and the status of host-defense mechanisms, an aminoglycoside must be added to an antipseudomonal penicillin or a third-generation cephalosporin.

Severe pyelonephritis with septicemia that is due to *P. aeruginosa* or Enterobacteriaceae can develop in hospitalized patients, especially those undergoing urologic intervention or those with urogenital anomalies, including obstruction, malformation, or neurogenic bladder. In these cases, the newer and less toxic third-generation cephalosporins offer a valuable alternative to the aminoglycosides (Horowitz et al., 1985; Preheim, 1985). However, combination therapy with an aminoglycoside is advised when multiply resistant organisms are isolated. It should be remembered that aminoglycosides can be inactivated by high urine concentrations of calcium and magnesium ions and by a low urinary pH (Minuth, Musher, and Thorsteinsson, 1976).

Endocarditis requires some special considerations. Approximately 80 to 90 percent of the endocarditis cases are caused by Gram-positive cocci, i.e., the enterococci, *viridans* streptococci, other streptococci, and coagulase-positive or coagulase-negative staphylococci. Although aminoglycosides are not intrinsically very active against these organisms, they are indicated in this situation because of the severity of the infection. The frequency of endocarditis caused by Gram-negative pathgens is about 5 percent and appears to be increasing (Finland and Barnes, 1970; Nastro and Finegold, 1973; Pelletier and Petersdorf, 1977; Geraci and Wilson, 1982; Wilson et al., 1982; Griffin et al., 1985; Kaye, 1985). Endocarditis exhibits special anatomic and functional features, such as an impaired local host-defense response with few phagocytes. In, addition, large bacterial populations of 10^8 to 10^{10} colony-

forming units per gram of tissue, with reduced metabolic activity, are protected from the antibiotics by a fibrin network.

For these reasons, corroborated by animal studies, endocarditis is generally treated with combined drugs: penicillin G plus streptomycin against enterococci, provided that the minimal inhibitory concentration for streptomycin is less than 2,000 mcg/ml. If the minimal inhibitory concentration is greater, penicillin G plus gentamicin is used. Penicillin G is combined with streptomycin against *viridans* streptococci when the minimal inhibitory concentration for penicillin exceeds 0.2 mcg/ml, or when the streptococci are fully sensitive (i.e., the minimal inhibitory concentration for penicillin is below 0.2 mcg/ml) and a short, 14-day course of therapy is planned. Gentamicin is combined with a penicillinase-stable penicillin for treatment of *S. aureus* endocarditis. Bacteremia is cleared more rapidly by drug combinations, but the clinical course is not substantially influenced. Gentamicin plus vancomycin plus rifampicin is used in patients with *Staphylococcus epidermidis* endocarditis involving prosthetic heart valves. Finally, an aminoglycoside is generally used with a beta-lactam antibiotic against Gram-negative endocarditis pathogens. In these cases, the best possible combination with regard to synergism should be sought *in vitro* (Reyes et al., 1979; Sande and Scheld, 1980; Drake and Sande, 1983; Karchmer, 1985; Wilson and Geraci, 1985).

Empiric therapy of intra-abdominal infections should be effective against a mixed flora, including enterococci, Enterobacteriaceae, *P. aeruginosa*, and strictly anaerobic organisms.

An aminoglycoside in combination with clindamycin, a 5-nitroimidazole, or cefoxitin has been effective in these situations. Among drugs used as monotherapy, piperacillin and imipenem have the broadest spectrums of activity. According to a review by Kager and Nord (1985), imipenem has already been proved very successful as monotherapy.

The third-generation cephalosporins are strong competitors of the aminoglycosides in the treatment of Gram-negative meningitis in patients of all ages (Landesman et al., 1981; Cherubin et al., 1982; Nelson, 1985). However, when combination therapy is needed to treat special pathogens, such as *P. aeruginosa*, or *Enterobacter* species, or when ventriculitis is present, additional routes of administration may be necessary. For example, injection of the aminoglycoside directly into the ventricle via an Ommaya or Rickham reservoir may improve its efficacy (Eigler et al., 1961; Kaiser and McGee, 1975; Wright et al., 1981). McCracken, Mize, and Threlkeld (1980) reported that intraventricular administration of gentamicin does not improve prognosis in newborns. Therefore, this form of therapy should not be used as routine treatment of neonatal meningitis caused by Gram-negative enteric bacilli.

In infectious arthritis or acute or chronic osteomyelitis develops in infants under one month of age or in patients more than 50 years of age, the differential diagnosis should consider Enterobacteriaceae and *P. aeruginosa* in addition to staphylococci as possible pathogens (Goldenberg and Cohen, 1976;

Pichichero and Friesen, 1982; Wheat, 1985). Empiric therapy should, therefore, consist of combining an aminoglycoside with a beta-lactamase-stable penicillin. After causative organisms are identified, therapy should be modified.

It is important to consider the spectrums of action of all available antibiotics especially those of beta-lactam antibiotics and aminoglycosides when choosing therapy for nosocomial septicemia. For newly developed substances such as monobactams, carbapenems, and 5-quinolones, there is still insufficient experience in this respect. Moreover, the therapeutic gaps with individual substances are important to know. For example, the aminoglycosides are inactive against anaerobes; tne new beta-lactam antibiotics exhibit only limited activity against penicillinase-producing strains of *S. aureus*; and the third-generation cephalosporins are inactive against enterococci and also show inadequate activity against *Bacteroides fragilis*. Most penicillins are inactive against *Klebsiella* species. In addition, clinical trials have shown that during monotherapy with third-generation cephalosporins and antipseudomonal penicillins, *Pseudomonas* strains often become resistant, causing therapeutic failures (Platt, et al., 1981; Neu, 1982; Gribble et al., 1983; Sanders et al., 1984; Hoogkamp-Korstanje, Erpecum, and van Kamp, 1985; Sanders and Sanders, 1985). Furthermore, the choice of substances has to be adapted based on the pathogens in an individual hospital. In general, a combination of a beta-lactam antibiotic with an aminoglycoside is used initially, then changed to monotherapy only after

identification of the pathogen in a patient with normal host-defense mechanisms. As mentioned previously, the aminoglycosides have new competition in the monobactams (e.g., aztreonam), the carbapenems (e.g., imipenem), and the 5-quinolones (e.g., ciprofloxacin, ofloxacin, and norfloxacin). Some of these agents possess even broader activity than the aminoglycosides, primarily because they attack anaerobes. It may eventually be possible to use them with sufficient safety as monotherapy in immunocompetent patients with septicemia.

9. Toxicity

The two most frequent and serious toxic effects occurring with aminoglycosides are ototoxicity and nephrotoxicity. Ototoxicity is a major limitation of their use because destroyed or damaged cochlear hair cells are unable to regenerate. Gentamicin is generally considered to be more toxic to the vestibular branch of the eighth cranial nerve more than to the auditory branch. Aminoglycoside-induced nephrotoxicity is usually reversible following discontinuance of the drug. Many risk factors have been suggested for both ototoxicity and nephrotoxicity in patients receiving aminoglycosides. Patients especially at risk are those whose condition may lead to raised plasma concentrations such as in patients with renal failure. Peak plasma concentrations of gentamicin above 10 to 12 mcg/ml and trough plasma concentrations, that is those immediately before the next dose, of above 2 to 4 mcg/ml may be associated with a greater risk of toxicity (Dahlgren, Anderson, and Hewitt, 1975; Goodman et al., 1975).

Other toxic effects attributed to gentamicin include neuromuscular blockade, and allergy, including cross-reactivity. Infrequent effects reported for gentamicin include anemia, purpura, convulsions, visual disturbances, increased serum aminotransferase values, and increased serum bilirubin concentrations.

9.1 Ototoxicity

Damage to the eighth nerve occurs in 10% of patients treated with gentamicin (Hewitt, 1974; Barza and Scheife, 1977; Smith et al., 1977), and is symptomatic in about 2% (Jackson and Arcieri, 1971; Giusti and Hayton, 1973). Ototoxicity does not correlate well with the development of symptoms; many patients found to have measurable ototoxicity are asymptomatic. In other words, the clinical prevalence of gentamicin ototoxicity in large series of patients is 2% to 3% (Jackson and Arcieri, 1971; Hewitt, 1974). However, evidence of damage may increase to 10% to 20% of selected groups of high - risk patients who are studied with detailed measurements of eighth nerve function (Nordstrom et al., 1973; Tjernstrom et al., 1973).

In humans, as in animal models, damage to the vestibular system predominates over cochlear impairment. In a major review of gentamicin ototoxicity, two thirds of the patients showing toxicity had evidence of vestibular damage alone (Jackson and Arcieri, 1971). Of the one third with hearing impairment, half had additional symptoms of vestibular toxicity. Thus, only 16% of patients with clinically ascertained ototoxicity had evidence of auditory dysfunction alone. Symptoms of vestibular toxicity include vertigo, ataxia, and nystagmus; these symptoms may progress to the

point that patient cannot walk unaided (Dayal, Smith, and McCain, 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 the 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 (Tjernstrom et al., 1973).

The evaluation of vestibular function by bedside clinical testing is often inadequate to detect early toxicity. Dysfunction may be less obvious in the bedridden, extremely ill person, and symptoms of vestibular toxicity can be compensated for by visual and proprioceptive adjustments (Tjernstrom et al., 1973). Auditory impairment is limited initially to high-tone frequency loss. Thus, caloric testing, electronystagmography, and audiometry have been recommended to document the frequency and early appearance of eighth nerve damage. The use of such testing imposes practical problems, especially in the critically ill, less cooperative patients who are most likely to suffer toxicity.

Of the several factors that have been related to an increased risk of ototoxicity, the most important is pre-existing renal impairment (Jackson and Arcieri, 1971; Nordstrom et al., 1973). In an extensive review by Jackson and Arcieri (1971), this was present in 64% of patients who experienced gentamicin-related ototoxicity. Similarly in both prospective and retrospective studies, patients with decreased renal function suffer a greater frequency of eighth nerve damage than those with normal function (Tjernstrom et

al., 1973; Dayal et al., 1974). Therefore, other risk factors for ototoxicity may be similar to those suggested for nephrotoxicity (Whelton, 1985) but may also include noise and increased susceptibility in the neonate and the elderly. However, the clinical value of many of these factors for predicting toxicity remains to be confirmed in clinical studies. Although concurrent use of ethacrynic acid, a potent loop diuretic, is considered to potentiate the auditory toxicity of the aminoglycosides (Mathog and Klein, 1969), the use of furosemide does not appear to be an important factor (Smith and Lietman, 1983).

In patients with renal dysfunction, the total dose of gentamicin per kilogram of body weight received and previous therapy with other ototoxic antibiotics correlate with ototoxicity (Gailiunas et al., 1976). Elderly patients may suffer more damage whether secondary to previous eighth nerve impairment or misjudgment of borderline renal function. A retrospective study by Moore et al. (1984b) indicated that duration of therapy, total aminoglycoside dosage, elevated body temperature, bacteremia, liver dysfunction, and the initial ratio of serum concentrations of urea nitrogen and creatinine were associated with increased risk of ototoxicity. Nevertheless, ototoxicity cannot be accurately predicted on the basis of the daily dose, total dose, or duration of gentamicin therapy or through use of serum levels of the drug. Early workers felt that high peak levels (> 10 mcg/ml) or excessive trough levels were associated with an increased prevalence of ototoxicity (Jackson and Arcieri, 1971; Nordstrom et al., 1973); others have not been able to correlate peak levels with eighth nerve damage (Wenk et al.,

1984) and some consider that ototoxicity is more commonly associated with sustained rather than peak concentrations (Phillips, 1982). In addition, other authors (Appel and Neu, 1978; Barza and Lauermann, 1978) recommended that ototoxicity related to the "total area under the serum concentration-time curve (AUC)" of drug administration.

The basic cellular mechanisms of ototoxicity remain to be ascertained. Hypotheses include interference with active ionic transport systems that maintain the chemical composition of the labyrinthine fluids and direct toxic effects of the aminoglycosides on neural cell metabolic processes (Neu and Bendush, 1976). Until these mechanisms are defined and until the exact relation of the administered dose and serum concentrations to the ototoxic effects of gentamicin are clarified, certain precautions are warranted. These include careful attention to dose administered, avoidance of prolonged potentially toxic levels (peak > 10 mcg/ml and trough > 2 mcg/ml) and prolonged high-dose therapy; awareness of the hazards of previous or concurrent use of other ototoxic drugs; close assessment of auditory and vestibular function, especially when prolonged therapy is contemplated; and, above all, close monitoring of renal function with appropriate adjustment of antibiotic dosage.

9.2 Nephrotoxicity

Aminoglycosides are freely filtered by the glomerular basement, while their nephrotoxic damage is specific to the lining cells of the renal proximal tubule. Animal studies have defined both the histopathology and characteristic features of gentamicin nephrotoxicity but not the mechanism of the renal damage.

In human beings, the frequency of gentamicin nephrotoxicity varies from 2% to 10% (Hewitt, 1974; Smith et al., 1977). Although the incidence of renal damage fell in the clinical trials of the Schering Corporation from 7.7% in 1965 to 1966 to 2.9% in 1970 to 1973, this probably reflects an increased recognition of patients at risk, closer monitoring of renal function, and the use of serum assays of gentamicin with subsequent adjustment of dose. Gentamicin nephrotoxicity appears to be dose-related (Fanning, Gump, and Jick, 1976) and is more common in elderly debilitated patients, in those with previous renal damage, and in those who have a contracted intravascular volume (Cabanillas et al., 1975; Fanning et al., 1976; Gary et al., 1976).

Clinically, mild proteinuria and granular cylindruria are often followed by the decline in renal function. Although gentamicin nephrotoxicity has been divided into two forms (a dose - related, nonoliguric form characterized by a gradual decrease in renal function, and the abrupt onset of oliguric renal failure), this division more likely reflects the overall clinical status of the patient at the time of drug administration than two distinctive patterns of nephrotoxicity (Appel and Neu, 1977). Nonoliguric renal failure secondary to gentamicin has been reported (Gary et al., 1976), and may represent a keener awareness of milder forms of damage or the more frequent use of this agent in less severely ill patients. Renal damage often follows a prolonged course of therapy with inadequate monitoring of renal function tests.

Serial measurements of serum creatinine and urea nitrogen concentrations may show continued deterioration in the

glomerular filtration rate after gentamicin has been withdrawn. Progressive renal dysfunction necessitating dialysis and leading to a uremic death has occurred (Cabanillas et al., 1975). However, both renal morphologic and functional changes are generally reversible if the renal damage is discovered early and the drug is promptly withdrawn, because the renal proximal tubular lining cells have tremendous regenerative capability. Return of renal function tests to base-line status is characteristically slow and may take many weeks to months, because renal damage is closely associated with both elevated and prolonged aminoglycoside tissue concentrations (Gary et al., 1976).

There is mounting evidence that aminoglycoside related proximal tubular damage is readily detectable 5 to 10 days prior to the initial rise in serum creatinine (Mondorf, Breier et al., 1978a; Schentag, Plaut et al., 1979b). The first clinical signs of nephrotoxicity may include an increase in the excretion of renal tubular enzymes in the urine (Patel et al., 1975; Stroo and Hook, 1977; Mondorf, Brier et al., 1978a; Mondorf, Zegelman et al., 1978b), proteinuria (Peterson, Ervin, and Berggard, 1969), and the appearance of urinary casts (Schentag, Gengo et al., 1979a) and although these signs have been used by some clinicians as an early indicator of renal toxicity their clinical usefulness remains to be determined (Reynolds, 1989).

Although nephrotoxicity has been associated with serum trough concentrations of gentamicin greater than 2 mcg/ml (Burton, Vasko, and Brater, 1985b), evidence from clinical studies is conflicting (Wenk et al., 1984) and nephrotoxicity may still

occur despite monitoring to ensure that trough concentrations are maintained below this value (Schentag, Cerra, and Plaut, 1982). The reason for this is that the drug may produce non-dose related renal damage. A number of other risk factors for developing aminoglycoside nephrotoxicity have been suggested by findings from *animal* studies including duration of therapy, dehydration, hypokalemia, age, sex, pre-existing renal dysfunction, liver disease, and recent prior therapy with aminoglycosides or other nephrotoxic agents (Appel and Neu, 1978; Reynolds, 1989). Although there is some confirmatory evidence from studies in humans for a number of these factors (Moore et al., 1984c; Whelton, 1985; Sawyers et al., 1986), some still consider they have limited value in predicting toxicity (Luft, 1984; Lam et al., 1986).

9.3 Ototoxicity and Nephrotoxicity in Comparative with Other Aminoglycosides

Gentamicin appears to have the most definite nephrotoxic potency compared with tobramycin, netilmicin, and amikacin (Kahlmeter and Dahlager, 1984). A large-scale, double-blind study in the United States showed that elevations of serum creatinine level were recorded significantly less frequently with tobramycin than with gentamicin (Smith et al., 1980). In the comparative assessment of ototoxicity, the investigators found no significant differences. Other studies have also established a lower nephrotoxicity rate for tobramycin when compared with gentamicin (Kumin, 1980; Schentag et al., 1982).

With regard to cochleotoxicity and vestibulotoxicity, the findings need to be interpreted with some cautions. Audiograms and vestibular investigations are harder to perform and assess in a standardized manner than are creatinine clearance measurements or an excretion analysis of tubular enzymes. In part, the discrepancies in results from various investigators reflect these technical difficulties. The comprehensive results of investigations by Kahlmeter and Dahlager (1984), who reviewed comparative aminoglycoside toxicity studies published between 1975 and 1982, showed that gentamicin, tobramycin, and amikacin were about equally vestibulotoxic, that amikacin was more cochleotoxic than gentamicin and tobramycin, and that netilmicin showed the lowest potency for both cochlear and vestibular toxicity. In directively comparative studies between individual aminoglycosides, these investigators obtained somewhat divergent results, due to the technical problems already mentioned. In seven comparative trials between gentamicin and netilmicin, the incidence of cochleotoxicity for gentamicin amounted to 2.1 percent; in contrast, in ten comparative trials between gentamicin and amikacin, cochlear toxicity with gentamicin was 11.4 percent.

9.4 Other Toxicity

Other side effects attributed to aminoglycosides include neuromuscular blockade, hypersensitivity reaction, and infrequent local gastrointestinal, hematologic, and central nervous system toxicity. Aminoglycoside antibiotics have produced a curare-like neuromuscular blockade leading to muscular paralysis and

respiratory depression. Most cases have been associated with the use of aminoglycosides in patients undergoing general anesthesia and those receiving other drugs with neuromuscular blocking activity (Warner and Sanders, 1971; Hall et al., 1972; Holtzman, 1976). Other patients at risk include those with renal failure, myasthenia gravis and other diseases affecting neuromuscular transmission. Blockade has usually been associated with intraperitonial or intrapleural instillation of large doses but there have been reports following parenteral administration in patients at risks, e.g., previous neurologic disease (Holtzman, 1976). The exact mechanism for the neuromuscular blockade remains to be determined but appears to be due to both pre- and postsynaptic action on acetylcholine release and uptake (Mastaglia, 1982); it may be reversed by administration of calcium salts or an anticholinesterase agent such as neostigmine or endrophonium, however, the use of neostigmine has been less successful.

Of 15348 hospital inpatients monitored by the Boston Collaborative Drug Surveillance Program between 1975 and 1982 allergic skin reactions were detected in 3 of 670 patients given gentamicin (Bigby et al., 1986). There have been isolated reports of gentamicin producing adverse effects on the blood including granulocytopenia, reversible agranulocytosis (Chang and Reyes, 1975), and thrombocytopenia. Infrequent effects reported for gentamicin include anemia, purpura, convulsions, visual hallucinations (Byrd, 1977), increased serum aminotransferase values, and increased serum bilirubin concentrations (Reynolds, 1989).