THE ANTIBIOTIC TREATMENT OF GRAM NEGATIVE BACTEREMIA - PHARMACOKINETICS AND DYNAMICS

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Dedicated to Anthoon and Nadi.

ABSTRACT

Felty and Keefer published the first report on gram negative bacteremia in 1924 and since then the incidence appeared to be on the increase. The mortality rate associated with gram negative bacteremia in the pre-antibiotic era was high. However, notwithstanding the introduction of effective antibiotics, the refinement of antibiotic dosage adjustments by means of pharmacokinetic techniques and the *in vitro* sensitivity testing of causative organisms, the mortality rates associated with gram negative bacteremia have not been drastically reduced.

The objectives of this study were:

- To review the epidemiology of gram negative bacteremia in a hospital.
- To determine population pharmacokinetic parameters of antibiotics used in the treatment of gram negative bacteremia.
- To identify clinical, pharmacokinetic and microbial parameters which may influence the outcome of gram negative bacteremia.

Epidemiology was reviewed retrospectively by studying patient records. The NONMEM (Nonlinear mixed effects model) programme was used to determine population pharmacokinetic parameters. Antibiotic plasma levels were determined either by HPLC (High pressure liquid chromatography) or the EMIT (Enzyme immunoassay method) assay. Standard microbiology laboratory techniques were employed to investigate bacterial characteristics *in vitro*. The parameters describing bacterial eradication were derived from killing curves of bacteria versus antibiotic concentrations of drugs. Multiple linear regressions were performed to establish the association between pharmacokinetic- and microbial parameters and the outcome of the disease.

The small number of patients in this study do not allow for specific conclusions. Certain trends were however noted. The incidences of gram negative bacteremia in the adult and neonatal populations investigated were respectively 5.8 and 66 per 1000 admissions. Mortality rates were 32% in the adult population and 66% in the neonatal population. *E.coli* and *P.aeruginosa* were the most common causative organisms in respectively the adult and neonatal population. Unexplained variation between subjects in clearance and volume of distribution was significantly reduced if these parameters were adjusted for weight. Multiple regression analysis revealed an optimum fit with the four variables, namely serum bactericidal activity (SBA),concentration at 50% effect (EC_{50}), bacterial rate (BR) and post-antibiotic effect (PAE) ($r^2 = 0,90$) allowing for the derivation of the following equation:

Days to eradication $= 2.91 - 0.51$ BR $- 0.81$ PAE(1hr) $+ 4.30\ EC_{50} - 0.06$ SBA

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INTRODUCTION

Gram negative bacteremia and septic shock occur primarily in hospitalized patients who usually have underlying diseases which render them susceptible to blood stream invasion (Dale and Petersdorf, 1987). The neonate in his immaturity is also highly susceptible to invasion by gram negative bacteria and at this age the incidence of gram negative bacteremia is higher than at any other period of life (Krugman *et al.*, 1977). The organisms most commonly associated with gram negative bacteremia are the enteric bacilli: *Escherichia coli* , and species of klebsiella, enterobacter, proteus and pseudomonas (Dale and Petersdorf, 1987). The incidence of gram negative bacteremia has been reported as high as 12 cases per 1000 admissions in some large urban hospitals.

The mortality rate associated with gram negative bacteremia is high. In one of the earliest articles on the subject of gram negative bacteremia published in the pre-antibiotic era, a mortality rate of 32 % was recorded (Felty and Keefer, 1924). More recently in the post -antibiotic era, mortality rates of 35 % and 20 % have been reported respectively in neonates and adults (Krugman *et al.*, 1977; Bryan *et al.*, 1983). It would therefore appear that notwithstanding the introduction of antibiotics into the therapeutic armamentarium of gram negative bacteremia, mortality rates have not altered dramatically. Inappropriate use of antibiotics may account for this phenomenon (Haddy *et al.*, 1987). Institution of appropriate antimicrobial therapy depends on three factors:

(1) The accurate and prompt identification of the causative organism.

(2) The introduction of methods for antimicrobial sensitivity testing which reflect *in vivo* susceptibility.

(3) The monitoring of antibiotic plasma concentrations with appropriate individualization of dosage adjustments when required.

To allow for all three these factors Schentag *et al.*, (1984) proposed a holistic approach to the treatment of infections. This approach designated dual individualization, involves the incorporation of *in vivo* drug pharmacokinetics and *in vitro* organism pharmacodynamic data into the formula for dosage adjustments.

All the aspects of gram negative bacteremia discussed above are encountered at Baragwanath Hospital, a large urban teaching hospital. The hospital contains 3000 beds, but the number of patients hospitalized frequently exceeds the number of beds. Daily admissions to Baragwanath Hospital are in the order of 500 patients per day.

SECTION A

THE EPIDEMIOLOGY OF GRAM NEGATIVE BACTEREMIA IN A COMBINED COMMUNITY AND TERTIARY HOSPITAL

1. INTRODUCTION

To enable a hospital to set some criteria for the treatment of a specific disease, epidemiological knowledge regarding the disease is necessary. At Baragwanath Hospital epidemiological information on gram negative bacteremia is lacking. A retrospective epidemiological study was therefore performed to determine the nature and extent of gram negative bacteremias in adults and neonates at Baragwanath Hospital. A secondary objective was to identify factors which could possibly determine the outcome of gram negative bacteremia in these patients.

2. LITERATURE REVIEW

2.1 DEFINITION

Gram-negative bacteremia may be defined as the invasion of the blood stream by gram-negative bacteria. Clinically it manifests as chills, fever, vomiting, diarrhea and prostration. Twenty to forty percent of patients with gram negative bacteremia develop septic shock. Septic shock is defined as a condition characterized by inadequate perfusion following bacteremia with gram negative enteric bacilli. Hypotension, oliguria, tachycardia, tachypnea and fever are observed in most of these patients. The symptoms and signs of septic shock are caused by the release of endotoxin, a specific component of the cell wall of gram negative bacteria. At present, gram negative bacteremia, particularly when associated with the development of septic shock is a major cause of morbidity and mortality in hospitals (Dale and Petersdorf, 1987).

2.2 ETIOLOGY

The most frequent causative organisms of gram negative bacteremia and septic shock are *Escherichia coli* (*E. coli*) and species of klebsiella-enterobacter, proteus, pseudomonas

and serratia (Dale and Petersdorf, 1987). These organisms belong to two families of gram negative bacteria namely enterobacteriaceae and pseudomonaceae (Freeman,1979; Boyd and Marr, 1980).

Enterobacteriaceae are recognized by their peritrichous flagella. With certain exception these organisms generally form part of the natural flora of the intestinal tract, and are therefore also called enteric bacteria. These organisms are not normally harmful to man and become pathogenic only if they invade tissues outside the intestinal tract. Pathogenicity is more profound in patients where the host defence mechanisms are impaired, for example in newborns, old age and patients on immunosuppressive drugs. (Freeman,1979).

Controversy exists regarding a formal classification of the Enterobacteriaceae. Two generally accepted classification schemes are presented in table A.I. The main difference between these two (one by Edwards and Ewing, 1972, and the other from the Bergey's Manual of Determinative Bacteriology, 1974) is that the former recognizes arizona as a genus while the Bergey's Manual refers to this genus as *Salmonella arizonae* (Edwards and Ewing, 1972; Buchanan and Gibbons, 1974). The latest update of these classifications was adapted in 1977 by the Enteric Section of the Center for Disease Control and is presented in table A.2 (Freeman, 1979). The most important modification was the alteration of the official designation *Klebsiella pneumoniae* to *Klebsiella oxytoca* (Freeman, 1979; Boyd and Marr, 1980).

A commonly used subclassification was implemented by Escherich in 1886 when he grouped together all the lactose fermenting organisms under the designation, coliforms (Boyd and Marr, 1980). At present the coliforms also include other gram negative organisms which do not necessarily ferment lactose but are related on other biochemical grounds. The remainder of this discussion will focus on the coliforms especially the enteric gram negative bacilli and pseudomonas.

TABLE A.1

Classification of the Enterobacteriaceae

Edwards and Ewing	Bergey's Manual (8th ed.)
FAMILY. Enterobacteriaceae	FAMILY. Enterobacteriaceae
TRIBE 1. ESCHERICHIEAE	
GENUS 1. Escherichia	GENUS 1. Escherichia
SPECIES. E.coli	SPECIES. E.coli
GENUS 2. Shigella	GENUS 2. Edwardsiella
SPECIES. S. dysenteriae	SPECIES. E. tarda
S. flexneri	GENUS 3. Citrobacter
S. boydii	SPECIES. C. freundi
S. sonnei	C. intermedius
TRIBE 2. EDWARDSIELLEAE	GENUS 4. Salmonella
GENUS 1. Edwardsiella	SPECIES S. choleraesius
SPECIES E. tarda	S. typhi
TRIBE 3. SALMONELLEAE	S. enteritidis
GENUS 1. Salmonella	GENUS 5. Shigella
SPECIES S. choleraesius	S. dysenteriae
S. typhi	S. flexneri
S. enteritidis	S. boydii
GENUS 2. Arizona	GENUS 6. Klebsiella
SPECIES A. hinshawii	SPECIES K. pneumoniae
GENUS 3. Citrobacter	K. ozaenae
SPECIES C. freundii	K. rhinoscleromatis
C. diversus	GENUS 7. Enterobacter
TRIBE 4. KLEBSIELLAE	SPECIES E. cloacae
GENUS 1. Klebsiella	E. aerogenes
SPECIES K. pneumoniae	GENUS 8. Hafnia
K. ozaenae	SPECIES H. alvei
K. rhinoscleromatis	GENUS 9. Serratia
GENUS 2. Enterobacter	SPECIES S. marcescens
SPECIES E. cloacae	GENUS 10. Proteus
E. aerogenes	SPECIES P. vulgaris
E. hafnia	P. mirabilis
E. agglomerans	P. morgani
GENUS 3. Serratia	P. rettgeri
SPECIES S. marcescens	P. inconstans
S. liquefaciens	GENUS 11. Yersinia
S. rubidaea	SPECIES Y. enterocolitica
TRIBE 5. PROTEEAE	Y. pestis
GENUS 1. Proteus	GENUS 12. Erwinia (plant pathogens)
SPECIES P. vulgaris	SPECIES E. herbicola
P. mirabilis	
P. morgani	
P. rettgeri	
P. providencia	
P. stuartii	
P. alcalifaciens	
TRIBE 6. Yersineae	
GENUS 1 Yersinia	
SPECIES Y. enterocolitica	
Y. pseudotuberculosis	
Y. pestis	
TRIBE 7. ERWINIEAE (plant pathogens)	
GENUS 1. Erwinia	
GENUS 2. Pectobacterium	

(Taxonomy and spelling according to Freeman,1979)

TABLE A.2

Changes in taxonomy and nomenclature adopted by the enteric section of the center control as of October, 1977

New Designation	Previous Designation
Klebsiella oxytoca	Klebsiella pneumoniae, indole positive, or indole and gelatin positive
Enterobacter sakazakii	Enterobacter cloacae, yellow pigment
Enterobacter gergoviae	
Hafnia alvei	Enterobacter hafnia
Citrobacter amalonaticus	Citrobacter freundii, malonate negative, H_2S negative, KCN and adonitol positive.
Providencia stuartii, urea positive	Proteus rettgeri, biogroup 5
Providencia stuartii, biogroup 4	Providencia alcalifaciens, biogroup 4
Providencia rettgeri	Providencia rettgeri, biogroup 1 - 4
Morganella morganii	Proteus morgani
Yersinia enterocolitica (typical)	Y. enterocolitica
Y. enterocolitica, sucrose negative	Y. enterocolitica
Y. enterocolitica, rhamnose positive	Y. enterocolitica
Y. enterocolitica, rhamnose and raffinose positive	Y. enterocolitica
Yersinia ruckeri	Red mouth bacterium

(Freeman, 1979)

2.3 Factors determining the invasion by gram negative bacteria

Following exposure to gram negative organisms the development of bacteremia and septic shock will depend on the virulence of the invading organism and the host's defence mechanisms.

2.3.1 Virulence of organisms

The virulence of an organism is determined by the antigenic structure of the specific organism and the ability to invade and cause tissue damage:

- Antigenic structure

  Antigens are present on the cell surface of most enterobacteriaceae and pseudomonaceae and are important for pathogenic and classification purposes. The antigens are classified as O (somatic), K (capsular), H (flagellar) antigens and pili or fimbriae. The gram negative organisms which possess either an O or K antigen

are more resistant to the bacteriolytic effect of human serum and are therefore more virulent. The K1 antigen strain of *E. coli* for example, is responsible for 70 % of neonatal meningitis. The K1 and K2 antigen strains of *K. pneumoniae* on the other hand are responsible for most respiratory tract infections (Boyd and Marr, 1980).

- Bacterial invasion of host

Firstly adherence occurs as a result of an interaction between "adhesins" on the fimbriae or other parts of the microorganisms and specific receptors on the host cell membranes. Bacterial proliferation follows adherence and may be inhibited by IgA or polymorphonuclear leucocytes.

- Tissue damage

Tissue damage is achieved either by direct destruction or by the release of toxins. Two types of toxins namely endotoxins and exotoxins are produced by bacteria. The differences between these are summarized in table A.3 (Jawetz *et al.*, 1982).

I Endotoxins

Endotoxin is a lipopolysaccharide substance that forms an integral part of the outer membrane of the bacterial cell wall and is released when the cell dies. This lipopolysaccharide is composed of lipid A, an attached core oligosaccharide and an O polysaccharide side chain. Lipid A is composed of a glucosamine disaccharide backbone apparently common to all Enterobacteriaceae. Lipid A is responsible for most of the biological effects of endotoxin and is very important in the pathophysiology of gram-negative bacteremia. The core oligosaccharide attached to lipid A is composed of inner and outer regions. The inner region appears to be similar in all Enterobacteriaceae but the outer region which is composed of hexose residues, can vary between different species.

II Exotoxins

In addition to endotoxins most of the enteric bacteria also produce exotoxins (sometimes referred to as enterotoxins) which are of considerable medical importance. These exotoxins can either be heat labile (LT), stable (ST) or both. The LT toxin is well characterized and has two subunits, A and B, with different actions. Subunit B facilitates the entry of subunit A where it then attaches to the gangliosides of some cells. The complex formed between subunit A and the cell membrane is referred to as the NAD/toxin complex. This complex activates adenylate cyclase and

leads to the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP).

TABLE A.3

Differences between exotoxins and endotoxins

Exotoxins	Endotoxins
Excreted by living cells, found in high concentrations in fluid medium	Integral part of microbial cell walls of gram negative organisms liberated upon their disintegration
Polypeptides, molecular weight 10 000 to 900 000	Lipopolysaccharide complexes. Lipid A portion probably responsible for toxicity
Relative unstable; toxicity often destroyed rapidly by heat over 60°C	Relative stable; withstand heat over 60°C for hours without loss of toxicity
Highly antigenic; stimulate the formation of high titer antitoxin. Antitoxin neutralizes toxin	Do not stimulate formation of antitoxin; stimulate formation of antibodies to polysaccharide moiety
Converted into antigenic, nontoxin toxoids by formalin, acid, heat, etc.	Not converted into toxoids
Highly toxic, fatal for laboratory animals in micrograms or less	Weakly toxic, fatal for laboratory animals in hundreds of micrograms
Do not produce fever	Produce fever in host

(Jawetz *et al.*, 1982)

2.3.2 Host defence mechanisms

Host defence mechanisms consist of physical and chemical barriers, inflammatory responses, the complement system and immune responses:

- **Physical and chemical barriers**

  When skin or mucous membranes are disrupted, invasion of the circulation by bacteria can occur. The normal flora may be altered by chronic diseases, alcoholism, diabetes mellitus or antibiotics and this may predispose to invasion by other microorganisms (Jawetz *et al.*,).

- **Inflammatory response**

  Any injury to tissue, such as that following the establishment and multiplication of microorganisms calls forth an inflammatory response. During this process polymorphonuclear leucocytes migrate to the area and chemical mediators of the inflammatory response including prostaglandins are released. The most common defect relating to the inflammatory response is neutropenia, which is characterized

by 3000 or fewer leucocytes per cubic milliliter. The lower the count becomes, the more susceptible is the host to infections. Bone marrow failure, peripheral destruction of cells, antineoplastic chemotherapy and neoplastic invasion of bone marrow are the main causes of leucopenia (Jawetz *et al.*, 1982).

- **Complement system**

  The complement system refers to a complex system of proteins and other factors found in normal serum of vertebrates. Activation of the complement sequence of reactions may occur by the classic pathway set off by antigen antibody reactions or by the alternative pathway which is activated by other substances including endotoxin. The sequence of reactions following activation of the complement system can lead to the production of biologically active factors (i.e. chemotactic factors) or cell damage (Jawetz *et al.*, 1982).

- **Immune response**

  Immune responses following contact with microorganisms produce a state of resistance to the host. Immune responses may be of the T-lymphocyte, B-cell or mixed T and B cell. Deficiency of these responses can cause a diminished defence of the host (Jawetz *et al.*, 1982).

2.4 THE PATHOGENESIS OF SEPTIC SHOCK

The mortality rate in patients with gram negative bacteremia is obviously higher if they develop septic shock. The manifestations of septic shock are largely a result of endotoxin release. The lipid A component of endotoxin reacts with cell membranes to liberate mediators of the inflammatory response including prostaglandins, leukotrienes and thromboxanes. These inflammatory mediators have a marked influence on vasomotor tone, microvascular permeability and the aggregation of leucocytes and platelets. Although the opposing actions and interactions of these substances are complex, their net effect in initiating the shock state appears to be very significant (Dale and Petersdorf, 1987). The lipid A component also activates the alternative complement pathway and the intrinsic coagulation system which further contributes to the manifestations of endotoxic shock which include the following:

- **Fever**

  Endotoxins, certain steroids and antigen-antibody complexes act on cells such as granulocytes and monocytes, releasing an endogenous pyrogen which activates the hypothalamic thermoregulatory center to produce fever.

- **Activation of mediators of the inflammatory response**

  Cell membrane phospholipases are activated by endotoxin and other bacterial products leading to the liberation of arachidonic acid and the synthesis and release of leukotrienes, prostaglandins and thromboxanes. In cells containing phospholipase A2 (e.g. neutrophils, monocytes and platelets) the platelet activating factor (PAF) is also generated. These mediators of inflammation have marked influences on vasomotor tone, microvascular permeability and the aggregation of leukocytes and platelets. Thromboxane A2 and prostaglandin F2α produce marked pulmonary vasoconstriction, leukotrienes D3 and D4 induce microvascular leakage and leukotrienes B4 and PAF promote neutrophil aggregation and activation.

- **Activation of the complement cascade**

  Microorganisms activate the classic complement pathway and endotoxins the alternative pathway. Complement activation, leukotriene generation and the direct effect of endotoxin on neutrophils lead to the accumulation of inflammatory cells in the lungs. The release of enzymes and the production of toxic oxygen radicals by these cells damage the pulmonary endothelium and initiate the acute respiratory distress syndrome (ARDS). The activation of the coagulation system leads to thrombin generation and platelet aggregation which occur in the microcirculation of many tissues.

- **Hypotension**

  Endotoxin stimulates the release of catecholamines and glucocorticosteroids from the adrenal glands, histamine from mast cells and serotonin from platelets. All these released substances in association with the effects of opioid peptide secretion in the central nervous system (CNS) and bradykinin generation from kininogen contribute to the hypotension which occurs with septic shock.

- **Leucopenia**

  Endotoxins cause early leucopenia which coincides with the temperature rise induced by the pyrogens.

- **Disseminated Intravascular Coagulation (DIC)**

  Activation of the coagulation system results in disseminated intravascular coagulation. The initial step is the activation of factor xii (Hageman factor) by endotoxin and this is followed by a cascade of reactions ultimately resulting in the deposition of fibrin - platelet aggregates in capillaries.

- **Impaired organ perfusion and acidosis**
  As a result of vascular reactions, hypotension and shock, the perfusion of vital organs (lung, heart, liver, brain, kidneys) is impaired leading to anoxia and inadequate functioning. Poor perfusion and anoxia lead to the accumulation of organic acids and metabolic acidosis may ensue. Poor perfusion of the kidneys also leads to oliguria. Perfusion disturbances elsewhere cause a sharp decrease in arteriovenous oxygen differences (Jawetz *et al.*, 1982) .

2.5 EPIDEMIOLOGY AND OUTCOME OF GRAM NEGATIVE BACTEREMIA

2.5.1 ADULT BACTEREMIA

2.5.1.1 Incidence and outcome

One of the earliest reports on gram negative bacteremia appeared in 1924 (Felty and Keefer, 1924). In this study which was performed in the pre-antibiotic era a mortality rate of 32% was recorded implying a recovery rate of 68%. The main causative organism was *E. coli* which accounted for 32% of cases. In the years that followed numerous articles on the subject of gram negative bacteremia appeared in the literature suggesting that the incidence of gram negative bacteremia was on the increase (du Pont and Spinks, 1969). An alarming finding was the apparent increase in mortality rates associated with gram negative bacteremia reported in 1950 after the introduction of antibiotic therapy (Young *et al.*, 1977; Bryan *et al.*,1983).

Considering referral cases in tertiary hospitals, Du Pont and Spinks (1969) reported an increase in the number of gram negative bacteremia cases from 4.9/1000 in 1958 to 8.1/1000 in 1969. This incidence is still on the increase and reached 12.8/1000 referral cases in tertiary hospitals in 1987 (Dale and Petersdorf, 1987). The number of cases of gram negative bacteremia appears to be higher at referral than at community hospitals, with 75% of the infections in referral hospitals being hospital acquired or nosocomial (Haddy *et al.*, 1987). The main reasons for the steady increase in the incidence of gram negative bacteremia and for the higher incidence in teaching/referral than community based hospitals can be summarized as follows:

- As a result of increasingly advanced laboratory facilities more blood cultures are performed and consequently more episodes are reported.

- The wide usage of broad spectrum antibiotics has lead to the emergence of highly resistant organisms.

- Patients in tertiary teaching hospitals are generally more severely ill and are subjected to more invasive diagnostic and therapeutic procedures. In addition their defence mechanisms are frequently compromised.

- Older people increasingly contribute to a larger percentage of the population and consequently there are now more patients with chronic illnesses.

- There has been an increase in the use of immunosuppressive agents for various indications.

In 1962 McCabe and Jackson published a seminal article in which they correlated the number of deaths of patients who developed gram negative bacteremia with the severity of the underlying disease. They divided these diseases into 3 categories:

- **Rapidly fatal**: (High possibility of death within a year) i.e. acute and chronic leukemia.

- **Ultimately fatal**: (possibility of death within 4 years) i.e. aplastic anemia, myeloma, lymphomas, metastatic carcinomas, cirrhosis and chronic renal disease.

- **Non- fatal**: i.e. diabetes mellitus, and genitourinary, gastrointestinal and obstetrical diseases.

With a few modifications this classification is presently still used to predict outcome of gram negative bacteremia. McCabe and Jackson (1962(a); 1962(b)) also pointed out that the survival rate was higher in patients receiving antibiotics than in those receiving none. This observation was substantiated by a number of reports which followed in later years (Du Pont and Spinks, 1969; Bryan *et al.*,1983).

It would therefore appear that the two most important factors determining the outcome of gram negative bacteremia are the severity of the underlying disease and the instigation of appropriate antibiotic therapy. What constitutes appropriate antibiotic therapy is however a subject of debate. Most authors define appropriate therapy as follows: A drug to which the organism is susceptible *in vitro*, started on the day when the first positive blood culture is reported and administered for 5 days (Kreger *et al.*, 1980(a);

Bryan *et al.*, 1983). Bryan *et al.*, (1983) showed a good correlation between outcome of bacteremia and the initiation of antibiotic therapy only after the first positive blood culture was obtained. However, in their study design patients who died from shock within the first 72 hours were not included. This may have accounted for the better than expected correlation considering the relatively late initiation of antibiotic therapy. Although authorities differ regarding the time at which antibiotic therapy should be started and whether combinations of antibiotics should be used or not , they all agree that some antibiotic therapy is better than none (Du Pont and Spinks, 1969; Bryan *et al.*, 1983). Mortality rates of 60% has been recorded when no antibiotic therapy was given (Haddy *et al.*, 1987). Kreger *et al.*, (1980(a); 1980(b)) showed a decrease in mortality rate in all three categories of underlying disease when appropriate therapy was given. The mortality rate with inappropriate and appropriate therapy in the rapidly fatal group was 77% and 29% respectively, in the ultimately fatal group 28% and 26% respectively and in the non-fatal group 29% and 10% respectively (Dale and Petersdorf, 1987).

Authors in favour of combination antibiotic therapy argue that it gives broader cover for mixed infections and resistant organisms and that the effect can be synergistic (Marples *et al.*, 1984). The arguments against combination therapy are that it is more expensive and that the risks of toxicity and development of resistant strains are higher. With the emergence of superinfections Dale and Petersdorf (1987) stated that single drug therapy is as effective as combination therapy provided the organism is susceptible to the single drug *in vitro*.

2.5.1.2 Causative organisms

E. coli is responsible for approximately 50% of gram negative bacteremias in community hospitals and 30% in referral hospitals. This organism is isolated from the urine in 95% of patients with bacteremia and is therefore responsible for most bacteremias that follow initial urinary tract infections.

K. pneumoniae is the second most common causative agent and accounts for 15% of cases, most of them nosocomial with the respiratory tract as the most common source. The respiratory tract normally becomes colonized with *K. pneumoniae* in patients with chronic illnesses, patients recently hospitalized and those who have recently received antibiotic therapy. Although *K. pneumoniae* is the most common organism, the respiratory tract can also become colonized with *E. coli* and *P. aeruginosa*. Bacteremia occurs in 15% of patients with pneumonia (McGowan *et al.*, 1975; Montgomery, 1979).

Species of proteus, enterobacter and pseudomonas each contribute approximately 10% to the overall number of bacteremias (McGowan *et al.*, 1975). Patients with serious disorders of the GIT (perforated appendix, perforated diverticulum and ischemic bowel peritonitis) may have local infections without bacteremia. If bacteremia occurs the particular species of micro-organisms isolated from the blood will usually reflect the pre-existing bowel flora. Polymicrobial bacteremia occurs in 10% of all episodes of gram negative bacteremia and is most frequently associated with an intra-abdominal source. Biliary tract disease especially when associated with obstruction or ascending cholangitis often results in bacteremia with *E. coli* and species of klebsiella and enterobacter.

In patients with burn wounds or cellulitis, more resistant causative organisms such as species of pseudomonas, serratia and acinetobacter are frequently found (Montgomery, 1979). The hypotension syndrome and "sepsis" in these patients are sometimes complicated by other opportunistic organisms and fungi. When bacteremia is caused by the introduction of intravascular devices the organisms isolated normally reflect the hospital ecology and often include more resistant species (McGowan *et al.*, 1975).

2.5.2 NEONATAL BACTEREMIA

2.5.2.1 Incidence and outcome

Neonatal bacteremias refer to bacteremias acquired within the first month after birth. The occurrence is 1-10/1000 live births in the USA with a mortality rate of 10-40% (Siegel and McCracken, 1981). In underdeveloped countries the mortality rate can be as high as 75% (Siegel and McCracken, 1981; Mir *et al.*, 1987).

Factors, which predispose to the development of neonatal bacteremia and determine its outcome are the following:

- **Age**

  Premature infants have a 3 - 10 fold higher incidence of neonatal bacteremia than full term infants (Plotkin, 1981). Infants born to mothers with infected amniotic fluid have a 1-5% chance of infection. Full term infants may be protected by the antibacterial activity of amniotic fluid which appears at 20 weeks of gestation and increases steadily thereafter (Schlievert *et al.*, 1977).

- **Host defence mechanisms**

Certain host defence mechanisms have been shown to be defective in neonates. These include a deficient production of neutrophils, a decrease in the anti-bacterial activity of leukocytes, impaired chemotaxis of neutrophils and monocytes and a deficiency of complement (C3) especially in low birth weight infants. (Mir *et al.*, 1987).

- **Sex**

  Male infants have an approximately 2-fold higher incidence of neonatal sepsis than female infants (Plotkin, 1981).

- **Time of onset**

  A distinction is drawn between early and late onset infections. Early onset infections occur within the first 7 days after birth and late onset infections after a week. The mortality rate is considerably higher when the neonate develops an infection within the first seven days of life.

- **Resuscitation and hospitalization**

  Nosocomial infections are easily acquired by neonates from the hands of staff or via hospital equipment such as catheters and feeding tubes. Infants requiring resuscitation at birth or having a prolonged stay in hospital as a result of prematurity or disease are at greater risk of acquiring infections which usually involve resistant organisms.

- **Causative organisms**

  E. coli is the most common cause of gram negative bacteremia in neonates (Sarff *et al.*, 1975). The GIT is the main source of *E. coli* in the neonate with the K1 strain being the most virulent and associated with the majority of neonatal infections especially meningitis. Vertical transmission from mother to child is considered to be a common mode of infection (Sarff *et al.*, 1975).

  Species of klebsiella and pseudomonas are the second most common causative organisms of neonatal bacteremia. Other gram negative organisms such as species of enterobacter, proteus, salmonella, acinetobacter and cinobacter are less frequent causes of neonatal bacteremia. These latter organisms are normally responsible for late onset nosocomial infections, they are more resistant and form part of the hospital environment (Plotkin,1981.)

- **Antibiotic therapy**

The choice of antibiotic depends largely on whether the causative organism is acquired from the mother or from the hospital environment. With nosocomial infections combination antibiotic therapy is advisable due to the number of resistant organisms in the hospital. A penicillin-aminoglycoside combination is advocated in this instance due to wider cover and synergistic effect (Mir *et al.*, 1987). Antibiotic toxicity manifests more frequently in the neonatal population as a result of the constantly changing metabolism through gestational and chronological ages. The monitoring of plasma levels of antibiotics are very important in these patients especially for drugs like aminoglycosides which have a small therapeutic index. The required duration of therapy is normally longer than in the adult population being an average of 10-14 days after symptoms have subsided (Mir *et al.*, 1987).

3. AN EPIDEMIOLOGICAL STUDY OF GRAM NEGATIVE BACTEREMIA AT BARAGWANATH HOSPITAL

3.1 OBJECTIVE

A retrospective epidemiological study was performed to determine the nature and extent of bacterial infections in an adult and neonatal population at Baragwanath Hospital. This study will be presented under two headings namely adult and neonatal gram negative bacteremia.

The primary objective of the study was to determine the epidemiology of gram negative bacteremias in adults and neonates at Baragwanath Hospital. A secondary objective was to identify factors which could possibly determine the outcome of gram negative bacteremias in adults and neonates.

3.2 LOCATION

This study was carried out at Baragwanath Hospital, Soweto, Republic of South Africa. Baragwanath Hospital is the largest hospital in the Southern hemisphere and serves a mixed first and third world population. The hospital functions as a teaching, referral and community hospital. Patients who are seriously ill are referred to the hospital from rural areas and even areas outside the borders of the Republic of South Africa. The community of Soweto is also served by Baragwanath Hospital and presents at the hospital with both minor and major diseases.

Microbiological investigations for Baragwanath Hospital are carried out at the laboratories of the South African Institute for Medical Research (SAIMR) which are located on the premises of Baragwanath Hospital.

3.3 APPROVAL OF THE STUDY

The study was approved by the Ethics Committee of Baragwanath Hospital.

3.4 STUDY DESIGN

All documented cases of gram negative bacteremia in adults from the medical wards and neonates from neonatal intensive care over a six month period were reviewed. The

methods of data collection were epidemiological rather than clinical in the sense that data was obtained from patient records. A retrospective investigation of bacteriological and clinical data was performed. In the first phase of the study the records of blood cultures were screened at the SAIMR laboratories at Baragwanath Hospital. All blood cultures positive for gram negative bacteria from the medical wards and neonatal ICU were recorded and the causative organism noted. In the second phase of the study the medical records of the patients who had positive blood cultures were traced. The third phase of the study involved analyses of recorded data.

- INCLUSION CRITERIA. Patients with a positive blood culture for gram negative bacteria were included in the study.

- EXCLUSION CRITERIA. Patients with a positive blood culture for salmonella, neisseria and haemophilus were not included in this study because of the the low mortality rate associated with these infections (Du Pont and Spinks, 1969).

The following populations were defined:

- **Overall population:** This population included all the patients for whom positive blood cultures were recorded at the SAIMR.

- **Study population:** This population included all patients with a positive blood culture for whom the medical records could be traced. It was not possible to trace the medical records of all patients for whom a positive blood culture was reported. There are a number of reasons for this discrepancy. Baragwanath Hospital is understaffed and handles a large number of patients. A manual non-computerized filing system is used which results in certain errors. The medical records of patients who die are sent to the mortuary and some records never reach the archives.

3.5 BACTERIOLOGICAL DATA

3.5.1 Identification of causative organism

Organisms were identified and classified according to standard microbiological techniques (Leinette *et al.*, 1980).

3.5.2 Antibiotic susceptibility

The Kirby Bauer disc diffusion technique was used to determine antibiotic susceptibility (Bauer *et al.*, 1966).

3.6 DATA DERIVED FROM MEDICAL RECORDS

The data gathered from medical records were different for the adults and neonates and will be discussed under the respective headings of adult gram negative bacteremia and neonatal gram negative bacteremia.

4 THE INVESTIGATION OF ADULT GRAM NEGATIVE BACTEREMIA

4.1 METHODOLOGY

This part of the study involved the surveillance of bacteriological and clinical data of patients admitted to the medical wards of Baragwanath Hospital from January to June 1988. The following data were derived from the medical records of patients with gram negative bacteremia:

- Age
- Sex
- Presence and severity of underlying disease
- Initial antibiotic therapy
- Mortality associated with gram negative bacteremia
- Acquisition of infection (community acquired or nosocomial)
- Route of infection

Some of these aspects required further definition:

- **Underlying disease**

  The patients were classified according to the presence or absence of underlying diseases. When present, such diseases were further categorized according to the criteria of McCabe and Jackson (1962(a) into rapidly fatal, (death anticipated within 1 year) ultimately fatal (death anticipated within 4 years) and non- fatal.

- **Nosocomial and community acquired infections**

  Nosocomial infections were defined as those confirmed by a positive blood cultures and which started on or after the third day of hospitalization (McGowan *et al.*, 1975; McCue, 1987). Any positive culture obtained before this was classified as a community acquired infection.

- **Mortality associated with gram negative bacteremia**

  In the absence of any other explanation bacteremia was considered as the cause of death if it occurred within 7 days of the last positive blood culture (Bryan *et al.*, 1983).

- **Appropriate antibiotic therapy**

  For the purpose of this study appropriate therapy was defined according to McFarlane and Nana (1985) i.e. an antibiotic to which the isolated organism was sensitive (*in vitro*), which was started on the day when the first blood culture was reported as positive, and continued for at least 5 days.

- **Initial antibiotic therapy**

  The first antibiotic that was administered to the patient was recorded as initial antibiotic therapy.

4.2 RESULTS

During the period January to June 1988 an average of 3000 patients per month were admitted to the medical wards. One hundred and four positive gram negative blood cultures were reported during this six month period. The medical records of 76 patients could be traced. Three of these patients had polymicrobial infections and therefore 76 patients and 79 organisms were investigated. Ampicillin was the initial antibiotic therapy in all the patients.

4.2.1 INCIDENCE OF GRAM NEGATIVE BACTEREMIA

With 104 positive gram negative blood cultures for 6 months (17.3 a month) and 3000 admissions per month reported, the incidence of gram negative bacteremia in the medical wards was therefore 5.8/1000 patients a month.

4.2.2 PREVALENCE OF CAUSATIVE ORGANISMS

The organisms isolated from patients with gram negative bacteremia are summarized in table A.4 and figure A.1. As can be seen from figure A.1 the frequency of occurrence of individual organisms did not differ markedly between the overall and study populations. The percentage of patients with *E. coli* (53%), proteus (9%), and acinetobacter (5%) were the same for the two groups. The incidence of klebsiella and pseudomonas differed between the two groups by one percent. The study population is therefore clearly representative of the overall group. In the study population (figure A.2), *E. coli* was the organism most commonly isolated (53%) followed by klebsiella (23%), proteus (9%) and pseudomonas (8%). The incidence of other organisms was 5% or less.

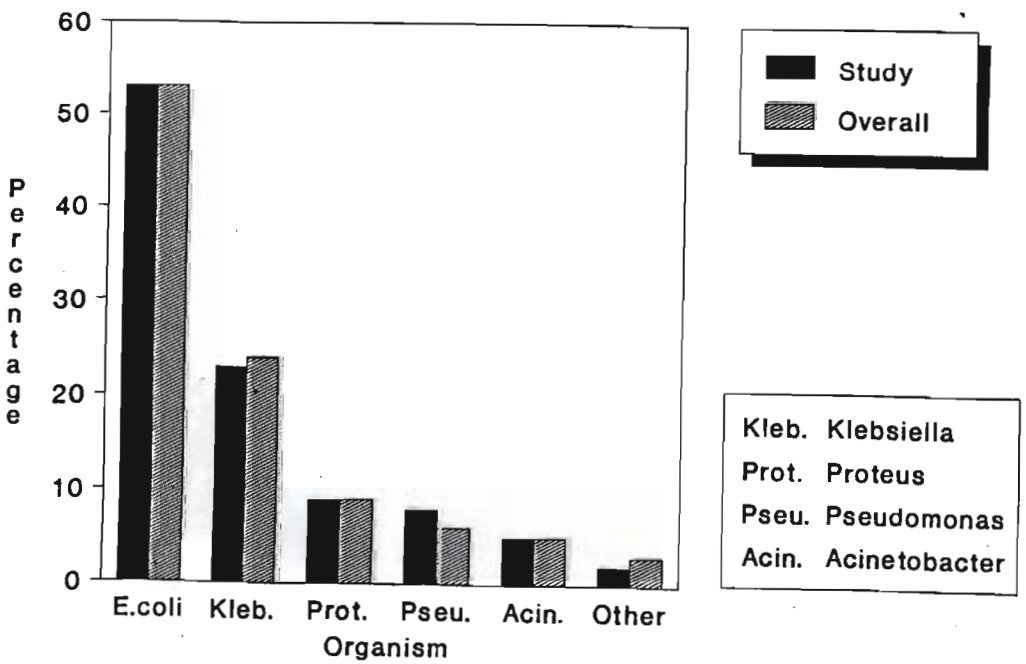


Fig A.1 Comparison between organisms in study and overall populations

TABLE A.4

Gram negative organisms isolated from the adult population with gram negative bacteremia

	Study population		Overall population	
	Number of isolates	Percentage	Number of isolates	Percentage
E. coli	42	53	55	53
Klebsiella	18	23	25	24
Proteus	7	9	9	9
Pseudomonas	6	8	7	7
Acinetobacter	4	5	5	5
Serratia	1	1	1	1
Yersinia	1	1	1	1
Citrobacter	0	0	1	1
Total	79	100	104	100

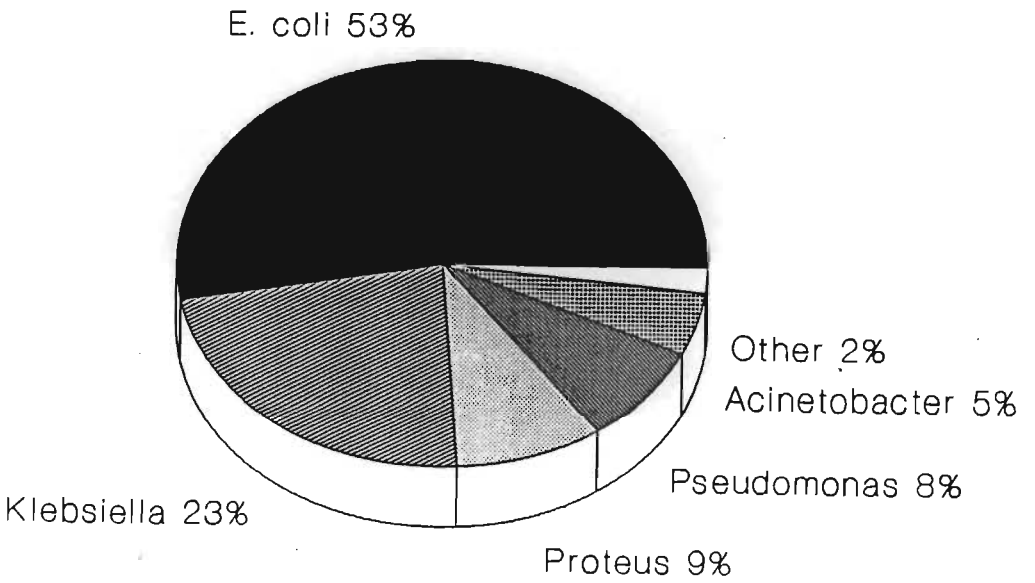


Fig A.2 Percentage distribution of organisms in adult study population

4.2.3 THE RELATIONSHIP BETWEEN THE CAUSATIVE ORGANISM, MORTALITY RATE, UNDERLYING DISEASE AND NOSOCOMIAL INFECTION

Data pertaining to the causative organisms, number of deaths, underlying diseases and nosocomial origin are summarized in table A.5 and figure A.3. The only patient with a serratia infection died. Aside from this the mortality rate for patients in the present study was the highest with klebsiella infections (39%), followed by *E. coli* (33%), proteus (29%) and acinetobacter (25%). For both pseudomonas and yersinia infections no deaths were reported. The percentage of patients with an underlying disease prior to bacteremia was the highest with proteus (86%), followed by pseudomonas (83%), klebsiella (78%), acinetobacter (75%) and *E. coli* (62%). Forty three percent of patients with proteus bacteremia contracted the infection nosocomially followed by 22% of patients with klebsiella, 17% with pseudomonas and 10% with *E. coli.*

TABLE A.5

Relationship between underlying disease, death and nosocomial origin of gram negative bacteremia in the adult population

Organism	Number of isolates	Under = lying disease	%	Deaths	%	Noso = comial origin	%
E. coli	42	26	62	14	33	4	10
Klebsiella	18	14	78	7	39	4	22
Proteus	7	6	86	2	29	3	43
Pseudomonas	6	5	83	0	0	1	17
Acinetobacter	4	3	75	1	25	0	0
Serratia	1	0	0	1	100	0	0
Yersinia	1	0	0	0	0	0	0
Total	79	54		25		12	

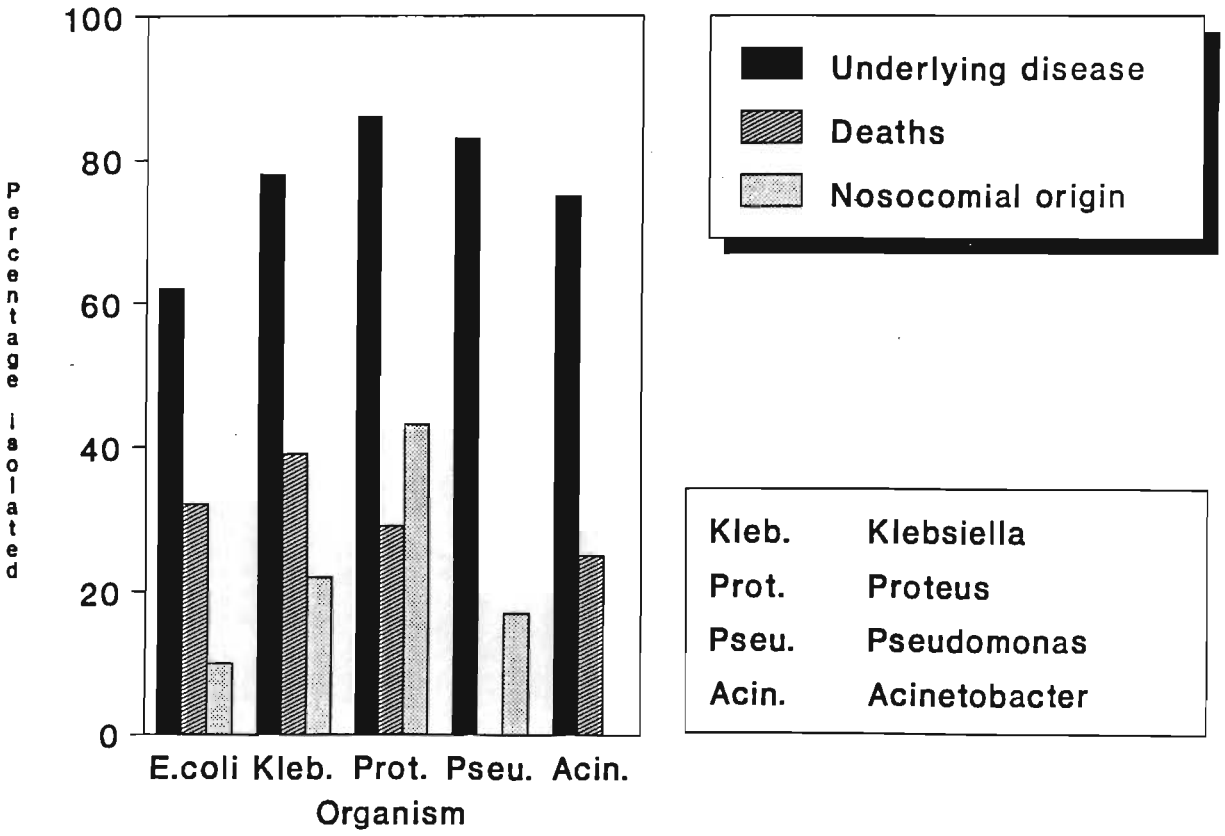


Fig A.3 Relationship between underlying disease, death and nosocomial origin

4.2.4 ROUTES OF INFECTION

The different causative organisms and their routes of infection are summarized in tables A.6, A.7 and figure A.4. The genitourinary tract (GUT) was the most common source of infection (34%), with *E. coli* the predominant organism (78%). Klebsiella (15%) was also found in the GUT but less frequently than *E. coli.* The lungs were the second most common route of infection (24%) where the predominant organisms were klebsiella and *E. coli* (32% each). *E. coli* was the only organism also found in the GIT and CNS. The GIT and CNS were the source of infection in 6 and 3% of the cases respectively. In 14% of the patients the skin was the route of infection and the organisms responsible were *E. coli* (45%), klebsiella (27%) and proteus (27%). In 16% of patients the route of infection was not identified: The organisms involved in these cases were *E. coli* (23%), klebsiella (31%) and 15% for both proteus and acinetobacter. Pseudomonas was the

only organism where all the routes of infection could be traced with no route reported as unknown. This study differentiate between IE end GIT. GIT denotes patients with diarrhea whereas IE refers to patients with other intestinal diseases.

TABLE A.6

Routes of infection in adult population

Organism	GUT	GIT	Lung	Skin	Bile	IE	CNS	Unk.
E. coli	21	5	6	5	0	0	2	3
Klebsiella	4	0	6	3	1	0	0	4
Proteus	0	0	2	3	0	0	0	2
Pseudomonas	1	0	4	0	0	1	0	0
Acinetobacter	1	0	1	0	0	0	0	2
Serratia	0	0	0	0	0	0	0	1
Yersinia	0	0	0	0	0	0	0	1
TOTAL	27	5	19	11	1	1	2	13

GUT Genitourinary tract
GIT Gastrointestinal tract
IE Intestine
CNS Central nervous system
Unk. Unkown

TABLE A.7

Percentage distribution of routes of infection in the adult population

Organism	GUT	GIT	Lung	Skin	Bile	IE	CNS	Unk.
E. coli	78	100	32	45	0	0	100	23
Klebsiella	15	0	32	27	100	0	,0	31
Proteus	0	0	11	27	0	0	0	15
Pseudomonas	4	0	21	0	0	100	0	0
Acinetobacter	4	0	5	0	0	0	0	15
Serratia	0	0	0	0	0	0	0	8
Yersinia	0	0	0	0	0	0	0	8

GUT Genitourinary tract
GIT Gastrointestinal tract
IE Intestine
CNS Central nervous system
Unk. Unkown

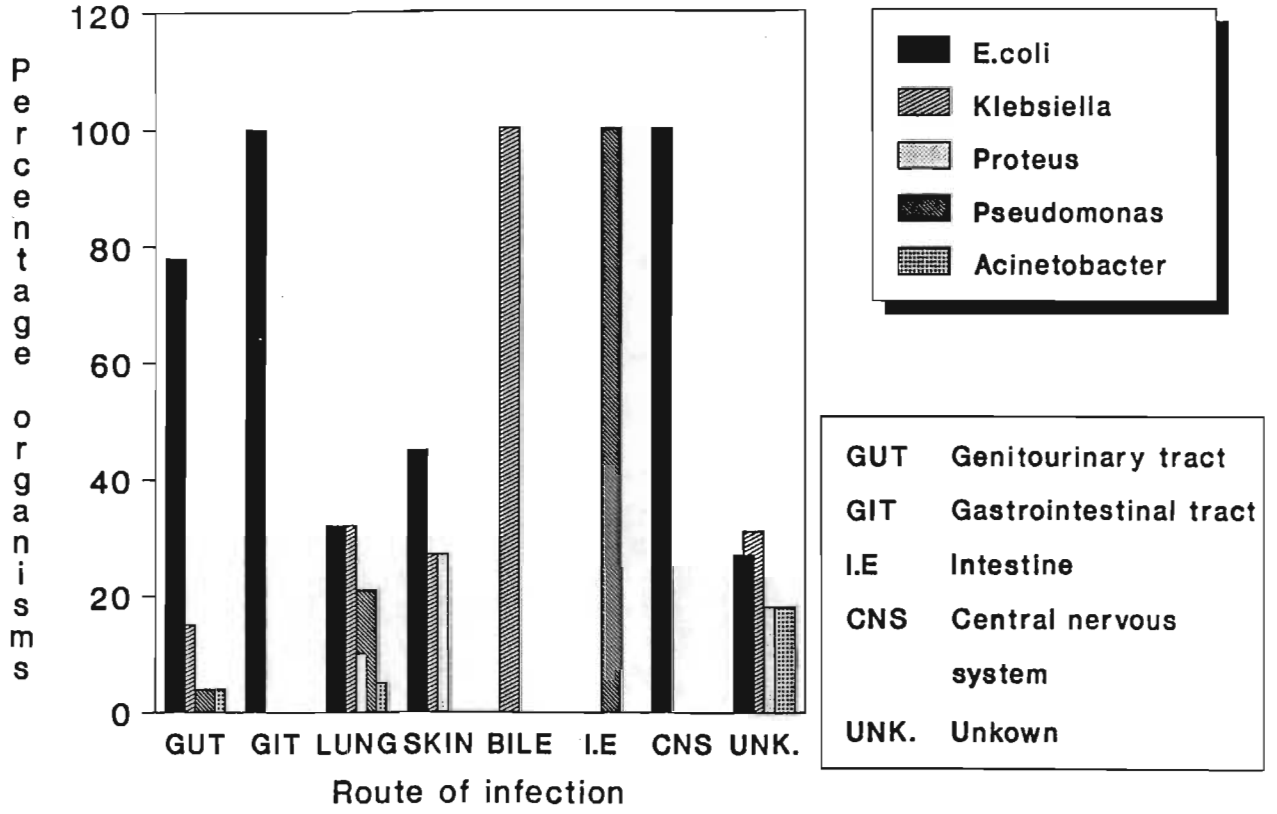


Fig A.4 Causative organism and route of infection in adult population

4.2.5 RELATIONSHIP BETWEEN AGE AND MORTALITY

Figure A.5 and Table A.8 show the incidence of gram negative bacteremia within various age groups and the associated mortality rates.

The average age of patients was 50.5 years with the highest incidence of bacteremia (20%) recorded in the 30-39 age group. The highest mortality rate (50%) occurred in the age group 70-79 years. 32% (24) of the 76 patients died. Of these 9 (38%) were younger than 50 years and 15 (63%) were older than 50 years.

TABLE A.8

Relationship between age and mortality in the adult population

Age	Number of patients	Number of deaths	Percentage of deaths
10 - 19	3	1	33
20 - 29	9	0	0
30 - 39	15	4	27
40 - 49	11	4	36
50 - 59	10	4	40
60 - 69	13	6	46
70 - 79	10	5	50
80 - 89	2	0	0
90 - 99	3	0	0

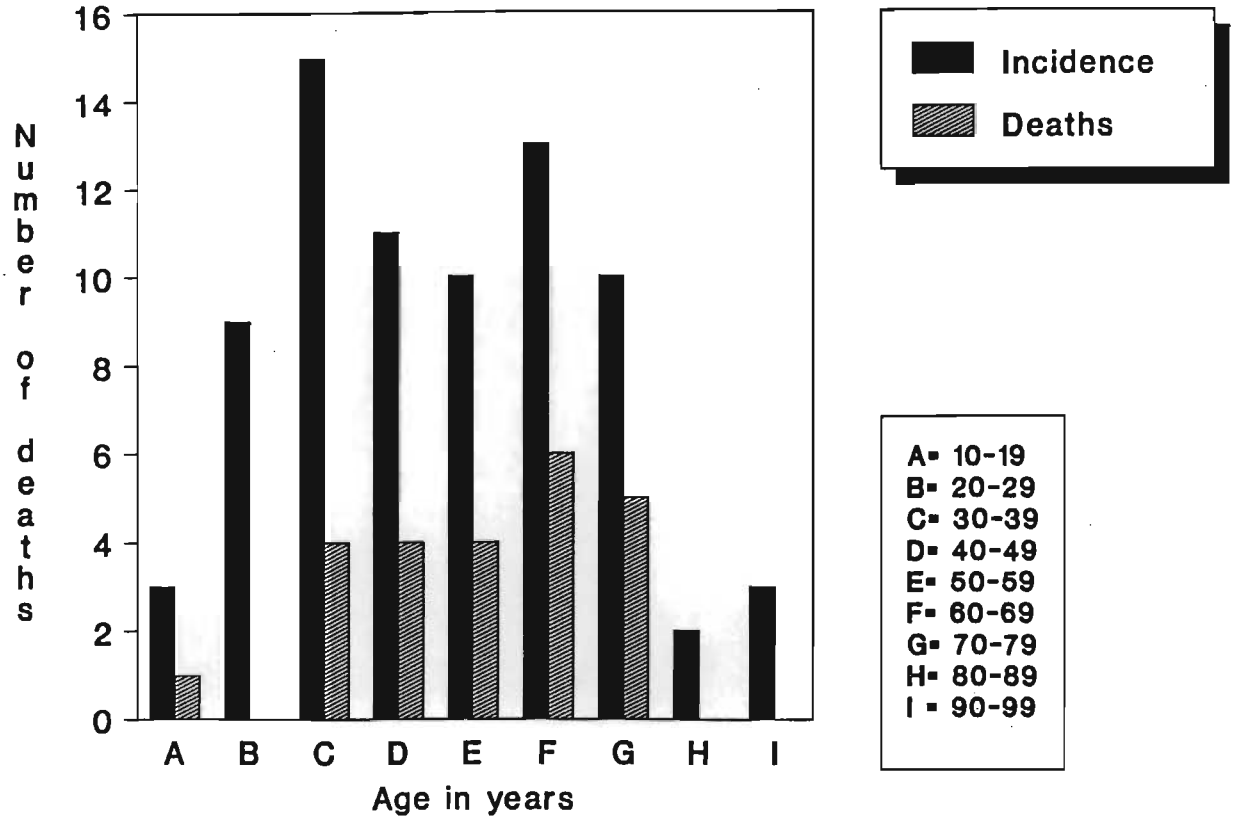


Fig A.5 Relationship between age and mortality in the adult population

4.2.6 SEVERITY OF UNDERLYING DISEASE

The relationship between severity of underlying disease and deaths are summarized in Table A.9 and figure A.6. Overall thirty two percent (24) died. Of these 16 (67%) died within the first 72 hours of admission. Sixty seven percent of the patients with a rapidly fatal, 48% with an ultimately fatal, 38% with a non-fatal and 8% with no underlying discase died.

TABLE A.9

Relationship between severity of underlying disease and death in the adult population (Classification according to McCabe and Jackson, 1962)

Severity of underlying disease	Number of episodes	Number of deaths	Percentage deaths	Deaths as % of total
Rapidly fatal	3	2	67	8
Ultimately fatal	21	10	48	42
Non - fatal	26	10	38	42
No underlying disease	26	2	8	8
TOTAL	76	24	-	100

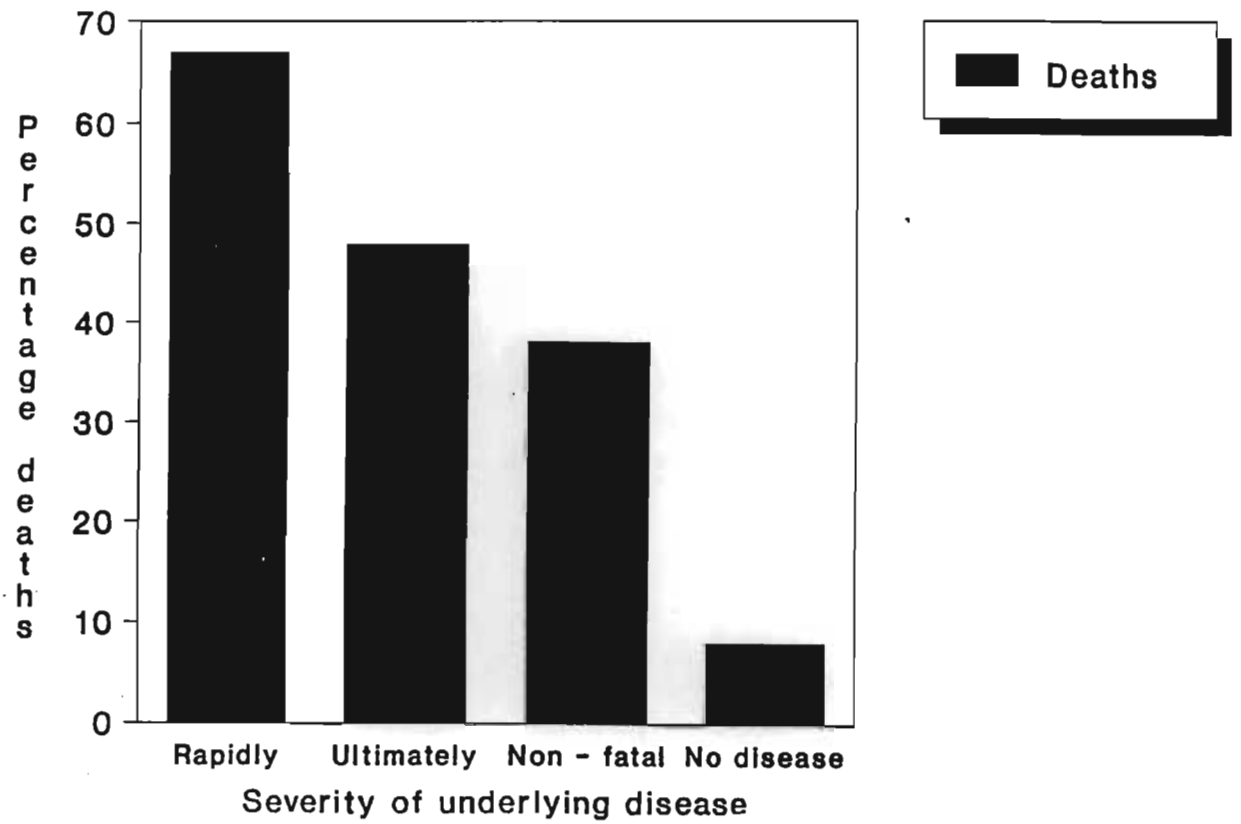


Fig A.6 Relationship between underlying disease and death in the adult population

4.2.7 ANTIBIOTIC RESISTANCE PATTERNS OF ORGANISMS ISOLATED

The resistance patterns of the organisms to antibiotics routinely used in the medical wards at Baragwanath Hospital are summarized in Table A.10 and figure A.7.

Seventy six percent of *E.coli*, 43% of proteus, 88% of klebsiella and 100% of pseudomonas were found to be resistant to ampicillin. Proteus was not tested against the aminoglycosides or the third generation cephalosporins. *E. coli* was the most susceptible to the aminoglycosides with only 2% being resistant to gentamicin and tobramycin and none to amikacin. Klebsiella showed no resistance to amikacin but a 29% and 18% resistance to gentamicin and tobramycin respectively. Pseudomonas showed a 17% resistance to all aminoglycosides.

E. coli and klebsiella showed susceptibility to all the third generation cephalosporins tested, but pseudomonas was resistant to cefotaxime (50%) and ceftriaxone (33%).

TABLE A.10

Percentage of organisms resistant to antibiotics routinely used in the medical wards of Baragwanath Hospital

Antibiotic	E. coli %	Proteus %	Klebsiella %	Pseudomonas %
Ampicillin	76	43	88	100
Piperacillin	33	0	53	17
Gentamicin	2	-	29	17
Amikacin	0	-	0	17
Tobramycin	2	-	18	17
Cefotaxime	0	-	0	50
Ceftriaxone	0	-	0	33

\- = not tested

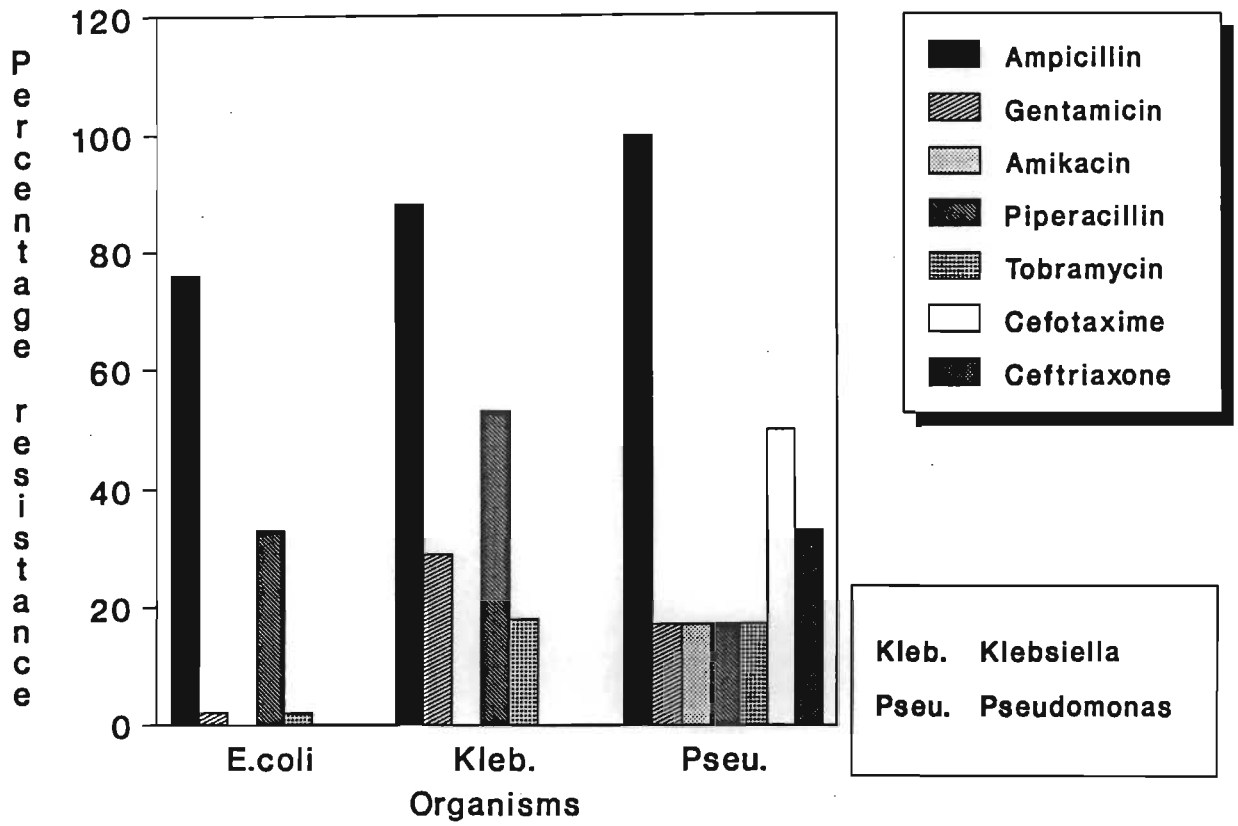


Fig A.7 Resistance patterns of organisms in the adult population

4.3 DISCUSSION

The case selection in this study was limited to patients admitted to medical wards and therefore more restricted than it was in a number of studies reported in the literature. Notwithstanding certain similarities were noted. The incidence of gram negative bacteremia (5.8/1000 patients) at Baragwanath Hospital corresponds with those reported for other centers which have varied from 4.7/1000 patients for community hospitals to 12.8/1000 patients for teaching hospitals (McCue, 1987; Dale and Petersdorf, 1987). The higher incidence of gram negative bacteremia in teaching hospitals is well documented and may be ascribed to a number of factors (Ashiru and Osoba, 1986). Patients admitted to teaching hospitals are generally severely ill, often immunosuppressed and frequently elderly with chronic diseases. Furthermore resistance of organisms to antibiotics occurs more frequently in teaching hospitals which complicates the effective eradication of particular organisms (Gatell *et al.*, 1988). The incidence of gram negative bacteremia at Baragwanath Hospital which serves as a combined teaching and community hospital could therefore be expected to be between that reported for community and teaching hospitals. Fifteen percent of these infections were hospital acquired.

At Baragwanath Hospital the highest mortality rate (39%), was associated with gram negative bacteremias due to klebsiella infections. Klebsiella associated mortality rates of 35.5% (Haddy *et al.*, 1987), 45% (Bryan *et al.*, 1983) and 57% (Du Pont and Spinks, 1969) have previously been reported. The mortality rate associated with klebsiella at Baragwanath Hospital therefore appears to correspond to the mortality rates reported from other centers.

Of the klebsiella infections recorded at Baragwanath Hospital 22% were hospital acquired and 78% community acquired. The incidence of nosocomial klebsiella infections at Baragwanath Hospital was substantially lower than the incidence (up to 50%) reported at other centers (Montgomery, 1979; Garcia de la torre *et al.*, 1985). The high incidences at these other centres were associated with sporadic epidemic outbreaks in intensive care units for neonates or adults, during winter months (Garcia de la torre *et al.*, 1985). The lower incidence of nosocomial klebsiella infections at Baragwanath Hospital may be ascribed to the design of the study which only included patients admitted to medical wards and which was carried out largely during summer and autumn months (January to June). Furthermore the medical wards at Baragwanath Hospital consists of individual physically separated units that restrict the spread of nosocomial infections.

Proteus infections was associated with the third highest mortality rate (29% at Baragwanath Hospital of which 43% was hospital acquired). In 86% of the patients with proteus bacteremia an underlying disease was present. Proteus species are rarely primary pathogens but produce disease in locations previously infected by other pathogens. Proteus species are able to establish reservoirs in the inanimate hospital environment and are a frequent cause of nosocomial infections (Bryan *et al.*, 1983). Furthermore these gram negative bacteria readily develop resistance to antibiotics which may account for the high mortality rate associated with proteus infections at Baragwanath Hospital.

E. coli accounted for 53% of all gram negative bacteremias in the medical wards and of these only 10% were hospital acquired. The *E. coli* associated mortality rate was however, slightly lower (33%) than the rate associated with klebsiella (39%). This association of a high incidence with a lower mortality rate in patients with *E. coli* bacteremia has been documented before (Kreger *et al.*, 1980(a); Kreger *et al.*, 1980(b)). In the majority of patients with *E. coli* bacteremia at Baragwanath Hospital the source of the infection was the GUT (50%). These patients were generally healthy prior to the infection and did not fall into the groups with rapidly fatal or ultimately fatal diseases. This may account for the lower mortality rate associated with *E. coli* bacteremia.

Pseudomonas accounted for 8% of gram negative bacteremias at Baragwanath Hospital. In 83% of these patients an underlying disease could be identified while 17% of the pseudomonas infections were nosocomial. No deaths were reported as a result of pseudomonas infections in the present series. This finding is contrary to the high mortality rates associated with pseudomonas infections in other series (Du Pont and Spinks, 1969). There are a number of possible explanations for the low mortality rate associated with pseudomonas infections in the present study. Serious infections with pseudomonas are almost invariably associated with local tissue damage or diminished host resistance. The present investigation did not include patients admitted to surgical wards or burn units. Furthermore only one of the patients with pseudomonas bacteremia was immunosuppressed and only 3 patients had a rapidly fatal underlying disease. Hospital strains of pseudomonas are generally more resistant to antibiotics. In the present study however, only one patient had a nosocomial pseudomonas infection.

In the present study the GUT (34%) was the most common source of gram negative infection. The lungs (24%), skin (14%), GIT (6%) and CNS (3%) were other sources of infection. In 16% of the cases the source was unknown.

The finding that the GUT was the most common source of infection was not surprising in view of the fact that *E. coli* was the predominant (53%) cause of bacteremias in the present series. *E. coli* is responsible for 79% of all urinary tract infections. Similar findings related to *E. coli* bacteremias have been reported in other studies (Du Pont and Spinks, 1969).

It has been reported that klebsiella is the most common cause of pneumonias associated with gram negative bacteremia. In the present study the incidence of klebsiella (32%) and *E. coli* (32%) pneumonias was equal. A possible reason for this discrepancy is that klebsiella infections predominate in intensive care units (ICU) and during epidemics. Patients in the ICU were not included in the present investigation and no epidemics occurred during the months that the study was carried out.

The severity of underlying disease as a contributing factor to mortality in patients with gram negative bacteremia is clearly shown in the present study. Sixty seven percent of the patients with a rapidly fatal disease, 48% with an ultimately fatal disease and 38% with a non-fatal disease died.

The majority of patients with a rapidly fatal disease died 72 hours or more after the diagnosis of gram negative bacteremia had been confirmed due to uncontrolled infections. There were a number of reasons for this: These patients were generally severely ill, frequently immunosuppressed and had been exposed repeatedly to antibiotics which predispose to the development of antibiotic resistance (Kreger *et al.*, 1980(a)).

The relatively high mortality rates of 48% and 38% for patients with ultimately fatal and non-fatal diseases respectively are in agreement with the study of Kreger *et al.*, (1980(b)). From this study it appears that these patients die of shock within 72 hours of the onset of the gram negative bacteremia rather than from a persistent uncontrolled infection as in the case of patients with a rapidly fatal disease. In the present study 16 patients (67%) died within 72 hours. It therefore appears that the outcome of gram negative bacteremia depends not only on the severity of the underlying disease but also on whether shock develops within 72 hours. Furthermore it seems that if patients, (with the exception of those with rapidly fatal underlying disease) survived the first 72 hours of bacteremia, the overall chance of survival was fairly good.

Review of the literature indicates that the outcome of gram negative bacteremia is in part dependent on the initiation of appropriate antibiotic therapy even though there is

some controversy as to what constitutes appropriate antibiotic therapy. In the present study initial antibiotic therapy consisted of ampicillin. As virtually all the isolates were resistant to ampicillin, this may have contributed to mortality rates.

4.4 CONCLUSIONS

Gram negative bacteremia occurred in 5.8 per 1000 patients admitted to the medical wards at Baragwanath Hospital. *E. coli* was the most common causative organism followed by klebsiella and proteus. Fifteen percent of these bacteremias were hospital acquired. The incidence of bacteremia and nosocomial infections and the fact that *E. coli* was the most common organism suggests that Baragwanath Hospital is consistent with the criteria of a combined community and tertiary hospital. These findings correlate with literature reports on community and tertiary hospitals.

Gram negative bacteremia was associated with a high mortality rate in the present study. Thirty two percent of the patients died with 67% of these deaths occurring within 72 hours. Shock has been identified both in the literature and present study as a possible cause of early deaths in patients with gram negative bacteremia (Moore *et al.*, 1987). Early and adequate treatment of shock is therefore advocated in the management of gram negative bacteremia.

All the gram negative organisms cultured from patients with gram negative bacteremia were resistant to ampicillin. It is therefore suggested that an aminoglycoside should be combined empirically with a suitable penicillin in high risk groups. It is further suggested that the initial dose of aminoglycoside be designed to achieve a high peak concentration since a high peak concentration is associated with a better outcome in gram negative bacteremia (Moore *et al* , 1984 (a)).

Optimum aminoglycoside dosing is best achieved by the application of therapeutic drug monitoring. The population pharmacokinetic parameters must be representative of the population and it is therefore suggested that a study be designed to calculate such values for a gram negative bacteremic population on gentamicin treatment.

5 NEONATAL GRAM NEGATIVE BACTEREMIA

5.1 METHODOLOGY

This part of the study involved the surveillance of bacteriological and clinical data of neonates admitted to the Neonatal Intensive Care Unit (NICU) at Baragwanath Hospital from January to June 1988. The NICU consists of 2 units, a transient and neonatal unit. The transient unit serves the infants of older gestational age and higher body weight. Premature, low birth weight infants are served by the neonatal unit. Approximately 150 infants are admitted, monthly to this 30 incubator NICU. The following data were obtained from the medical records of neonates with gram negative bacteremia:

- Age (gestational/chronological)
- Sex
- Time of onset of infection
- Route of infection
- Initial antibiotic therapy
- Birth weight
- Method of birth

Some of the data necessitate further definition:

- **Early and late onset infections**
  Early onset was defined as an infection which started within the first 7 days after birth. Late onset infections started after the seventh day.

- **Appropriate therapy**
  Appropriate therapy was defined as an antibiotic to which the organism was sensitive (*in vitro*) and which was started on the day when the first blood culture was reported as positive and then administered for at least 10 to 14 days.

- **Preterm infants**
  Infants with a gestational age of less than 40 weeks.

- **Full term infants**

  Infants with a gestational age of at least 40 weeks.

STUDY DESIGN

A retrospective investigation of bacteriological and clinical data was performed. In the first phase of the study the records of blood cultures were screened at the SAIMR laboratories at Baragwanath Hospital. All blood cultures positive for gram negative bacteremia were recorded. In the second phase of the study the medical records of the infants who had had positive blood cultures were traced. The third phase of the study involved analyses of the recorded data.

- INCLUSION CRITERIA: All infants with a positive blood culture for gram negative bacteria were included in the study.

Categorization of patients

The following populations were defined:

- **Overall population:** This population included all infants for whom a positive gram negative blood culture was reported.

- **Study population:** This population included all infants with a positive blood culture of whom the medical records could be traced. (Reasons for inability to trace all records are the same as for adult population - see 3.4.)

5.2 RESULTS

Sixty positive gram negative blood bacterial cultures were reported for the six month period. The medical records of only 32 of these patients could be traced.

5.2.1 INCIDENCE OF GRAM NEGATIVE BACTEREMIA

An average of 150 neonates per month were admitted to the NICU during the study period. During the six month period 60 blood cultures positive for gram negative bacteria were reported (10 per month). The incidence of gram negative bacteremia was therefore 66/1000 neonates.

5.2.2 PREVALENCE OF CAUSATIVE ORGANISMS

The organisms isolated from neonates with gram negative bacteremia are summarized in table A.11 and figure A.8. As can be seen from table A.11 the frequency of occurrence of individual organisms did not differ markedly between the overall and study populations. In the study population (see figure A.9), pseudomonas was the organism most commonly isolated (44%), followed by klebsiella and carynobacter (19% each), *E. coli* (9%), bacillus species (normally *B.subtalis*) (6%) and enterobacter (3%).

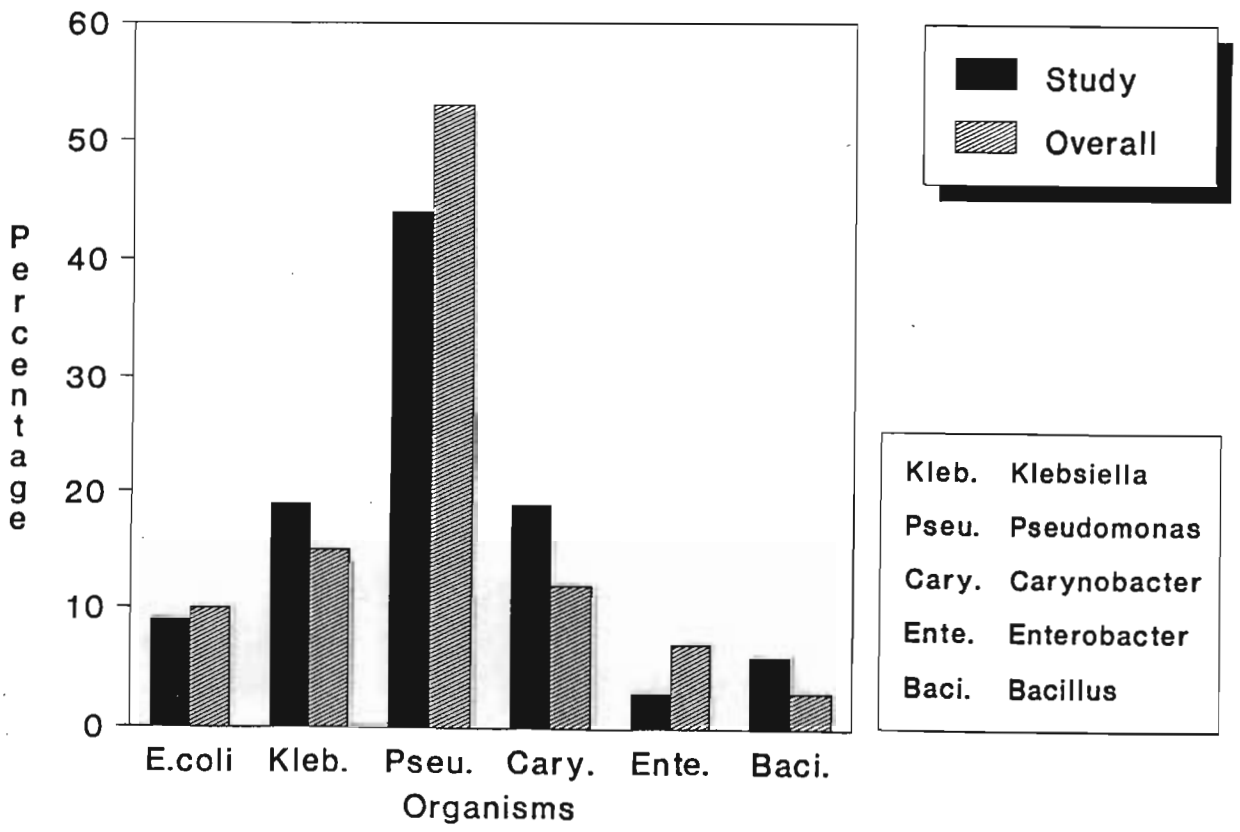


Fig A.8 Comparison between study and overall neonatal populations

TABLE A.11

Organisms isolated from the neonatal population with gram negative bacteremia

	Study population		Overall population	
	Number of isolates	Percentage	Number of isolates	Percentage
E. coli	3	9	6	10
Klebsiella	6	19	9	15
Pseudomonas	14	44	32	53
Carynobacter	6	19	7	12
Enterobacter	1	3	4	7
Bacillus species	2	6	2	3
Total	32	100	60	100

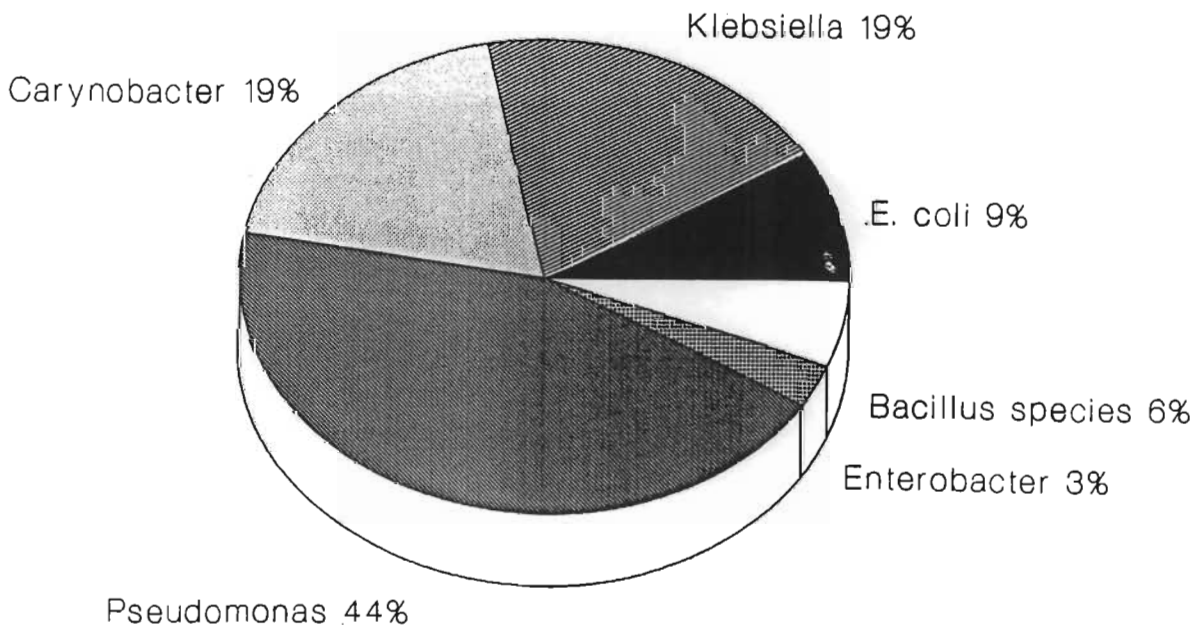


Fig A.9 Distribution of organisms in neonatal study population

5.2.3 RELATIONSHIP BETWEEN CAUSATIVE ORGANISM AND MORTALITY RATE

Data pertaining to the relationship between the causative organisms and mortality are summarized in table A.12 and figure A.10. The overall mortality rate for neonates with gram bacteremia was 66%. The highest mortality rate was associated with pseudomonas (71%) followed by klebsiella (67%) and *E. coli* (67%).

TABLE A.12

Relationship between the causative organism and death in the neonatal population

	Organism	Number of deaths	Percentage of deaths
E. coli	3	2	67
Klebsiella	6	4	67
Pseudomonas	14	10	71
Carynobacter	6	3	50
Enterobacter	2	1	50
Bacillus species	1	1	100
Total	32	21	

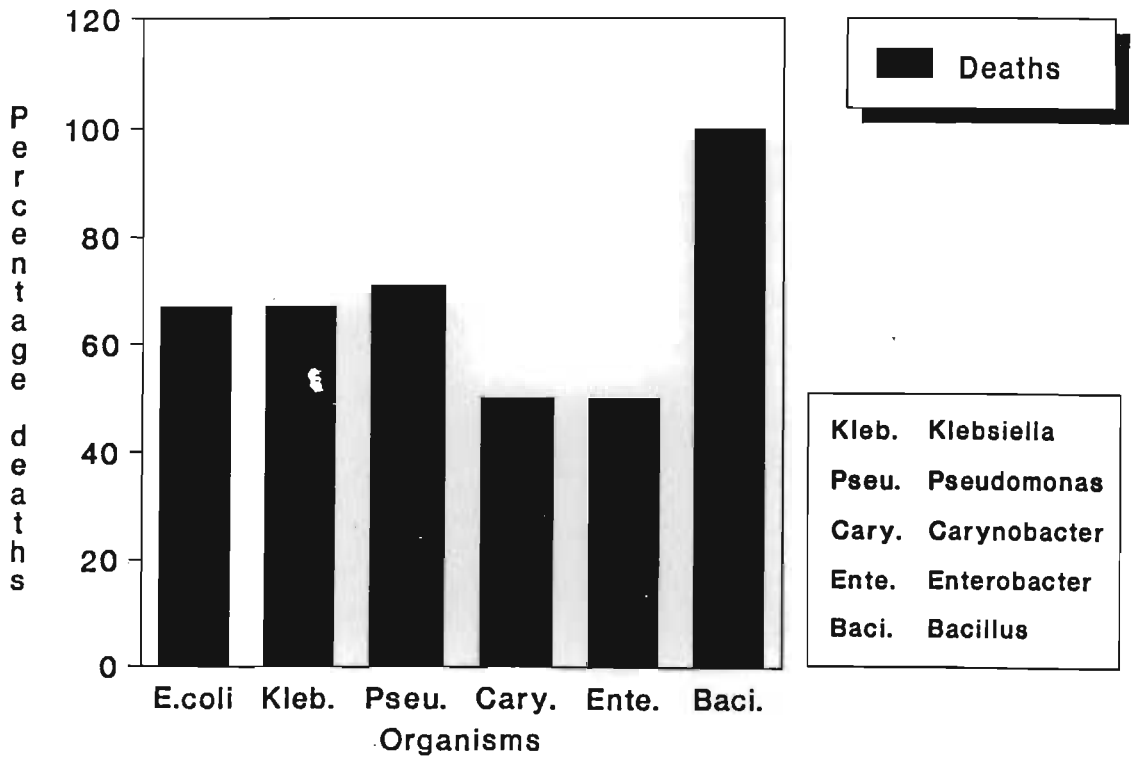


Fig A.10 Relationship between organisms and percentage deaths in neonatal population

5.2.4 FACTORS WHICH MAY INFLUENCE THE PROGNOSIS IN NEONATES WITH GRAM NEGATIVE BACTEREMIA

The relationship between mortality rate and factors which may influence prognosis (age, sex, gestational age, method of birth and time of onset of infection) is summarized in table A.13 and fig A.11.

- Weight

  Of the 32 patients in the study group 29 weighed less than 2.5 kg and of these 21 (72%) died. All the neonates (3) weighing more than 2.6 kg survived.

- Sex

  An equal number of male and female neonates contracted bacteremia. However, 75% of the male and 56 % of the female neonates died.

- Onset of infection

  Forty seven percent of neonates with an early and 82 % with a late onset of infection died.

- Method of birth

  The incidence of death was slightly higher in bacteremias following complicated births (71% versus 61%).

- Gestational age

  The incidence of death was higher in infants with a gestational age less than 40 weeks in comparison with full term infants (80% versus 14%).

TABLE A.13

Relationship between mortality due to gram negative bacteremia and other factors which may influence outcome

Factors		Number	Number of deaths	Percentage of deaths
Weight	< 2.5kg	29	21	72
	> 2.5kg	3	0	0
Sex	Male	16	12	75
	Female	16	9	56
Onset	Early	15	7	47
	Late	17	14	82
Birth	Normal	18	11	61
	Complicated	14	10	71
Gestational age	Term	7	1	14
	Preterm	25	20	80

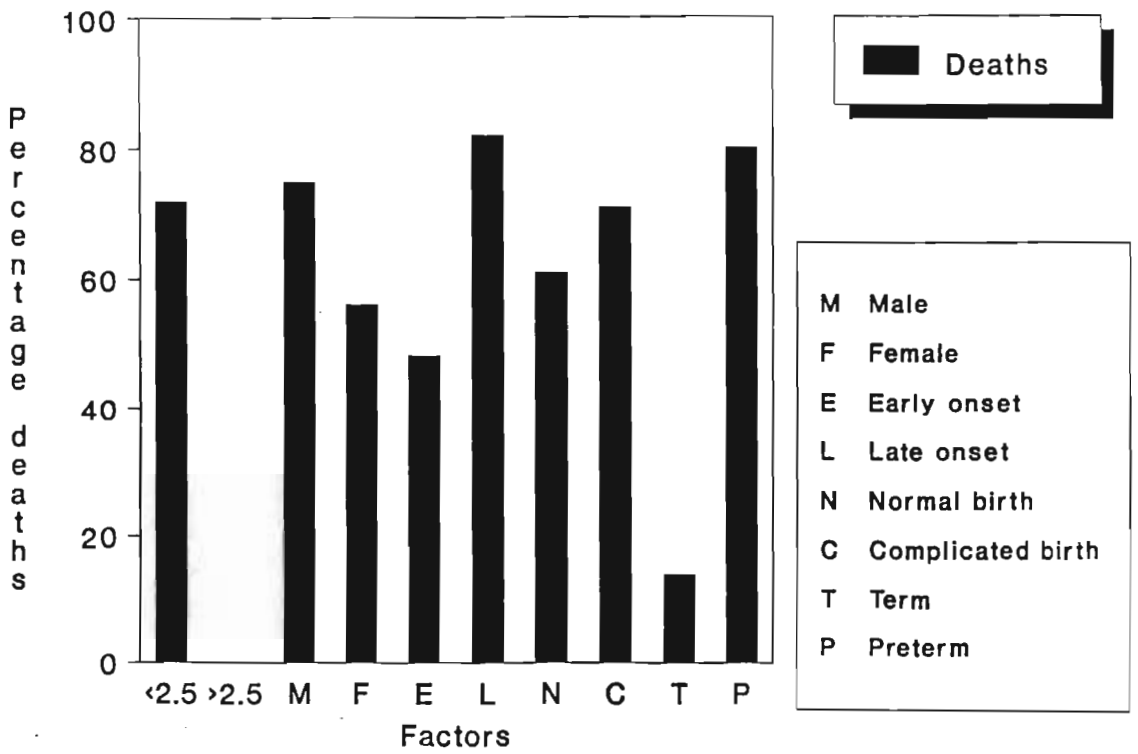


Fig A.11 Relationship between associated factors and mortality in neonatal population

5.2.5 ANTIBIOTIC RESISTANCE PATTERNS OF ORGANISMS ISOLATED

The resistance patterns of organisms to antibiotics routinely used at NICU are summarized in table A.14 and figure A.12. Sixty seven percent of *E. coli*, 78 % of klebsiella and 100 % of pseudomonas organisms were resistant to ampicillin. *E. coli* was susceptible to all the aminoglycosides and third generation cephalosporins tested. Klebsiella showed varying degrees of resistance to the aminoglycosides, amikacin (11%), tobramycin (13%) and gentamicin (56%) as well as to ceftazidime (56%). Pseudomonas was resistant to some extent to all aminoglycosides and third generation cephalosporins tested. With respect to pseudomonas, the most effective of the aminoglycosides and third generation cephalosporins were amikacin (10% resistance) and cefotaxime (72% resistance) respectively.

TABLE A.14

Percentage resistance of organisms to antibiotics routinely used in the neonatal population

Antibiotic	E. coli %	Klebsiella %	Pseudomonas %
Ampicillin	67	78	100
Gentamicin	0	56	50
Amikacin	0	11	10
Tobramycin	0	13	37
Cefotaxime	0	0	72
Ceftazidime	0	56	100
Ceftriaxone	0	0	94

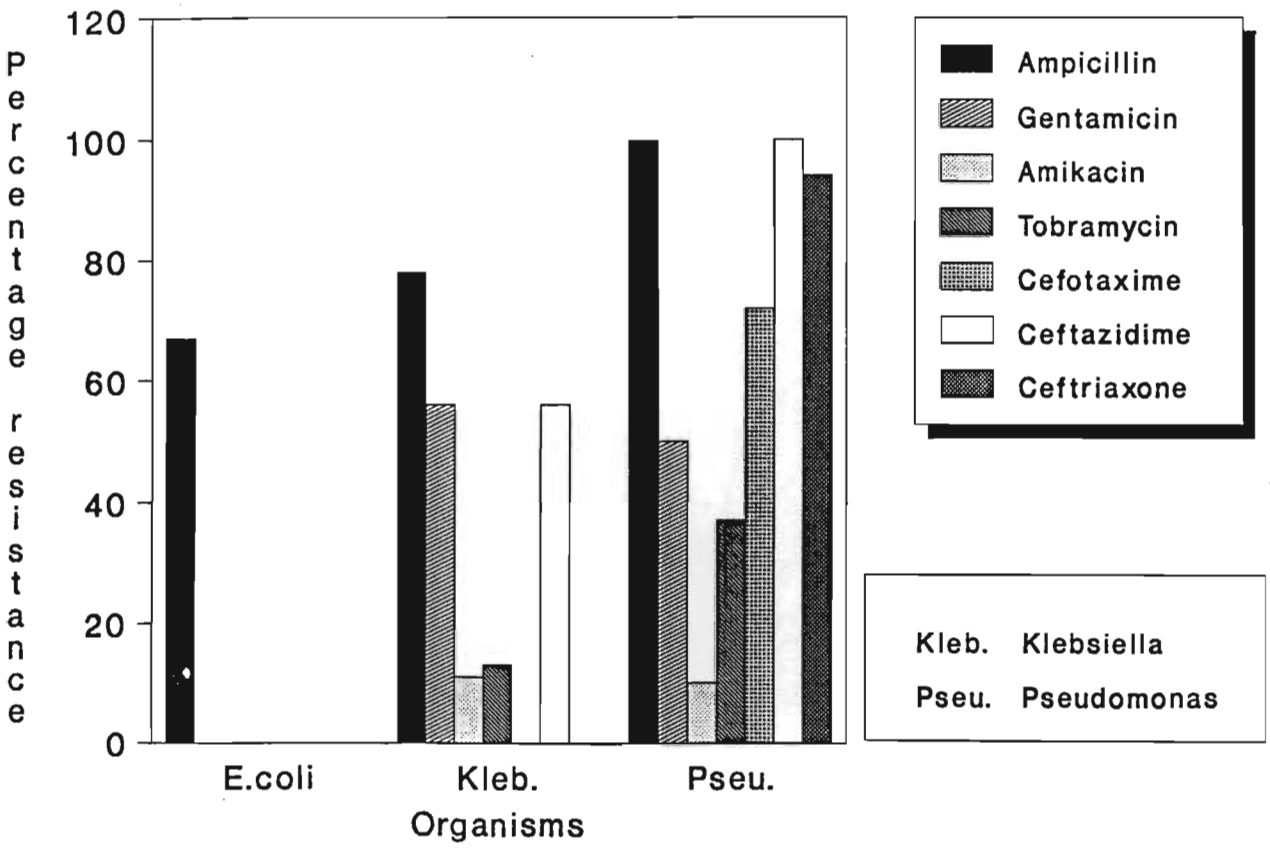


Fig A.12 Resistance patterns of organisms in the neonatal population

5.3 DISCUSSION

The small numbers of patients used in this study do not allow for specific conclusions. Certain trends were however noted. The incidence of gram negative bacteremia (66/1000 neonates) at the NICU of Baragwanath Hospital was higher than the incidence of 1 to 10/1000 reported in the literature (Siegel and McCracken, 1981). The populations studied by these investigators consisted of normal live birth groups, implying 40 weeks gestation and a birth weight of 2.5 kg or more. The high incidence (66/1000) and mortality rate (66%) associated with gram negative bacteremia in the present study could be accounted for as follows: The study population with respect to gestational age (25 infants at 28 to 40 weeks of gestation) and weight (29 of 32 neonates had a weight of less than 2.5 kg) was a high risk group. As the immune system only attains maturity at 40 weeks gestation in the majority of neonates in the present study the immune system was underdeveloped. This could have contributed to the higher incidence of gram negative bacteremia and the higher mortality rate.

A 2:1 ratio of male to female deaths associated with gram negative bacteremia has previously been reported (Plotkin, 1981). This finding was confirmed in the present study where a preponderance of male deaths (75% compared to 56% of females) was also observed. The preponderance of male deaths is not adequately explained in the literature but Washburn *et al.* (1965) have postulated a genetic origin that relates to X and Y chromosomes of male neonates.

At the NICU the highest mortality rate was associated with pseudomonas infections (71%), and pseudomonas was also the most commonly isolated organism. Only nine percent of the isolated organisms were *E. coli* with an associated mortality rate of 67%. These observations are in contrast to findings reported in the literature. In other studies *E. coli* appears to account for 50 % of bacteremias in neonates (Chow *et al.*, 1974). *E. coli* infections are usually acquired from the maternal genital tract during delivery and frequently occur in full term infants (Chow *et al.*, 1974; Sarff *et al.*, 1975). In the present study 25 infants had a gestational age ranging from 28 to 33 weeks. These infants born prematurely required prolonged hospitalization and multiple invasive procedures and were therefore more likely to develop nosocomial infections. This could account for the lower incidence of *E. coli* and higher incidence of pseudomonas bacteremias in the present study. Pseudomonas organisms require minimal nutrients and are able to establish reservoirs in the inanimate hospital environment. Furthermore resistance due to the acquisition of plasmids by pseudomonas organisms frequently occurs in the hospital environment. The incidence of resistant pseudomonas strains in the present study is in

agreement with an incidence of 64 to 100 % of resistant strains for pseudomonas in hospital environments (Mir *et al.*, 1987).

Sporadic outbreaks of infection with highly resistant strains of klebsiella appear to have accounted for the high incidences of klebsiella bacteremias reported in other neonatal intensive care units (Marples *et al.*, 1984 ; Garcia de la torre *et al.*, 1985; Mir *et al.*, 1987). In the present study the incidence of klebsiella bacteremia (19%) was only second to pseudomonas bacteremia and was associated with a 67 % mortality. Klebsiella strains in the present study were also associated with a high incidence of antibiotic resistance.

An important factor influencing the outcome of gram negative bacteremia in the neonate is appropriate antibiotic therapy (Mir *et al.*, 1987). All the gram negative organisms in the NICU were resistant to ampicillin which was routinely used as first line antibiotic therapy. Klebsiella was resistant only to cefotaxime and ceftriaxone and pseudomonas resistant to all antibiotics tested.

5.4 CONCLUSIONS

Gram negative bacteremia occurred in 66 per 1000 neonates admitted to the neonatal intensive care unit at Baragwanath Hospital. In these infants bacteremia was associated with a high mortality rate (66%).

The present study confirms the observations that a lower gestational age, lower birth weight and male gender influence the outcome of gram negative bacteremia in neonates.

The only discrepancy between this study and others was the higher incidence of late onset infections with pseudomonas as the most common causative organism. In the NICU at Baragwanath inappropriate initial therapy with ampicillin (to which all isolated organisms were resistant) may have contributed to the high mortality rate. Another problem identified was the highly resistant nature of pseudomonas and klebsiella strains in the NICU.

It is therefore recommended that resistance patterns in the NICU be monitored monthly and that initial antibiotic therapy is modified accordingly.

The use of aminoglycosides in the treatment of neonatal gram negative bacteremia seems logical. Representative population values for these drugs used in neonates are not well documented in literature. A study to calculate such values is therefore indicated.

SECTION B

THE DETERMINATION OF POPULATION PHARMACOKINETICS OF SELECTED ANTIBIOTICS EMPLOYED IN THE TREATMENT OF GRAM NEGATIVE BACTEREMIA

1. INTRODUCTION

Different factors which can influence the outcome of gram negative bacteremia were identified and discussed in section A. The high resistance patterns of organisms to ampicillin and inappropriate therapy were highlighted as possible drug related reasons for treatment failure. The use of aminoglycosides as first line therapy was advocated in both adult and neonatal populations. The combination of an aminoglycoside with cefotaxime was also discussed in the section on neonatal bacteremia.

Aminoglycosides, in particular, are toxic drugs which need accurate monitoring during treatment. Representative pharmacokinetic parameters for a specific population enables more appropriate prediction of a target dose in an individual. Data describing the population pharmacokinetics of aminoglycosides and third generation cephalosporins in neonates with gram negative bacteremia are lacking.

The non-linear mixed effect model (NONMEM) computer programme estimates population pharmacokinetic parameter values from routinely collected data in a specific population. Using NONMEM, parameters can be estimated which represent a specific group of patients - eg. neonates or patients with renal dysfunction.

The objective of this study was to determine population pharmacokinetic parameters of amikacin, gentamicin and cefotaxime in patients with gram negative bacteremia. Both an adult and a neonatal population were investigated.

2. LITERATURE REVIEW

2.1 INDIVIDUALIZATION OF DRUG DOSAGE REGIMENS

2.1.1 APPLIED PHARMACOKINETICS

Applied pharmacokinetics, "therapeutic drug monitoring" or clinical pharmacokinetics describes the process of using drug concentrations, pharmacokinetic principles, and pharmacodynamic criteria to optimize drug therapy for individual patients (Evans, 1986).

These disciplines originated with the work of Torsten Teorell published in 1937 (Teorell, 1937). The term pharmacokinetics did not appear until 1953 and is attributed to Dost (Dost, 1953). Until the late 1960's pharmacokinetics remained an academic discipline used primarily by those involved in drug research. In 1966 Kruger-Theimer provided a comprehensive discussion of the application of pharmacokinetic theory to the design of drug dosage regimens (Kruger-Theimer, 1966).

This study will focus on population pharmacokinetics and not as such on the individualization of a dosage regimen. For this reason definitions of the main pharmacokinetic parameters will be outlined briefly (Peck and Rodman, 1986):

- **Bioavailability (F)**

  Bioavailability can be defined as the rate of and extent to which the drug reaches the general circulation in an active form.

- **Desired plasma concentration**

  The first task in designing a therapeutic regimen is to choose the therapeutic objective or endpoint. Studies of drugs which show some relationship between plasma concentration and the effect, has served to establish usual "therapeutic ranges" defined by a minimum effective concentration (MEC) and maximum safe concentration (MSC). The therapeutic range however only acts as a guide and the clinical assessment of the patient remains of primary importance.

- **Volume of distribution** (Vd)

Volume of distribution is simply the size of the compartment necessary to account for all the drug in the body if it were present throughout the body in the same concentration as in the sample measured.

- **Clearance (Cl)**

  The clearance of a drug (Cl) is a proportionality constant relating the rate of elimination (Ro) to plasma concentration (Cp). Clearance refers to the volume of fluid (e.g. plasma) cleared of drug per unit time.

- **Elimination rate constant (kd) and half-life (t½)**

  The elimination half-life (t½) is the time taken for the plasma concentration to decline by 50% after all the absorption and distribution processes are completed. Half-life is therefore dependent on the elimination rate constant (kd), which is a constant relating the amount of drug in the body (Ab) with the rate of elimination (Ro) (Winter, 1980).

2.2 POPULATION PHARMACOKINETICS

The individualization of a dosing regimen is not possible without the aid of population pharmacokinetic data. If absolutely no prior information about a drug's disposition is available, then its initial use in a patient would constitute an entirely new experiment, the consequences of which would be unpredictable. Only after a complete **individual pharmacokinetic** experiment (a complete profile of plasma concentrations in the patient) would the pharmacokinetics of the drug in the patient be available for determination of the dosage regimen. Clinically this approach is impractical, however, if average population pharmacokinetics is available this provides some idea of what to expect of the individual on average. Thereafter, one or just a few drug concentrations taken from the patient early in the course of therapy and interpreted in the context of population data enables one to estimate individualized pharmacokinetics which may lead to further refinement of the dosage regimen (Peck and Rodman, 1986). There are two requirements for applying population data to the individual:

- a relevant population pharmacokinetic data base, and

- a framework for linking the individual patient to the population.

Population pharmacokinetics, therefore, entails the summary of pharmacokinetic studies in groups of individuals and the establishment of relationships between individual patient characteristics and pharmacokinetic parameters. Studies of drug disposition in a number of individuals generally reveal that the essential pharmacokinetic parameters (e.g. bioavailability, volume of distribution, clearance) lie within a restricted range of values. While this is especially true if the study group is homogeneous with regard to individual characteristics that influence drug disposition, it is not always the case for a group with a greater extent of variability. Population pharmacokinetics describes this variability in terms of a number of factors designated as fixed and random effects.

The fixed effect or population typical value parameters represent the population average values of pharmacokinetic parameters which may in turn be a function of various patient characteristics such as (a) age, weight, height and sex; (b) underlying pathology such as renal or hepatic impairment and (c) other influences on drug disposition such as concomitant drug therapy, smoking habits and alcohol intake (Beal *et al.*, 1986).

The random effects quantify the amount of pharmacokinetic variability which is not explained by fixed effects i.e. inter- and intrasubject variability. This interindividual deviation, (i.e. how the individual differs from the study group) is also termed the population variability value for the parameter (Peck and Rodman, 1986; Whiting *et al.*, 1986). Current methods of population pharmacokinetic analysis entail calculation of the standard deviation as the population variability value. Population studies involve the estimation of population typical (i.e. mean) and population-variability (i.e. standard deviation) values for each pharmacokinetic parameter. The population mean and standard deviation thus summarize the population distribution. Estimation of these fixed and random effects allows for:

- the design of dosage regimens which will, in general, suit patient groups who are at particular risk e.g. the elderly or those with impaired renal and hepatic function

- the design of individual dosage regimens and their optimization by means of Bayesian feedback techniques.

Population pharmacokinetics should be studied in a heterogeneous group of individuals exhibiting a range of patient characteristics which are thought to influence drug disposition: For example a group of patients of varying weight, age, and degree of renal dysfunction. This is done deliberately to establish relationships between individual patient characteristics and distribution of population pharmacokinetic parameter

distributions. The relationship discovered may be categorically quantitative as in the observation that smokers (an individual characteristic) tend to have typical theophylline clearance values about 50 to 60% higher than those of non-smokers. Alternatively a continuous quantitative relationship may be discerned as in the linear relationship between creatinine clearance and aminoglycoside clearance.

2.2.1 METHODS OF DETERMINING POPULATION PHARMACOKINETIC PARAMETERS

2.2.1.1 Two stage method, the traditional approach

The traditional method of determining population pharmacokinetic parameters consists of undertaking intensive experimental studies of the pharmacokinetics of a drug in a small number of individuals. These individuals are normally healthy volunteers. The study is designed to reveal maximum information about the individual's pharmacokinetics, and it involves many samples (often more than 20) per patient (Sheiner and Beal, 1984).

In stage 1 the individual's data is analyzed by curve stripping, log-linear regression or nonlinear least squares regression whereby individual pharmacokinetic parameters are estimated iteratively by fitting the data to a pharmacokinetic model (Peck *et al.*, 1984(a); Peck *et al.*, 1984(b)). In stage 2a, the parameters are summarized by calculating the means and standard deviations. These may be taken as estimates of the population-typical (fixed) and population-variability values (random). In stage 2b, relationships between patient characteristics and the estimated pharmacokinetic parameters are established by categorization or regression techniques.

The main drawbacks of this method are:

- Accurate and precise stage I estimates of individual pharmacokinetic parameters require multiple, appropriately-timed blood samples that involve costly contrived experiments. In this type of study the volunteers generally have to be reimbursed. In addition the temporary hospitalization on a clinical research basis can be costly.

- These studies are most readily performed in groups of healthy volunteers and are practically impossible to execute in large numbers of patients undergoing routine therapy.

- The number of patients is normally small and not a true reflection of a whole population. This may lead to population parameter estimates which deviate substantially from true population values. This is especially so for the random interindividual-effect parameter.

- Careful control of diet, study conditions etc. all undertaken in the interest of obtaining data with low variability can actually prevent the discovery of unexpected but important influences on kinetics.

- In order to be able to use the method of ordinary least squares for the initial analysis, some assumptions regarding the error between "true" drug level and observed level are necessary.

These assumptions are that :

- the different errors are independent of the other

- the errors are additive

- the errors are of the same typical order of magnitude.

These assumptions are problematical for the following reasons:

- Absolute measurement errors are often not of constant magnitude. The error may differ with low and high drug concentrations

- The population parameters may be biased.

Apart from all the disadvantages the traditional two stage approach remains important during the development of new drugs (Peck and Rodman, 1986)

2.2.1.2 Mixed effect modeling

(Sheiner et al., 1977; Whiting et al,. 1986; Peck and Rodman, 1986).

The mixed effect model treats the population and not the individual as the unit of analysis. Mixed effect modeling allows direct estimation of population pharmacokinetic parameters in a single stage of analysis applied simultaneously to data from many individuals. In this method, an individual's pharmacokinetic parameters are not directly determined. Rather, a generalized form of least-squares regression, known as extended

least squares, is used to estimate fixed effect and random-effects parameters. Fixed effect parameters include the population typical values (means) as well as the coefficients of regression relationships between individual patient characteristics and population-typical values for the pharmacokinetic parameters. Random-effect parameters are the population-variability values (standard deviations) representing interindividual deviation from fixed-effect parameter estimates after population relationships and residual random error have been taken into account.

A powerful feature of the mixed effect modeling technique is the ability to accommodate patient pharmacokinetic data as it arises in the course of routine clinical therapy. For example, only one or two drug concentrations per patient, drawn virtually at random, may provide a suitable data base for mixed effects modeling. It has the ability to accommodate scanty data from actual clinical therapy (e.g. a specific disease state), thus enhancing the use of clinical data for incorporation into techniques of drug regimen design and clinical pharmacokinetic forecasting. The principle aim of population pharmacokinetic analysis is therefore to account for the inherent kinetic variability within a population of patients in terms of a number of readily identifiable factors. These may be physiological, pathological, environmental or genetic. One important outcome should be the provision of rational dosage guidelines for specific risk groups e.g. the very young, the elderly and patients with impaired renal, cardiac or hepatic function.

2.2.1.2.1 Nonlinear mixed effect model approach (NONMEM)

(Beal and Sheiner, 1980, 1982; Sheiner and Grasela, 1984).

The NONMEM computer programme is at present the only programme available for derivation of population pharmacokinetic parameters from routine clinical data as described in the previous section. The NONMEM approach was developed and implemented by Sheiner and Beal in 1977. It describes the observed concentration time data in terms of:

1. A number of fixed effect parameters (θ_K) which include mean values of the relevant structural pharmacokinetic model parameters or a number of parameters which relate the structural model parameters to demographic and pathophysiological variables.

2. Two types of random effect parameters. These can be distinguished as:

- the variances of the θ_K parameters, i.e. the intersubject variability within the population ($\omega\theta_K^2$)

- the residual intrasubject variable due to random fluctuations in an individuals parameter values, measured error and all sources of error not accounted for by the other parameters (σ_ε^2)

Because of the marked difference between the NONMEM approach and the old traditional approach several points require clarification:

- **Reliability of NONMEM analysis**
  The first question addresses the accuracy of these parameters which are calculated from routinely collected data in comparison to the more controlled evaluation of a traditional pharmacokinetic analysis. Sheiner *et al.* (1977) found a good correlation between their population values for digoxin (clearance and volume of distribution) and previously reported values obtained by traditional approaches. The same was true for phenytoin (Grasela *et al.*, 1983; Vozeh *et al.*, 1981) and procainamide (Grasela and Sheiner, 1984). Values for mexiletine and lignocaine results presented by Vozeh *et al.* (1982) were also in general agreement with those reported by other investigators.

- **Number of data points per subject**
  It is difficult to establish from the literature how many data points per subject will be sufficient for a NONMEM analysis. It is however true that a high degree of intersubject variability can only be explained if a relatively large number of subjects expressing that variability is studied (Maitre *et al.*, 1987). Moreover, an estimate of the intrasubject component can only be obtained by collecting several (3 or 4) samples per subject from the majority of subjects. The intrasubject estimate will, however, always be confounded by other sources of variability such as assay error and model mis-specification (Whiting *et al.*, 1986).

- **The nature of the population parameter distributions**
  The NONMEM approach is based on parameter distributions that are unimodal (and probably normal after an appropriate transformation). This assumption on the parameter distributions may be the single biggest disadvantage of this programme.

  It is especially important when a new drug is studied, in the absence of any knowledge about an underlying genetic polymorphism, not to constrain the pa-

rameter distributions. Grasela and Sheiner (1984) also documented this flaw in the programme when they were not able to use a unimodal estimate of clearance to discriminate between fast and slow acetylators in their study on procainamide (Whiting *et al.*, 1986).

2.2.1.2.2 Non-parametric maximum likelihood approach (NPML)

The non-parametric maximum likelihood approach (NPML) needs no prior assumption about the parameter distribution and this may overcome the restrictions of NONMEM. The method gives rise to discrete distributions for the likelihood function and the parameter distributions. These are then smoothed to give continuous distributions, which may be skewed or multimodal. The NPML approach has been tested successfully on simulated data but remains to be proven in practice, where the proportion of data in one or more modes may be small (Whiting *et al.*, 1986).

2.2.2 DATA REQUIRED FOR DETERMINATION OF POPULATION PHARMACOKINETIC PARAMETERS USING NONMEM

Although population pharmacokinetics with NONMEM is based on routinely collected data, an informal study needs to be designed. It is, however, still important to collect appropriate data. This has considerable bearing on the success of population pharmacokinetic studies and determines: (a) what can be learned from existing data, and (b) what prospective data are required to answer specific questions.

Two types of data namely kinetic and demographic, are required for population pharmacokinetics.

2.2.2.1 Kinetic data

As determination of population pharmacokinetic parameters using NONMEM is not confined to structured studies, a greater degree of flexibility in the collection and recording of kinetic data is possible. There are two kinds of kinetic data:

- Data specifying the dosage regimen which is associated with a particular concentration measurement, e.g. the dose, route of administration, dosage interval, whether steady-state has been achieved and if not, details of the preceding dosage history.

- Concentration-time data, i.e. concentration measurement(s) and the time(s) between sampling and the preceding dose.

The following plasma concentration data can be considered:

- **Steady state trough concentrations**
  Trough levels yield minimal information as they do not represent the "average steady-state" concentrations. These levels can therefore not be used in the standard steady-state equation to calculate clearance. A mathematical relationship between trough concentrations and dosing rate must therefore be established before analysis. Several steady-state trough concentrations per patient plus information on the dosing interval are necessary.

- **Average steady-state concentrations**
  The relationship between dose rate and an average steady state concentration can be formulated with a clearance term or, in the case of non-linear kinetics with Michaelis-Menten parameters.

- **Concentration measured at any time after an oral dose**
  If concentrations defining the various parts of the entire concentration-time profile are available, the average population values of the usual pharmacokinetic parameters such as rate constants and half-lives can be obtained. Steady-state and non-steady-state data can be used provided the documentation of the dosage history is complete. The kinetic data normally consists of 3 or 4 concentration-times pairs per patient, ideally spread out so that all aspects of the relevant profile are covered. The randomization of time points may be difficult to achieve in practice but one approach is to divide a dosage interval into equal or unequal periods (where $n > 2$) and to acquire one (or more) samples from each patient within each period, but not necessarily within the same dosage interval.

- **Concentration measurements at any time after both intravenous and oral doses**
  Applying the same design criteria as described above, the average population values of volume of distribution, clearance and bioavailability can be obtained if both intravenous and oral data are available. The confidence with which these parameters are estimated, however, will depend very much on the mix of intravenous and oral data available. These data should be collected from each patient within a period where intraindividual variability is minimized or where changes in important demographic factors are carefully recorded.

The data requirements presented thus far only serve as general guidelines. Basically any data collected routinely can be used to derive population pharmacokinetic parameters using NONMEM. The results of the analysis, however, will reflect the type of data collected. For instance a predominance of "steady-state concentrations" will provide good information about clearance (or the ratio of clearance to bioavailability) but little information about volume of distribution.

2.2.2.2 Demographic data

Since data may be collected over lengthy periods of time, possible changes in pathophysiology within as well as between patients must be taken into account. There are two kinds of demographic data:

- That obtained at the beginning of a study which defines the pathophysiological status of patients at that time, and include age, sex, weight, height, smoking habits, alcohol consumption, nature and severity of disease, concurrent medication and biochemical and hematological indices.

- That obtained during any dosage interval of interest to account for changes which may have occurred during the course of treatment (Whiting *et al.*, 1986).

2.3 METHODS OF INDIVIDUALIZING PATIENT DOSAGE REGIMENS

A patient's actual pharmacokinetic response to a particular dosage regimen may differ from the predicted response even though the initial dosing regimen was based on typical population values. Although the aim of population pharmacokinetics is to estimate values as representative of the population as possible, interindividual variation still exists in the population. The following discussion will present the various approaches for the determination of the individual patient's pharmacokinetics :

i. Least squares method (LS)

This method fits blood sample data using the statistical estimation known as maximum likelihood. A least squares analysis involves a computer search for parameters values of the pharmacokinetic model (M) which minimizes the objective function (OBJ) described below:

$$OBJ = \sum_{i=1}^{n} \frac{(Cm - Ci)^2}{\sigma_1^2} \qquad (b.1)$$

Cm = measured drug concentration

Ci = predicted drug concentration

σ_1 = standard deviations from the random error model (E) for i = 1 to n available concentrations.

The computer search selects values for M that yield estimates of Ci which most closely correspond to the measured concentrations. The σ_1 can either be entered in the fitting procedure as "known" values or estimated automatically in the procedure under explicit assumptions about the functional form of the error model (E) Peck *et al.*, 1984(a); Peck *et al.*, 1984(b)). The need to weight observations with the appropriate σ_1, stems from the varying absolute error for different values of concentrations measured. For example, if an assay has a constant coefficient of variation of 10% and values of 1 to 10 units are measured, the absolute error would range from 0.1 (10%) to 1 (100%) units.

The following are limitations of the LS method:

- All of its information regarding the pharmacokinetic parameters is derived from the patient's drug concentration data. Thus any prior knowledge of the patient's pharmacokinetic values are excluded from the LS analysis.

- It requires multiple, well timed drug concentrations to provide accurate and precise estimates of the parameters. The minimum number of drug concentrations for least-squares estimation is determined by the number of parameters in the model. For example, the one-compartment model commonly used for intravenous aminoglycosides or theophylline has two parameters (volume and elimination rate or clearance), thus in such cases at least two observations are required.

- If fewer drug concentrations are available the LS method can be modified to accommodate them by fixing one or more parameters at assumed values - leaving fewer parameters for individualized estimations.

ii. Bayesian method

The Bayesian Method combines Bayes' theorem and maximum-likelihood estimations. Bayes' theorem estimates individual pharmacokinetic parameters as follows:

$$\text{prob (P/C)} = \frac{\text{prob (P)} \cdot \text{prob (C/P)}}{\text{prob (C)}} \qquad \text{(b.2)}$$

prob (P/C) = Probability distribution (Bayes mean estimate ± SD) of the patient's pharmacokinetic parameters (P) taking into account the measured drug concentration (C)

prob (P) = The probability of the patient's parameter within the assumed population parameter distribution.

prob (C/P) = The probability of the measured concentrations in the context of pharmacokinetic model (M) and random (measurement) errors.

prob (C) = Unconditional probability distribution of the observed levels.

When the population distributions of population pharmacokinetic parameters are approximately Gaussian, application of the method of maximum-likelihood estimation of the above expression of Bayes' theorem results in the following objective function:

$$OBJ_{bayes} = \sum_{j=1}^{P} \frac{(PJ - Pj)^2}{\sigma_{pj}^2} + \sum_{i=1}^{N} \frac{(CI - Ci)^2}{\sigma_i^2} \qquad \text{(b.3)}$$

PJ and Pj = Population and individual's j = 1 to p pharmacokinetic parameters

σ_{pj} and σ_i^2 = Population parameter standard deviations

CI and Ci = Observed and predicted i = 1 to n available drug concentrations

Minimization of the Bayesian objective function (OBJ Bayes) results in estimates of pharmacokinetic parameters, closer to the patients real value which take into account

the measured and predicted drug concentration along with the information on measurement error and the typical variability values of pharmacokinetic parameters in the population.

It is important to note that the Bayesian method can also be used without any measured level in which case only the first summation term remains. This in effect is simply predicting the serum concentration using population pharmacokinetic parameters. More importantly, however, if more concentrations in the individual are known the second summation becomes more predominant and the estimated parameters become more representative of the individual.

The appeal of this approach, in contrast to the intuitive or least-squares approach which relies either entirely on prior expectations or depends solely upon measured drug concentrations, is that it mimics human thinking as follows:

- The initial therapy is targeted, using population-based parameters adjusted for the patients characteristics.

- Plasma drug concentrations are measured at informative times and compared with expected values.

- Individualized pharmacokinetic parameter estimates are made cautiously taking into account both (i) the expected drug levels and their variability (based upon the average parameter values and variability in the population) and (ii) the measured drug levels and their expected variability (due to measurement error and other sources of random variability) (Whiting *et al.*, 1986).

2.4 ASPECTS OF NEONATAL PHARMACOKINETICS RELEVANT TO INTRAVENOUS DOSING

2.4.1 DRUG DISTRIBUTION (Vd)

The age-related differences in the composition of fat and water compartments may have an important impact on the distribution of some drugs. The Vd for more water soluble drugs such as the aminoglycosides is closely related to the extracellular water (ECW) and total body water (TBW). As a result the volume will increase as the water compartment increases.

Neonates have a larger proportion of their body mass made up of water than do older infants and adults. Similarly the premature infant (85% TBW) has more body water than the full term infant (75% TBW). The relative volume of ECW and the ratio of extracellular to intracellular water (ICW) is also higher in neonates, than in infants and children.

During normal development as the TBW content decreases, there is an increase in body fat. Fat accounts for 16% of body weight in the full-term neonate whereas premature babies are practically devoid of any fat. The Vd of a more lipophilic drug like diazepam is smaller in neonates and infants (1.3 to 2.64 ℓ/kg) than in adults (1.6-3.24 ℓ/kg) (Milsap and Szefler, 1986).

2.4.1.1 Protein binding

Greater variability in protein binding of drugs exists during the neonatal than adult period. The plasma protein concentration of the neonate is approximately 80% of that of an adult. The principle drug binding protein is albumin which shows an increase in concentration as well as increased affinity for acidic drugs with an increase in gestational age. (Morselli *et al.*, 1980).

The lower affinity of albumin for acidic drugs in the neonate may be related to competition for binding sites by increased concentrations of endogenous substances such as bilirubin and free fatty acids. (Morselli *et al.*, 1980).

The concentration of α1-acid glycoproteins which bind basic drugs such as lignocaine and propranolol is also reduced in the neonate (Morselli *et al.*, 1980).

2.4.2 METABOLISM

The capacity to metabolize drugs varies throughout development. The liver is the major site of the four principle pathways of drug metabolism, namely oxidation, reduction, hydrolysis (all Phase I reactions) and conjugation (a Phase II reaction). Studies indicate that virtually all of the enzymatic microsomal systems for drug biotransformation are present at birth but also that their capacities increase with advancing age (Rane and Jomson, 1980; Assael, 1982). Decreased hepatic uptake, low concentration of intracellular carrier proteins and decreased production of bile, diminish enzyme activity in neonates (Rane and Jomson, 1980). The insufficiency of one pathway may lead to metabolism via alternative pathways. For example, the methylation of theophylline to

caffeine occurs in neonates but eventually becomes insignificant in older children and adults (Morselli *et al.*, 1980).

UDPG-glucuronyl transferase activity responsible for the conjugation of various endogenous substances and drugs (e.g. bilirubin, morphine and chloramphenicol) is depressed at birth and reaches adult values at an age of three years. Agents such as chloramphenicol which are totally dependent on this pathways for elimination are potential toxins in neonates.

2.4.3 RENAL EXCRETION

Renal function (with respect to renal plasma flow, glomerular filtration, concentrating and acidifying abilities, tubular absorption and tubular secretion), when normalized for body surface area, is significantly lower in infants and small children than in adults. Although often only 20% to 40% that of older children, renal function is usually adequate to maintain normal homeostasis. During stressful situations such as infection, acid-base imbalance and dehydration, the immaturity and lack of functional reserve of the kidney may become apparent in the neonate and young child (Milsap and Szefler, 1986).

At birth, kidney blood flow is characterized by increased vascular resistance and a preferential intrarenal flow away from the outer kidney cortex. During the postnatal period, both increases in cardiac output and decreases in intrarenal vascular resistance occur which dramatically increase kidney perfusion. Glomerular function is more advanced than tubular function at birth and this imbalance may persist until six months of age. Premature infants have lower filtration rates than full term infants and also develop their filtration capacity more slowly postnatally. In premature infants tubular function is even more reduced at birth and is associated with decreased clearance of glucose, phosphate, bicarbonate, urea and other nitrogenous wastes. Penicillins which depend mainly on tubular secretion for elimination are cleared slowly by the neonate. Passive tubular reabsorption may be reduced in the infant and neonate and the relatively low urinary pH of this population may also influence the rate and extent of tubular reabsorption of some drugs (Morselli *et al.*, 1980).

2.5 PHARMACOKINETIC PRINCIPLES OF CEFOTAXIME

2.5.1 COMPARTMENTAL KINETICS

After IM and IV administration, cefotaxime kinetics can be described by a simple two compartment open model with a rapid distribution phase (15-20 min) followed by a more prolonged apparent elimination phase (0.8-1.34 hours). In neonates the distribution and elimination phase have been reported to be respectively 5.6 minutes and 4.8 hours (Kearns *et al.*, 1989). It has been shown that cefotaxime obeys linear dose dependent kinetics up to 2 g but becomes non-linear at higher doses (Carmine *et al.*, 1983). After a 1000 mg intravenous bolus dose, mean peak plasma concentrations range between 81 and 102 μg/mℓ in adults. Concomitant administration of probenecid increases the plasma concentration of cefotaxime (Carmine *et al*, 1983). Both glomerular filtration and tubular secretion appear to be involved in the renal excretion of cefotaxime (Carmine *et al*, 1983).

2.5.2 DISTRIBUTION

Because of its low lipophilicity cefotaxime like the other cephalosporins, generally cannot gain entry into cells. The physiological volume to which the cephalosporins distribute is the volume of the extracellular water (= 212 mℓ/kg.). After I.V. infusion of 1 g cefotaxime over 30 minutes the apparent volume of distribution at steady state is 21.6 ℓ/1.73 m^2. The extent of protein binding of cefotaxime ranges from 30 to 50% which is generally lower than that for the other cephalosporins. Concentrations of cefotaxime are low in the cerebrospinal fluid if the meninges are not inflamed, but are between 3 to 30 μg/mℓ in children with meningitis. Inhibitory concentrations (0.2-5.4 μg/mℓ) for most gram-negative bacteria, are attained in purulent sputum, bronchial secretions and pleural fluid after doses of 1 or 2 g. Concentrations likely to be effective against most sensitive organisms are also attained in female reproductive organs, otitis media effusions, prostatic tissue, intestinal fluid, renal tissue, peritoneal fluid and the gallbladder wall after usual therapeutic doses. High concentrations of cefotaxime and desacetyl-cefotaxime are attained in both the common bile duct and the gallbladder. At any time concentrations in the gallbladder wall are about one-fifth to one-tenth of those in the bile (Soussy *et al.*,1980).

2.5.3 ELIMINATION AND METABOLISM

Cefotaxime is partially metabolised prior to excretion. The main metabolite is the microbiologically active product, desacetyl-cefotaxime. Two other inactive metabolites M2 and M3 (isomers of desacetyl-cefotaxime lactone in which the β-lactam ring has opened) are generally not detected in the serum of healthy volunteers but are found in higher concentrations in patients with renal failure. Of an intravenous dose 50 to 60% is excreted unchanged, and 24% as desacetyl-cefotaxime in the urine. The mean half-life of cefotaxime is reported to be 0.9 to 1.14 hours and the mean half -life of desacetyl-cefotaxime is 1.3 hours (Mandell and Sandle, 1985; Wise and Wright, 1981).

2.5.4 FACTORS INFLUENCING CEFOTAXIME PHARMACOKINETICS

2.5.4.1 Age

- **Neonates**

In neonates, the pharmacokinetics of cefotaxime are significantly influenced by gestational and chronological age as well as by birth weight. In preterm and low birth weight neonates, the elimination half-life is prolonged relative to that of full term or normal birth weight neonates of the same age. Half-lives of 5.7 hours for preterm babies less than one week old and of 3.4 hours for full term babies of the same age have been reported (Kafetzis *et al.*, 1982; Kearns *et al.*, 1989).

Kearns *et al.* (1989) were not able to show any difference in the half-lives of cefotaxime in babies who weighed less or more than 1000 g respectively but a significant linear correlation was found between gestational age and half-life or total body clearance.

The elimination of the active metabolite desacetyl-cefotaxime is markedly slower in premature neonates than in children and adults. The metabolic pathways responsible for the formation of desacetyl-cefotaxime are active by 27 to 28 weeks of gestation and as the metabolite is largely excreted by renal mechanisms the prolonged t½ may be due to developmental immaturity of both glomerular filtration and active renal tubular secretion (Kearns *et al.*, 1989). The volume of distribution of cefotaxime under steady-state conditions is larger in newborns than in adults (0.31-0.35 ℓ/kg in neonates and 0.21-0.29 ℓ/kg in adults).

- **Elderly patients**

  An increase in the half-life of cefotaxime in the elderly has been reported (Carmine *et al.*, 1983). The study of Ludwig *et al.* (1988) confirmed this finding but they demonstrated that the decrease in elimination of cefotaxime in the age group 60-80 years was slight and only reached significance in the age group over 80 years. Cefotaxime therefore appears to be a safe drug in the elderly only requiring dosing adjustment for age in patients over the age of 80 years.

2.5.4.2 Diseases

- **Liver diseases**

  Patients with drug-induced hepatocellular damage showed no marked changes in cefotaxime blood levels but had lower serum concentrations of the desacetyl metabolite (Carmine *et al.*, 1983). The serum half-lives of cefotaxime and desacetyl-cefotaxime were significantly increased in patients with advanced cirrhosis (Balant *et al.*, 1985).

- **Renal disease**

  Severe renal dysfunction was associated with a decrease in total plasma clearance, renal clearance and the urinary recovery of cefotaxime and desacetyl-cefotaxime. The effect on the metabolite was more marked. In these patients the half-life of cefotaxime only increased to ± 5.5 hours but the half-life of desacetyl-cefotaxime increased to as much as 10-15 hours. The effects of renal failure on the clearance of both cefotaxime and its metabolite are even more pronounced when renal failure is accompanied by other illnesses such as heart failure, pulmonary edema and septicemia (Carmine *et al.*, 1983; Kearns *et al.*, 1989).

2.6 AMINOGLYCOSIDE PHARMACOKINETICS

2.6.1 ABSORPTION

Due to their polarity the aminoglycosides are poorly absorbed from the intestinal tract, with only 0.3% to 1.5% of an administered dose appearing in the urine. Nevertheless repeated oral or rectal administration may lead to toxic concentrations in patients who have severe renal insufficiency. Peritoneal absorption can be substantial and can lead to serious side effects. Aminoglycosides are generally well absorbed after intramuscular injection. In patients with severe gram-negative sepsis, perfusion of the intramuscular

administration site may be reduced because of hypotension, and the rate of absorption may be substantially reduced. Repeated injections at the same site may also impair absorption and result in variations in the serum levels of the aminoglycosides.

Peak serum concentrations are generally achieved within 30 to 120 minutes after an intramuscular injection. In younger patients with normal renal function the peak concentration occurs 30 to 60 minutes after administration with very little variation between individuals. In patients older than 40 years the elimination of aminoglycosides becomes more variable, with a wider variation in peak times. In patients with renal impairment the peak occurs as late as two to three hours after administration.

Aminoglycosides can also be administered intravenously by bolus injection, by 30 to 60 minute infusions or by continuous IV infusion but an intermittent infusion over 30 to 60 minutes is thought to be safer (Rotschafer *et al.*, 1983).

A higher incidence of toxicity may occur with continuous infusions and besides, the relationship between bacterial killing effect and time of exposure to antibiotic indicates that aminoglycosides need only be in contact with the bacteria for a very short period of time (Zaske, 1986).

2.6.2 DISTRIBUTION

Because of their polar nature, the aminoglycosides are largely excluded from most cells, the central nervous system and from the eyes. They distribute well into most body fluids including synovial, peritoneal, pleural fluids and ascites and slowly into the bile, feces, the prostate and amniotic fluid. Binding to serum proteins is less than 10% and is not considered to be clinically relevant. These agents cross the placenta and achieve fetal serum concentrations of 21% to 37% of maternal serum concentrations (Zaske, 1986).

Aminoglycoside antibiotics distribute primarily to a space similar to the extracellular fluid compartment. In normal volunteers the extracellular fluid compartment approximates 20-25% of body weight. This physiological space is susceptible to changes during gram-negative sepsis as a result of e.g. dehydration and congestive heart failure. Frequently, patients in the initial phases of gram-negative sepsis are febrile, vomiting and consequently become dehydrated. As a result the extracellular fluid compartment and drug distribution are decreased. On the other hand patients who have congestive heart failure or peritonitis, patients immediately postpartum and patients receiving intravenous hyperalimentation show an increase in distribution volume (Zaske, 1986).

The newborn infant with a larger extracellular fluid volume per unit body weight has a distribution volume in the range of 50-70% body weight. The distribution volume displays marked interpatient variation and may even vary in the same patient during the course of therapy with an aminoglycoside. This is especially true for patients who are markedly dehydrated in the initial phase of sepsis (small distribution volume) and then after administration of intravenous fluids to replace the fluid deficit show an increase in distribution volume.

These changes in distribution volume are independent of any change in renal function but will influence the drug's half-life if the total body clearance remains constant.

Pharmacokinetic model

Aminoglycoside antibiotics are characterized by linear kinetics and under the right set of experimental conditions demonstrate a triphasic decay of serum concentrations with time.

The third phase (confirming the presence of a tissue compartment) can easily be demonstrated in patients with compromised renal function. This phase probably results from tissue redistribution of aminoglycoside and may explain the accumulation of the drug in patients receiving aminoglycosides (Schentag and Jusko, 1977). In a clinical setting, however, considering all variables it can be concluded that a one or two compartment model will give a reasonable approximation. The infusion rate and serum sampling strategies can generally be modified to provide reliable estimates of drug elimination and distribution volume with substantially fewer samples.

2.6.3 ELIMINATION

Aminoglycosides are eliminated primarily unchanged by the kidney via glomerular filtration. Active secretion may account for a small amount of drug elimination. Elimination by the kidney accounts for approximately 55-95% of the dose administered. Small amounts of these drugs have been found in the bile which thus represents an additional route of elimination.

Wide interpatient variation in elimination exists between patients with normal and impaired renal function and this variation is even greater in patients treated for gram-negative sepsis. In volunteers with normal renal function, the half-life of gentamicin was reported to vary between 2.5 and 4 hours and the half-life of amikacin between 0.8 to

2.8 hours. When a group of patients with gram-negative sepsis were studied the half-life for gentamicin ranged from 0.4-3.27 hours in 855 patients who had normal serum creatinine levels. The total body clearance of the aminoglycosides in this group also displayed considerable patient to patient variability (Rotschafer *et al.*, 1983; Zaske *et al.*, 1982).

2.6.4 Factors influencing the pharmacokinetic parameters of aminoglycosides

- **Renal function**

  In patients with normal renal function the variation in elimination of aminoglycoside can be explained by the small variations in renal function. On the other hand only 50% of variation in elimination can be explained by the difference in renal function in a population with sepsis (Kaye *et al.*, 1974; Barza *et al.*, 1975). In patients with sepsis the variation in plasma aminoglycoside levels are therefore not totally the result of differences in renal function which further complicates the dosing regimen of patients with gram-negative bacteremia.

- **Age**

  In healthy adults cardiac output, renal blood flow, and glomerular filtration decrease with increasing age. The rate of aminoglycoside elimination (primarily cleared by glomerular filtration) therefore continually decreases with increasing age. Endogenous production of creatinine decreases with increasing age, and serum creatinine concentrations may be a misleading indicator of glomerular filtration and aminoglycoside elimination.

- **Distribution volume**

  Different disease states can either increase or decrease the distribution volume of the aminoglycosides. Edema and congestive heart failure increase the volume of distribution while this volume can decrease in patients who are dehydrated. For the aminoglycosides there is a significant relationship between half-life and distribution volume. The distribution volume is probably a physiological marker of the extracellular fluid compartment and when either the elimination rate increases or the half-life diminishes, it is an indication that the distribution volume has decreased or that the Cl has increased. Elderly people who develop severe gram-negative sepsis

may develop congestive heart failure and the increase in extracellular fluid and the edema may result in a higher distribution volume and half-life of aminoglycosides (Sandle and Mandell, 1985).

- **Fever**

Fever also seems to be an important factor influencing the serum concentrations and elimination of aminoglycosides . In dogs pretreated with endotoxin, a 25% decrease in serum gentamicin concentrations was observed at 60 minutes post-injection suggesting an increase in elimination rate (Zaske, 1986). In sick febrile human volunteers, serum concentrations of gentamicin were reduced by 40% at one, two and three hours after intramuscular injection. Physiologically, fever may change the elimination of aminoglycosides by increasing heart rate and cardiac output thereby increasing renal blood flow and glomerular filtration.

- **Ideal body weight**

Due to their polar nature aminoglycosides were originally thought to distribute solely into ideal body mass, and prediction of serum concentrations was thought to be improved if methods used ideal body weight rather than total body weight. Later data suggested that these agents also distribute into adipose tissue (Bauer *et al.*, 1980). Gentamicin was found to distribute into 5-6% of excess weight. Thus the drug's distribution volume increases with excess weight, presumably due to distribution into the extracellular water within adipose tissue.

- **Gender**

The elimination rate constant and clearance of aminoglycosides were significantly faster in females than in males. Similarly half-lives were shorter and distribution volumes were smaller in females than in males (Zaske,1986).

- **Pregnancy**

The extracellular fluid compartment, total body water, cardiac output, renal blood flow, and glomerular filtration are all increased during the later phase of pregnancy. The equilibrium is usually reestablished two to five days after delivery. As aminoglycosides distribute into the extracellular fluid and are dependent upon glomerular filtration for excretion, their serum concentrations are markedly

influenced by pregnancy. During pregnancy the half-life of aminoglycosides was reported to be shortened (Zaske *et al.*, 1980).

- **Burn patients**

The caloric expenditure of burn patients can be as high as 10 000 calories daily and can even be higher with concurrent gram-negative sepsis and fever. Hemodynamic changes secondary to burn wounds appear to explain why burn patients have an extremely rapid rate of elimination of aminoglycosides. In addition, the extracellular fluid compartment in burn patients can be markedly enlarged immediately after the injury. Consequently, the occasional burn patient who develops gram negative sepsis early in the course of resuscitation may have an extremely high distribution volume and a prolonged half-life, even though renal function tests are normal (Zaske, 1986).

- **Paediatric patients**

The elimination rate of aminoglycosides in paediatric patients is rapid and the half-life is shorter than in adults. This rapid rate of elimination is even more apparent in paediatric patients with cystic fibrosis, burns or leukemia.

- **Ascites**

An expanded extracellular fluid volume explains the increase in distribution volume of aminoglycosides in patients with ascites. The aminoglycosides distribute rapidly into the ascites fluid, and a large loading dose is necessary to achieve therapeutic serum levels. The half-lives of aminoglycosides are prolonged in these patients due to the large extravascular distribution volume.

- **Surgical patients**

Surgical patients after trauma or patients with peritonitis, or pancreatic pseudocyst, have marked changes in the physiological parameters which affect the disposition of aminoglycoside antibiotics. Surgical patients may also be hypermetabolic secondary to trauma or surgical intervention resulting in an increased elimination rate of aminoglycosides. These changes have a substantial effect on the clearance of aminoglycosides with consequent marked variations in plasma levels (Schentag and Jusko, 1977).

- **Neonates**

In the newborn, especially the premature patient dynamic changes occur in physiologic parameters such as cardiac output, renal blood flow, renal function and extracellular fluid volume. The glomerular filtration remains relatively constant at low rates until 34 weeks of gestation (coinciding with the completion of glomerulus formation) after which it increases. The increase in glomerular filtration rate is therefore dependent on postconceptual rather than postnatal age. Hindmarsh *et al.* (1983) studied gentamicin kinetics in very low birthweight infants who required 2 to 3 successive courses of gentamicin for suspected sepsis. The mean postnatal ages at the time of second and third administration periods were 19 ± 9 and 68 ± 26 days, respectively. Gentamicin clearance was found to correlate well with creatinine clearance and with postconceptual age during all 3 administration periods. The mean clearance values for the 3 age groups were 0.38 ± 0.14, 0.44 ± 0.18; and 1.21 ± 0.39 mℓ/kg/min, respectively. The clearance in the infants with a mean postnatal age of 68 days was significantly greater than in the other age groups, whereas a statistically significant difference was not observed between the infants studied at birth and at 19 days of age. The $t_{½\beta}$ of the aminoglycosides varies inversely with renal clearance, gestational age, and postconceptual age. Gentamicin half-lives of 8.86, 6.62 and 5.12 hours in infants of post- conceptual ages 30 weeks or less, 40 to 37 weeks, and 37 weeks or more, respectively were reported (Besunder *et al.*, 1988(a); Besunder *et al.*, 1988(b); Szefler *et al.*, 1980).

3. METHODOLOGY

The population pharmacokinetics of aminoglycosides and cefotaxime, a third generation cephalosporin, used in the treatment of gram negative bacteremia were determined.

3.1 PATIENT DATA COLLECTION

Neonates and adults with gram negative bacteremia were investigated. The study was performed at Baragwanath Hospital and approved by the Ethics Committee of the hospital. The blood samples were collected between January 1987 and June 1988. The following data relevant to pharmacokinetic principles were obtained from the medical records of patients:

3.1.1 Adult population

- Underlying disease
- Age
- Sex
- Weight
- Serum creatinine concentration

3.1.2 Neonatal population

- Age (gestational and postnatal)
- Sex
- Weight
- Height
- Serum creatinine concentration

Inclusion criteria were as follows:

- **Diagnoses**

Only patients in whom the diagnosis of a gram negative bacteremia was confirmed by a positive blood culture were included in the study.

- **Hospital status**

Only patients hospitalized in the neonatal intensive care unit (neonates) or in the medical wards (adults) of Baragwanath Hospital were included in the study.

- **Sex**

Both males and females were included in the study.

- **Age**

Premature infants (gestational age from 28 tot 33 weeks and postnatal age from 4 to 23 days) were included in the neonatal population. The adult population included only patients over the age of 20 years.

- **Informed consent**

Informed consent was obtained from the adults and the parents of the infants included in the study.

3.2 COLLECTION OF PLASMA SAMPLES FOR THE DETERMINATION OF ANTIBIOTIC LEVELS

- **Antibiotic therapy**

  The neonates received amikacin (aminoglycoside) and cefotaxime (third generation cephalosporin). The adults received gentamicin (aminoglycoside).

- **Route of administration**

The antibiotics were administered intravenously and intramuscularly respectively to the neonates and the adults.

- **Dosing regimens**

Amikacin 15mg/kg/day was administered in divided doses at 12 or 8 hourly intervals. The dosing interval was determined by the renal status of the patient. Scr concentrations are routinely used in the adjustment of aminoglycoside blood levels. Scr concentrations do not reflect renal function as accurately as creatinine clearance. Creatinine clearance determinations do however require the collection of a 24 hour urine specimen which was not practical in the present study. When the serum creatinine level therefore exceeded normal values the dosing interval was increased from 8 to 12 hours. Cefotaxime 100mg/kg/day was administered in divided doses.

Gentamicin 3 or 4 mg/kg/day was administered in divided doses at 8 or 12 hourly intervals. The normal dosing interval was 8 hours but if the serum creatinine value exceeded the normal 80 - 120 μmol/l range, the dosing interval was increased to 12 hours.

- **Plasma sample size**

Five ml and 2 ml samples were collected from the adults and neonates respectively.

- **Plasma sample collection protocols**

Blood for determination of amikacin and gentamicin levels was drawn routinely when toxicity was suspected or when the clinical response was unsatisfactorily. The standard protocol for the collection of aminoglycoside plasma levels at the Baragwanath Hospital was therefore used. Cefotaxime plasma levels were collected for research purposes and therefore a specific protocol was drawn up for this drug. The following requirements were set out in the protocols:

AMINOGLYCOSIDES

- Blood samples were drawn only after steady state had been reached usually after 3 doses.

- Two samples, a peak (one hour after dosing) and trough level (just before the next dose) were collected. The peak level was standardized as one hour after the dose so that it would be appropriate to both the I.M. and 5 minute I.V. bolus methods of administration.

CEFOTAXIME

- Blood samples were drawn only after steady state had been reached. This was usually after 3 doses of the drug had been administered at six hourly intervals.

- Three samples, at an half an hour after dosing, 3 hours after dosing and 6 hours after dosing were collected.

- Provided that the clinical status of the patient allowed blood collection two mℓ samples were collected at each sampling time.

3.3 DETERMINATION OF PLASMA CONCENTRATIONS OF DRUGS

- A high pressure liquid chromatographic (HPLC) method was used to determine cefotaxime concentrations.

  This method was developed by Dr. Seifart (Department Pharmacology, Tygerberg Hospital). The validation of the method and the determinations of cefotaxime levels were carried out by them at Tygerberg Hospital. The procedures used in the HPLC assay and a characteristic HPLC chromatogram of one of the samples are shown in Appendix A.

- An enzyme immunoassay method (EMIT) was used to determine amikacin and gentamicin concentrations (Eppel, 1978).

  The EMIT assay which is commercially available is a homogenous enzyme immunoassay technique used for the analysis of low concentrations of specific compounds in biological fluids. Sensitivity with a coefficient of variation(CV) as low as 10% is reported for the EMIT assay. Concentrations as low as 0.1 μg/mℓ for gentamicin and 1 μg/mℓ for amikacin are measurable with the EMIT method.

3.4 STATISTICAL ANALYSIS OF DATA WITH NONMEM

The population pharmacokinetic analysis was performed on a main frame computer (ICL 2988) at the Unversity of Durban-Westville using Double Precision NONMEM 77 - version II level 1.4 together with the PREDPP package (ADVAN 1, ADVAN 2, TRANS 2 and SS2) (Beal and Sheiner, 1980-1986)

3.4.1 Pharmacokinetic models utilized during analyses

3.4.1.1 Amikacin and cefotaxime

Amikacin and cefotaxime were administered as bolus intravenous injections. The time course of the plasma concentrations of these drugs can be adequately described by an one compartment open linear model with first order absorption where:

$$C_p = \frac{D}{Vd} \cdot \frac{e^{-k}}{1 - e^{-k\tau}} \qquad \text{(b.4)}$$

$$C_m = \frac{D}{Vd} \cdot \frac{e^{-k\tau}}{1 - e^{-k\tau}} \qquad \text{(b.5)}$$

Cp = peak plasma concentration
Cm = trough plasma concentration
D = dose
Vd = volume of distribution
τ = dosing interval
$k = \frac{Cl}{Vd}$
Cl clearance

3.4.1.2 Gentamicin

Gentamicin was administered as an intramuscular injection. The time course of the plasma concentration for gentamicin can be adequately described by an one compartment open model with first order absorption:

$$Cp = \frac{ka \cdot D}{Vd\ (ka - k)} \cdot (e^{-kt} - e^{-kat}) \qquad \text{(b.6)}$$

Cp = peak
D = dose
ka = absorption constant
Vd = volume of distribution
$k = \frac{Cl}{Vd}$
Cl clearance

3.4.2 Statistical models utilized during analyses

3.4.2.1 Interindividual variability (η)

The error structures for interindividual variability (η) can be described either by an exponential or an additive model. With the exponential model it is assumed that the variability in pharmacokinetic parameters are distributed in a log normal fashion. It has previously been shown that a log normal rather than a normal distribution describes the η appropriately since the distributions of individual parameters in a patient population are generally skewed (Grevel *et al.*, 1988). Other advantages of an exponential error model are the following:

- The individual fixed parameters will always be positive and greater than zero.
- If a symmetrical distribution is assumed, the distribution of individual parameters is skewed to the right, which enables a more conservative prediction of plasma levels (i.e. a prediction which is always slightly higher than the actual measured level) (Sheiner and Beal, 1980).

For the reasons set out above it was decided to implement the exponential error model in the present study. The error models for interindividual variability in, for example, the jth individual were:

$$Cl_j = Cl \cdot \exp(n^jCl) \qquad (b.7)$$

$$Vd_j = Vd \cdot \exp(n^jVd) \qquad (b.8)$$

$$Ka_j = Ka \cdot \exp(n^jVd) \qquad (b.9)$$

Where η has a mean value of zero and variance $\eta\sigma^2$. Due to the exponential nature of the error model the interindividual variance was given as a variance σ^2 and was documented as coefficient of variation.

3.4.2.2 Intraindividual variation (ε)

The intraindividual variation (ε) or residual variability in the ith concentration of the jth individual was modelled as follows:

$$Cij^0 = Cij \cdot \exp(\varepsilon ij) \qquad (b.10)$$

Cij^0 = measured concentration
Cij = predicted concentration
εij = Independent statistical error between individual j and the specific plasma concentration ij. Where ε has a mean value of zero and variance of $\varepsilon\sigma^2$ (expressed as $\sqrt{\sigma^2}$ x 100 in results).

This intraindividual error model allows for:

- all uncertainty caused by intraindividual time variation in Clj and Vdj.
- pharmacokinetic model misspecifications
- analytical error in Cij°
- error in Cij.

The standard error (SE) of the parameters Cl, Vd and Ka and the variances (SE var), σ^2 and Ω were estimated by NONMEM. The standard error of the inter- and intraindividual variability was approximated using equation b.11

$$SEvar = \sqrt{\sigma^2} + SFvar - \sqrt{\sigma^2}$$

$$SE = \left(\frac{SEvar}{\Theta_1}\right) \cdot 100 \qquad (b.11)$$

3.4.3 NONMEM regression models

The purpose of the analysis was to relate Cl and Vd to relevant patient characteristics. The following models were therefore implemented:

1. $Cl = \theta_1$
   $Vd = \theta_2$

2. $Cl = \theta_1 \cdot WT$
   $Vd = \theta_2$

3. $Cl = \theta_1$
   $Vd = \theta_2 \cdot WT$

4. $Cl = \theta_1 \cdot WT$
   $Vd = \theta_2 \cdot WT$

5. $Cl = (\theta_1 \cdot WT + \theta_3 \cdot Scr)$
   $Vd = \theta_2 \cdot WT$

6. $Cl = \theta_1 \cdot BSA$
   $Vd = \theta_1 \cdot BSA$ (only for amikacin and cefatoxime)

7. $Cl = \theta_1 \cdot POST$
   $Vd = \theta_2$ (cefotaxime only)

8. $Cl = (\theta_1 \cdot WT) + (\theta_3 \cdot POST)$ (Cefotaxime only)
   $Vd = \theta_2 \cdot WT$

WT	=	weight
Scr	=	serum creatinine
BSA	=	body surface area
POST	=	postnatal age
θ_3	=	Ka Additive for gentamicin in models 1 to 5.

3.4.4 Criteria for testing superiority of one model over another

(Grevel et al., 1988)

- Each NONMEM run provides in its output the value of its objective function, which is 2 times the negative logarithm of the likelihood function. The difference in the objective function (DOBF) obtained is approximately chi square distributed with degrees of freedom equal to the number of fixed parameters minus one. For cefotaxime and amikacin (2 fixed effect parameters) a DOBF of more than 3.8 ($p < 0.05$) was regarded as significant and for gentamicin (3 fixed effect parameters) a DOBF of more than 8 ($p < 0.005$) was regarded as significant.

- A lack of correlation between parameters by inspection of the correlation matrix of the estimate provided in the NONMEM output.

- Small standard errors of estimates.

- Weighted residuals which are randomly scattered around zero when plotted against the predicted concentration.

- Smaller inter-individual variances.

4. RESULTS

4.1 CLINICAL AND THERAPEUTIC DATA

The clinical data used for population parameter estimations are summarized in tables B.1 and B.2.

Data from the neonatal population on cefotaxime and amikacin are presented in table B.1. Eleven infants received both amikacin and cefotaxime while one received cefotaxime only. Amikacin was administered as 15 mg/kg/day in divided doses at 12 hourly intervals to 10 patients and at 8 hourly intervals to one patient. Cefotaxime was administered as 100 mg/kg/day in divided doses at 6, 8 or 12 hourly intervals.

Gestational ages varied from 28 to 34 weeks (average 31.1 ± 1.9) and postnatal age from 4 to 23 days (average 11.6 ± 5.8). Five patients displayed an elevated white blood cell count (higher than 10,000 mm^3). Serum creatinine concentrations varied from 39 to 86 $\mu mol/\ell$ with an average of 65 ± 14.7 $\mu mol/\ell$ The average height was 36.7 ± 3.6 cm (30 to 44 cm) and the average weight was 1.4 ± 0.3 kg (0.930 to 1.765 kg). Forty-six plasma samples were collected for analysis (25 for cefotaxime and 21 for amikacin levels.) Two peak level samples (after one hour) with concentrations of 34.2 and 22.9 $\mu g/m\ell$ were collected for amikacin and 4 peak level samples (after half an hour) with an average concentration of 123.0 ± 9.7 $\mu g/m\ell$ (112.8 to 136.16 $\mu g/m\ell$) for cefotaxime.

Table B.2 summarizes the data from the adult population receiving gentamicin: Eleven patients (7 females and 4 males) were included in the study. Age varied from 21 to 70 years (43.6 ± 18.2 years) and weight from 46 to 79 kg (61.5 ± 9.1 kg). Ten of the 11 patients had an elevated white blood cell count. The serum creatinine concentration varied from 52 to 187 $\mu mol/\ell$ with an average of 102.0 ± 34.2 $\mu mol/\ell$ Twenty eight plasma levels were collected. Of these, nine were peak levels (after one hour) which varied from 3.5 to 6.3 $\mu g/m\ell$ (average 4.9 ± 1.1 $\mu g/m\ell$). Only one patient had a trough level concentration higher than 2$\mu g/m\ell$.

TABLE B.1

Clinical profiles from neonates on amikacin and cefotaxime treatment

Patients	GA (weeks)	PA (days)	WBC (10^3 cells /mm^2)	Scr (μmol/ℓ)	Urea	Height (cm)	Weight (kg)	Drug	Dose (mg)	Dosing interval (hours)	Sample time (hours)	Conc. (μg/mℓ)
1	32	10	8.5	51	1.6	37	1.305	Cefotaxime	70	12	3.03 6.00	58.32 27.68
								Amikacin	10	12	3.00 6.00	6.5 4.0
2	28	11	11.3	72	8.1	37	1.230	Cefotaxime	60	12	0.53 3.53 5.00	121.76 87.35 33.82
								Amikacin	20	12	0.53 3.52	24.6 6.2
3	28	6	8.3	78	5.9	34	1.140	Cefotaxime	60	12	0.52	136.16
								Amikacin	8	12	0.52 6.0	31.4 6.5
4	32	19	9.9	50	3.7	40	1.765	Cefotaxime	45	6	2.42 5.02 6.00	55.28 35.77 22.41
								Amikacin	12	12	0.53 1.90 3.00	24.2 14.9 7.5
5	32	10	9.5	39	3.3	44	1.470	Cefotaxime	35	6	2.92 6.00	23.43 11.05
								Amikacin	11	12	3.50 9.00	9.1 2.0
6	32	4	30.7	63	4.8	37	1.260	Cefotaxime	32	6	0.50 3.50	121.29 24.06
								Amikacin	10	12	0.50 3.50	27.4 14.4

TABLE B.1 (cont.)

Clinical profiles from neonates on amikacin and cefotaxime treatment

Patients	GA (weeks)	PA (days)	WBC (10^3 cells /mm^2)	Scr (μmol/ℓ)	Urea	Height (cm)	Weight (kg)	Drug	Dose (mg)	Dosing interval (hours)	Sample time (hours)	Conc. (μg/mℓ)
7	32	9	9.1	86	4.8	36	1.40	Cefotaxime	70	12	3.02 4.03 11.75	84.68 58.08 9.31
								Amikacin	10	12	1.50	19.6
8	29	6	10.5	52	3.6	32	1.120	Cefotaxime	35	8	3.00 6.00	57.49 12.87
								Amikacin	8	12	0.50 6.52	27.8 10.0
9	32	23	11.5	70	3.2	30	0.930	Cefotaxime	25	6	0.92	86.05
								Amikaxin	7	12	0.92	34.2
10	32	17	12.0	64	3.0	39	1.600	Cefotaxime	75	6	4.00	21.77
								Amikacin	10	12	0.92 4.00	22.9 7.0
11	30	9	5.0	85	1.5	37	1.740	Cefotaxime	60	12	3.58 4.02	46.45 43.58
								Amikacin	8	8	0.50 1.00	28.0 23.5
12	34	15	5.3	70	1.0	37	1.400	Cefotaxime	60	12	0.52 3.03 6.02	112.80 47.00 15.75

GA Gestational age

WBC White blood cell count

Conc. Concentration

PA Postnatal age

Scr Serum creatinine

TABLE B.2

Clinical profiles from adult patients on gentamicin treatment

Patients	Age (years)	Sex	WBC ($10^3 cells/mm^2$)	Scr ($\mu mol/\ell$)	Weight (kg)	Dose (mg)	Dosing interval (hours)	Sample time (hours)	Conc. ($\mu g/m\ell$)
1	67	F	6.9	72	58	80	8	1.00 7.75	6.2 1.5
2	70	F	15.4	105	46	80	12	0.25 0.50 11.75	7.1 8.5 2.4
3	45	M	15.0	116	72	80	8	1.00 7.98	6.3 0.9
4	21	F	13.6	95	61	80	8	0.98 7.98	4.5 0.4
5	21	M	10.4	52	60	80	8	1.03 7.98	6.0 0.4
6	44	F	20.7	115	60	80	8	1.03 7.75	5.1 0.4
7	57	F	14.0	79	62	80	8	0.25 0.50 1.00 7.75	2.2 3.4 4.3 0.2
8	29	F	11.1	103	79	120	8	1.00 1.54 6.03 7.98	3.7 3.2 1.9 1.1
9	31	F	18.0	187	65	60	12	0.98 11.75	4.4 0.1
10	32	M	18.1	105	63	80	8	0.25 0.50 7.75	3.8 6.4 0.4
11	63	M	19.1	93	50	80	8	7.98 0.98	0.9 3.5

WBC White blood cell count
Scr Serum creatinine concentration

4.2 NONMEM DATA ANALYSES

4.2.1 Amikacin

The different models used and the hypothesis testing applied in the NONMEM data analysis are presented in tables B.3 and B.4.

Model 1 serves as the basis of all comparisons and is modeled simply as an average Cl and Vd with differences between subjects ascribed to inter-individual variation. Adjusting Cl for weight (Model 2) significantly decreased the unexplained intersubject variation (DOBF = 8.74). Adjusting Vd for weight (model 3) similarly significantly decreased the OBF (DOBF = 5.6). When both Cl and Vd were adjusted for weight simultaneously (model 6) the decrease in OBF was significant when compared to model 1 (2 degrees of freedom) and model 3 (1 degree of freedom) but not when compared to model 2. However, model 6 was better than model 2 on the grounds of a reduction in the intersubject variation of 3.8% to 2.5%.

The use of BSA (model 4) instead of weight (model 6) to adjust Cl and Vd did not significantly decrease the OBF. The inclusion of serum creatinine in addition to weight (model 5) did not decrease the OBF significantly (model 6). The interindividual variation (η) for Vd remained approximately constant in all the models and even increased in the model where Vd was corrected for weight (model 3). The intraindividual variation (ε) decreased when both Cl and Vd were corrected for weight or BSA (27 to 21%).

TABLE B.3

Influence of different factors on amikacin clearance (Cl) and volume of distribution (Vd)

ID	Model	OBF	Cl	η (%)	Vd	η (%)	ε (%)
1	Cl = Θ_1 (ℓ/h) Vd = Θ_2 (ℓ)	100.10	0.0947	27	0.304	39	27.0
2	Cl = Θ_1* WT (ℓ/h/kg) Vd = Θ_2 (ℓ)	91.36	0.0706	3.8	0.303	30	26
3	Cl = Θ_1 (ℓ/hr) Vd = Θ_2* WT (ℓ/kg)	94.50	0.0683	25.0	0.249	49	21
4	Cl = Θ_1 * BSA (ℓ/hr/1.73 m²) Vd = Θ_2 * BSA	87.57	0.8470	1.1	2.920	37	20.6
5	Cl = [(Θ_1* WT) + (Θ_3*Scr)] (ℓ/h/kg) Vd = Θ_2* WT (ℓ/kg)	86.86	Θ_1 = 0.117 Θ_3 = 0.071	14.5	0.259	36	20
6	Cl = Θ_1 * WT (ℓ/h/kg) Vd = Θ_2 * WT (ℓ/kg)	89.45	0.0683 (0.0102)	2.5 (54.6)	0.238 (0.024)	38 (26)	21 (25)

*Standard error is shown in parenthesis

TABLE B.4

Hypothesis testing for amikacin

ID	Hypothesis	Models compared	DOBF	p - value	Conclusion
1	Did WT influence Cl alone ?	1 + 2	8.74	$p < 0.05$	Yes
2	Did WT influence Vd alone ?	1 + 3	5.6	$p < 0.05$	Yes
3	Did WT influence both Cl and Vd?	1 + 6	10.65	$p < 0.05$	Yes
4	Did BSA influence Cl and Vd?	1 + 4	12.53	$p < 0.05$	Yes
5	Did Scr influence Cl ?	5 + 6	2.59	$0.2 < p < 0.1$	No
6	Did BSA influence Cl and Vd more than WT ?	4 + 6	1.88	$0.2 < p < 0.1$	No

4.2.2 Cefotaxime

The different models used and the hypothesis testing applied in the NONMEM data analysis are presented in tables B.5 and B.6. Model 1 serves as the basis of all comparisons and is modeled simply as an average CL and Vd with differences between subjects ascribed to inter-individual variation. Adjusting Cl for weight (model 2), Vd for weight (model 3) or Cl for postnatal age (model 4) did not significantly decrease the OBF. When both Cl and Vd were adjusted simultaneously for weight (model 7) or BSA (model 6) the decrease in OBF was significant when compared to model 1 (2 degrees of freedom). The inclusion of serum creatinine in addition to weight (model 5) did not decrease the OBF of models 6 or 7 significantly.

The η for Vd decreased when Cl was corrected for postnatal age (model 4), stayed the same when both Cl and Vd were corrected for BSA (model 6) and increased in all the other models. From these results it was not possible to obtain a standard error for the Vd parameter. This suggests that either the difference in the population was too small or that not enough information regarding the standard error could be obtained from the data.

TABLE B.5

Influence of different factors on clearance (Cl) and volume of distribution (Vd) of cefotaxime

ID	Model	OBF	Cl	η (%)	Vd	η (%)	ε (%)
1	Cl = Θ_1 (ℓ/h) Vd = Θ_2 (ℓ)	159.08	0.121	8.60	0.392	9.9	28.8
2	Cl = Θ_1* WT (ℓ/h/kg) Vd = Θ_2 (ℓ)	155.92	0.089	2.26	0.425	17.5	24.7
3	Cl = Θ_1 (ℓ/h) Vd = Θ_2* WT (ℓ/kg)	157.56	0.1260	11.4	0.296	10.3	26.8
4	Cl = Θ_1 * POST (ℓ/h/age) Vd = Θ_2 (ℓ)	158.94	0.115	12.0	0.390	4.5	29.0
5	Cl = [(Θ_1* WT) + (Θ_3*Scr)](ℓ/h/kg) Vd = Θ_2* WT (ℓ/kg)	151.479	Θ_1 = 0.0911 Θ_3 = 0.00007	13.3	0.311	13.3	23.0
6	Cl = Θ_1 * BSA Vd = Θ_2 * BSA	151.605	1.1203	8.75	3.750	9.6	24.7
7	Cl = Θ_1 * WT (ℓ/h/kg) Vd = Θ_2 * WT (ℓ/kg)	151.483	0.0919 (0.0053)	15.3 (22)	0.312 (0.019)	14 (neg= lible)	23.4 (16)

*standard error is shown in parenthesis

TABLE B.6

Hypothesis testing for cefotaxime

ID	Hypothesis	Models compared	DOBF	p - value	Conclusion
1	Did WT influence Cl alone ?	1 + 2	3.16	$0.1 < p < 0.05$	No
2	Did WT influence Vd alone ?	1 + 3	1.52	$p < 0.1$	No
3	Did POST influence Cl ?	1 + 4	0.14	$p < 0.1$	No
4	Did WT influence Cl and Vd?	1 + 7	7.6	$p < 0.05$	Yes
5	Did BSA influence Cl and Vd more than weight?	6 + 7	0.0	$p < 0.1$	No
5	Did Scr influence Cl ?	5 + 7	0.0	$p < 0.1$	No

4.2.3 Gentamicin

The different models used and the hypothesis testing applied in the NONMEM data analysis are presented in tables B.7 and B.8. Model 1 serves as the basis of all comparisons and is modeled simply as an average CL and Vd with differences between subjects ascribed to inter-individual variation. Adjusting Cl for weight (model 5) significantly decreases the unexplained intersubject variation (DOBF - 10.78). When both Cl and Vd were adjusted for weight simultaneously (model 3) the decrease in OBF was significant when compared to mode 1 (2 degrees of freedom). When Vd was however adjusted for weight (model 2) the OBF increased. The inclusion of serum creatinine in addition to weight (model 4) did not decrease the OBF of either model 3 or 5 significantly.

The η for Cl decreased markedly when Cl was corrected for weight (59 versus 25%). Due to limited data regarding Vd it was not possible to obtain a η value for Vd. The ε value decreased only when Cl alone was corrected for weight (29.4 versus 10%.)

Due to inadequate data regarding the absorption phase, the absorption constant (Ka) was calculated by manual iteration using NONMEM. A value for ka of 2.4 hr^{-1} produced the smallest minimum objective function and was subsequently used as fixed value in all data analyses.

TABLE B.7

Influence of different factors on clearance (Cl) and volume of distribution (Vd) of gentamicin

ID	Model	OBF	Cl	η (%)	Vd	ε (%)
1	Cl = Θ_1 (ℓ/h) Vd = Θ_2 (ℓ)	51.41	3.22	59	12.80	29.4
2	Cl = Θ_1 (ℓ/h) Vd = Θ_2* WT (ℓ/kg)	55.53	0.223	57	0.223	25.7
3	Cl = Θ_1* WT (ℓ/h/kg) Vd = Θ_2* WT (ℓ/kg)	42.83	0.0544	44	0.218	28.7
4	Cl = [(Θ_1* WT) + (Θ_3*Scr)](ℓ/h.kg) Vd = Θ_2* WT (ℓ/kg)	40.63	Θ_1 = 0.0619 Θ_3 = 0.0028	40	0.218	32.8
5	Cl = Θ_1 * WT (ℓ/h/kg) Vd = Θ_2 (ℓ)	40.63	0.0588 (0.008)	25 (20)	12.5 (1.51)	10 (17)

*standard error is shown in parenthesis

Θ_3 = Ka was fixed at 2.4

η was unobtainable for Θ_2

TABLE B.8

Hypothesis testing for gentamicin

ID	Hypothesis	Models compared	DOBF	p - value	Conclusion
1	Did WT influence Cl alone?	1 + 5	10.78	$p < 0.05$	Yes
2	Did WT influence Vd alone?	1 + 2	4.12 (increase)	unobtainable	No
3	Did WT influence Cl and Vd?	1 + 3	8.58	$p < 0.05$	Yes
4	Did Scr influence Cl?	3 + 4	2.20	$0.4 < p < 0.3$	No

5. DISCUSSION

5.1 AMIKACIN

The best fit of the model to the data was obtained when both Vd and Cl were adjusted for either weight or body surface area (BSA). Since BSA and weight are equally good predictors of clearance it would be more convenient simply to use weight. The inability of serum creatinine levels to improve the prediction of Cl further in a neonatal population was also documented by Kelman *et al.* (1984). Reasons for this could be the homogeneous nature of serum creatinine concentrations in the specific population or the fact that serum creatinine is not a true reflection of kidney function below 35 weeks gestation.

A Cl value of 0.847/ℓ/h/1.73 m^2, approximately 50% lower than reported values, was obtained in this study (Kasik *et al.*, 1985). In a study where only postnatal and not postconceptual age was documented, Cl values of 1.35/ℓ/h/1.73 m^2 and 1.48/ℓ/h/1.73 m^2 were reported for infants younger than 1 week weighing less than 2000 g and infants older than 1 week weighing more than 2000 g respectively (Zaske, 1986). The direct correlation between renal clearance of drugs and postconceptual age has been ascribed to immaturity of the glomeruli until 35 weeks of gestation (Kasik *et al.*, 1985).

The population studied in the present investigation had an average gestational age of 31 ± 1.9 weeks (range 28-34 weeks) which suggests that the glomeruli were not yet fully developed. The difference in gestational age may explain the discrepancy between clearance values reported in other studies and the values obtained in the present study.

The volume of distribution in the present study was 0.238 ± 38% ℓ/kg compared to a value of 0.45 ± 40% ℓ/kg reported for a postnatal age of 1-2 weeks (Zaske, 1986). Furthermore, for infants of gestational age of less than 32 weeks the Vd for most drugs appear to be larger than 0.4 ℓ/kg and only approaches a value of 0.25 ℓ/kg at an age of 3 months after birth (Kelman *et al.*, 1984; Kasik *et al.*, 1985).

The infants in the present study were acutely ill with gram negative bacteremia which was associated with fever and dehydration. The latter could account for the observed lower Vd. In a study by Marik *et al.*. (1990) a Vd value of 0.58 ℓ/kg was reported for neonates with bacteremia at Baragwananth Hospital which compares favourably with the results of the present study.

5.2 CEFOTAXIME

The best fit of the model to the data for cefotaxime was obtained when both Vd and Cl were adjusted for either weight or BSA, just as for amikacin. Since BSA and weight are equally good predictors of clearance it would be more convenient simply to use weight.

Serum creatinine levels was also unable to improve the prediction of Cl further and again correlates with the findings by Kelman *et al.* (1984). The Cl value of 0.0919(0.0053) ℓ/h/kg in the present study compared favourably to the Cl value of 0.074(0.003) ℓ/h/kg reported by Kearns *et al.* (1989) and can be related to the fact that gestational age was similar in both instances. In the Kearns study the average postconceptual age was 28.4 weeks and in the present study 31 weeks. The slightly higher value of 0.0919 can be explained by the older age of the study population group. The standard error was 0.003 and 0.005 respectively for Kearns and the present study. The strength of a NONMEM analyses featured strongly with this observation if it is kept in mind that the present analysis was performed on routinely collected data (maximum of three samples) in comparison with 10 samples over a 12 hour period in the Kearns study. The Cl (1.5 ml/min) obtained in the present study corresponds to expected values of 2 to 3 ml/min for glomerular filtration rate in neonates under 34 weeks of gestation. This suggests that glomerular filtration and possibly active renal tubular secretion were primarily responsible for the Cl of cefotaxime (McCracken *et al.*,1982).

The Vd value of 0.312 (0.019) ℓ/kg was also in keeping with literature reports (Balant *et al.*, 1985; Kearns *et al*,. 1989). The larger Vd in neonates compared to adults suggests that the dosing interval in neonates can be increased because of the associated longer half-life of the drug.

5.3 GENTAMICIN

The best fit of the model to the data for gentamicin was obtained when Cl was adjusted for weight alone or both weight and serum creatinine. Unlike weight alone serum creatinine was however unable to improve the prediction of Cl further. This observation that serum creatinine did not improve Cl predictions was contrary to literature reports (Rodvold *et al.*, 1990). The small population group and homogenous nature of serum creatinine concentrations could account for this discrepancy. Only one patient in the group, with a serum creatinine value of 163 μmol/ℓ, had a degree of renal failure. When no variation in a particular parameter is present the parameter becomes less relevant.

To obtain a true reflection of the role of serum creatinine values more patients with impaired renal function need to be investigated.

It was therefore difficult to compare the Cl value of this study with values reported in the literature where serum creatinine clearance were incorporated in the calculations:

- 0.815 Cl_{Cr} + 0.0417 (mℓ/min/kg) (Rodvold, 1990).
- 0.73 Cl_{Cr} + 0.06 (mℓ/min/kg) (Benet and Sheiner, 1985).

In the present study a Cl parameter could be calculated according to the formula (0.0619 * Wt) + (0.00277 * Scr). When this Cl value was compared to the Cl values obtained according to the formulation of other investigators (Rodvold, 1990; Benet and Sheiner, 1985) for an average man 60 yrs of age weighing 70 kg and a Scr value of 1 mg %, the following Cl values were obtained:

- Rodvold 3 ℓ/h
- Benet and Sheiner 2.79 ℓ/h
- Present study 4.33 ℓ/h.

The higher Cl value for this study can be explained by the fact that all the patients were acutely ill with gram negative bacteremia associated with pyrexia. Schentag *et al.* (1986) documented that pyrexia can increased Cl.

When Vd was corrected for weight a Vd value of 0.218 ℓ/kg was obtained which compared favourably with the values of 0.25 ℓ/kg reported in the literature (Rodvold *et al.*, 1990). The OBF value however increased from 51.41 to 55.53 when Vd was corrected for weight in the present study. Whiting *et al.* (1986) stated that steady state concentrations will provide good information about Cl but little about Vd in a NONMEM analysis. More blood samples in a drug level profile are therefore required for good Vd estimations.

6. CONCLUSIONS

Population parameter values for amikacin and cefotaxime in neonates and for gentamicin in an adult bacteremia population were determined in this study.

Clearance values obtained for cefotaxime and gentamicin but not for amikacin were comparable to values reported in the literature. The reason for the discrepancy with amikacin was due to the fact that the literature values were not corrected for gestational age. The importance of correction of clearance for gestational age featured strongly in this study.

Steady state data in a small population can therefore be sufficient to estimate representative clearance values. The strength of NONMEM also featured strongly with the similarities obtained between the results in this study and literature reports on single dose studies with multiple samples.

The estimation of Vd in all three populations was satisfactorily but interindividual variation values and standard errors in Vd was not satisfactorily. To be able to calculate better Vd values more data is needed and another study designed to collect more blood samples over the drug profile will have to be undertaken.

The similarity of cefotaxime and amikacin parameter values to those reported in the literature suggests that the ideal models of both these drugs can be used in this clinical setting. The following models for the prediction of Cl and Vd are therefore proposed:

- **Amikacin**
  Cl (ℓ/h/kg) = 0.0683 · WT
  Vd (ℓ/kg) = 0.238 · WT

- **Cefotaxime**
  Cl (ℓ/h/kg) = 0.0919 · WT
  Vd (ℓ/kg) = 0.312 · WT

- **Gentamicin**
  Cl(ℓ/h/kg) = 0.588 · WT
  Vd(ℓ) = 12.5

In the neonate both drugs are administered rapidly over a few seconds and Cp maximum and Cp minimum can be calculated using a bolus model :

$$Cp\ max = \frac{\frac{FD}{V}}{1 - e^{-k\tau}} \quad (b.12)$$

$$Cpmin = Cp\ max\ e^{-k\tau} \quad (b.13)$$

For a neonate of 1.5 kg receiving 10mg amikacin 12 hourly a peak of 27μg/ml and trough of 9.8 μg/ml can be expected.

With a dose of 50mg cefotaxime a peak of 109μg/ml and trough of 2μg/ml can be expected in the same neonate.

The population estimates for gentamicin are limited to patients with normal renal function and need to be verified in a study where more patients with a broad spectrum of serum creatinine values are incorporated. That implies a study where patients with some degree of renal failure are included.

SECTION C

DUAL INDIVIDUALIZATION:

AN INVESTIGATION INTO PHARMACOKINETIC - PHARMACODYNAMIC RELATIONSHIPS WHICH MAY IMPROVE GENTAMICIN THERAPY IN PATIENTS WITH GRAM NEGATIVE BACTEREMIA

1. INTRODUCTION AND OBJECTIVES

The pharmacokineticists of the 1990's are faced with the challenge of dual individualization in the optimization of the treatment of diseases. Dual individualization may be defined as the phenomenon whereby both pharmacokinetic and pharmacodynamic data are incorporated into the process of dosage adjustment. The ultimate goal of dual individualization is to optimize the dosing regimen for an individual patient. Such optimization may have a number of advantages including, lowered mortality rates, shortened hospitals stays and more cost effective management of patients.

The application of dual individualization to antibiotic therapy is complex because concurrent interrelationships exist among the drug, the host and the causative organism as depicted in fig C.1 (Schentag *et al.*,1986). Aminoglycoside therapy has been vastly improved by the current methods of dosage adjustment based upon pharmacokinetic principles. However, the classical pharmacokinetic approach tends to oversimplify the interactions between the causative organism and the host (fig C.1). Present

aminoglycoside dosage techniques assume that concentrations within the desired therapeutic range kill susceptible bacteria at an equal rate. Based upon this assumption, current dosage individualization considers only pharmacokinetics, efficacy in terms of effective range and toxicity. This approach has been studied in detail in patients. Little attention has been paid to simultaneous consideration of all variables relevant to drug treatment of infection such as pharmacodynamics, resistance, toxicity, host defences and pharmacokinetics. It therefore becomes logical to attempt to develop methods whereby the concentration dependent effects of the antibiotic on the organism (pharmacodynamic parameters) are incorporated in mathematical formulae designed to achieve optimum therapy in man.

The objective of this study was to identify certain pharmacodynamic parameters for gentamicin which can be incorporated in mathematical formulae for optimizing dosage regimens. These pharmacodynamic parameters were derived from an investigation in patients with gram negative bacteremia due to the organisms, *K. pneumoniae* and *E. coli*.

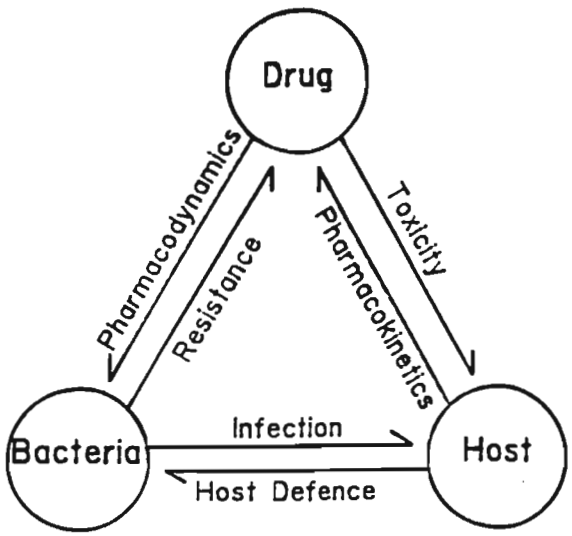


Fig C.1 Schematic representation of the interaction among bacteria, host and antibiotic

2 LITERATURE REVIEW

2.1 PHARMACODYNAMIC MODELING

(Holford and Sheiner, 1981; Schwinghammer and Kroboth, 1988)

Pharmacokinetic models generally describe drug concentration as a function of both dose and time. In contrast pharmacodynamics are in essence independent of time and describe pharmacodynamic equilibrium (time-independent) relationships between concentration and effect. The pharmacodynamic parameter of maximum effect may occasionally be time dependent but this is not always so. The only common feature in pharmacokinetic and pharmacodynamic models namely the drug concentration can be used to combine the two models and describe the overall dose effect relationship:

Dose → PK → CP → PD → Effect

PK = pharmacokinetics

CP = plasma concentration

PD = pharmacodynamics

The concentrations in the pharmacodynamic model have to reflect the concentration of the drug at the effect site (biophase). The pharmacokinetic models alone may not be able to predict such values directly so further modeling is required in order to link them:

Dose → PK → CP → PKCP → CE → PD → Effect

PKCP = pharmacokinetic - plasma concentration link

CE = effect site concentration

The final parameter in this model is the measured effect. For example the observable effect for an antihypertensive agent is the lowering of blood pressure, but the mechanistic effect may be a direct action namely relaxation of vascular smooth muscle. The pharmacodynamic model should refer to the direct effect of the drug on the blood vessel and a physiological model is needed to relate this action to the observed effect of lowering the blood pressure

Dose → PK → CP → PKPD → CE → PD → E → PE → Effect

E = observable effect

PE = physiological effect at tissue level

The rest of this discussion will deal with different pharmacodynamic models since the relevant pharmacokinetics of aminoglycosides were discussed in Section B.

2.1.1 Pharmacodynamic models

2.1.1.1 Types of models

- **Fixed effect model**

  A fixed effect is an observed effect which is either present or absent, for example the absence or presence of seizures after the administration of antiepileptic drugs. A fixed effect can also be defined by specific criteria, for example, the effect of an antiarrhythmic drug could be considered to be present when 70% suppression of ventricular extrasystoles occurs. With a fixed effect the extent of the response is immaterial - it is only important whether it occurs or not. The presence or absence of the effect is then related to a particular concentration in a statistical fashion. A good example of a fixed effect study is that of Beller *et al.* (1971). They routinely collected data on digoxin toxicity in a number of patients receiving digoxin. They defined the effect as the presence or absence of toxicity. They were then able to calculate that the probability of toxicity with digoxin was 50% at a concentration of 2ng/ml. In such studies the probability of an effect can then be expressed over a range of concentrations and when this is plotted a sigmoid curve is obtained.

- **Emax model**

  The Emax model is the simplest model which adequately describes drug effect over a whole range of concentrations and is formulated by the following equation sometimes referred to as the Langmuir model:

$$E = \frac{Emax \cdot C}{EC_{50} + C} \qquad (c.1)$$

E = effect

C = concentration
EC_{50} = concentration producing 50% of the maximum effect
Emax = maximum effect

The use of the Emax model for pharmacodynamic phenomena can be justified on theoretical grounds but empirically it is useful for the following reasons:

- It predicts the maximum effect a drug achieves
- It predicts no effect when no drug is present
- It allows for the saturation of effect and demonstrates futility of further increasing the dose once maximal effect is achieved.

The Emax equation can also be adapted to measure an effect which is observed as the inhibition of biological phenomenon:

$$E = \text{Nodrug} - \frac{\text{Emax} \cdot C}{IC_{50} + C} \qquad \text{(c.2)}$$

Nodrug = effect where no drug is present (for example, resting heart rate before a β-adrenergic antagonist is given)
IC_{50} = the concentration producing 50% inhibition of the maximum effect

- **Linear model**

When the drug concentration is low in relation to EC_{50} the effect becomes proportional to concentration :

$$E = S \cdot C \qquad \text{(c.3)}$$

S = slope of line relating effect to concentration
C = concentration

The disadvantage of this model is that it lacks the ability to define maximum effect. The model also does not allow for the saturation of the effect. The linear model can only be used over low concentration ranges which may however at times be the larger part of the therapeutic concentration ranges of particular drugs.

- **Sigmoid Emax model**

  Hill (1910) showed that not all concentration effect curves can be presented by the hyperbolic form of the Emax model. He added an extra parameter which can alter the simple hyperbolic form:

$$E = \frac{Emax \cdot C^N}{EC_{50} + C^N} \qquad (c.4)$$

  N = number that influences the slope of the curve
  N = 1 = hyperbolic curve
  N > 1 = sigmoid curve with a steeper slope
  N < 1 = sigmoid curve, shallower in its central part, steeper at low concentrations and more shallow at higher concentrations.

The larger the value of N, the greater the change in response with concentrations around the EC_{50} value. Generally the value of N lies between 1 and 3. Occasionally it is much greater, in which case the effect appears almost as an all-or-none response, because the concentration associated with minimal and maximal responses becomes so narrow (Rowland and Tozer, 1989).

2.1.1.2 Baseline effect

Many observable drug effects reflect changes from some baseline effect. For example blood pressure, may change after the administration of a drug from a specific pressure to something else. In this instance the baseline effect needs to be incorporated in pharmacodynamic models describing drug effect.

Equation c.5 is the Emax model with E_o the baseline effect. Equation c.6 is the linear model with E_o the baseline effect:

$$E = \frac{Emax \cdot C}{IC_{50} + C} + E_0 \qquad (c.5)$$

$$E = S \cdot C + E_0 \qquad (c.6)$$

2.1.2. Pharmacokinetic-pharmacodynamic models

2.1.2.1 The pharmacokinetic-independent model

In this model a concentration and effect are directly linked by taking simultaneous measurements of the effect and the concentration at the effect site.

Disadvantages of the pharmacokinetic independent model are:

- requirements for sampling increase when the concentration is changing rapidly
- the necessity of ignoring measurement error in the concentration value when relating it to effect
- the necessity for sampling directly from effect site.

2.1.2.2 The pharmacokinetic-compartment model

The implementation of an appropriate pharmacokinetic model will result in the more accurate prediction of plasma concentrations at specific times.

The time course of drug concentrations in plasma, urine or other biological fluids is usually modelled by compartmental analysis. The concentration of the drug in one of the compartments which is not directly accessible to sampling may be predicted from analysis of plasma concentrations.

Advantages of the pharmacokinetic-compartment model are:

- simultaneous measurements of concentration and effect are not necessary. The pharmacokinetic model provides information over the whole blood sample profile
- timing of sampling and effect measurement can be optimized, independently according to the expected model
- plots of concentration versus effect using the concentration predicted by a pharmacokinetic model for the central compartment are useful for the recognition of equilibration delays between plasma and effect site concentrations.

The major disadvantage of the pharmacokinetic-compartment model is the prerequisite that the time-course of effect site concentration parallels the distribution of the drug to those tissues that determine the multi-exponential character of the plasma concentration -time course.

2.1.2.3 The effect-compartment model

Provided the drug enters and leaves the effect site by a first order process it is possible to predict the time course of drug accumulation at the effect site as plasma concentration changes. For example, if a plasma concentration changes from 0 to a new value of C, the equilibrium effect corresponding to this concentration (C) and becomes EC. The rate constant (Ke_o) describes drug loss from the effect site and controls the onset of the effect.

Equations describing the effect site concentrations for a number of pharmacokinetic models have been presented in the literature and can be used to determine the actual concentration at effect site (Sheiner *et al.*, 1979).

2.2 METHODS OF MEASURING BACTERIAL GROWTH AND DEATH

Different methods for measuring the effect of antibiotics on microorganisms were devised during the course of the present study. In order to describe these methods, the following concepts need elaboration.

2.2.1 Bacterial growth

2.2.1.1 Definition of growth

Growth can be defined as the orderly increase in all the components of an organism. Cell multiplication is a consequence of growth. In unicellular organisms multiplication leads to an increase in the number of individuals making up a population or a culture (Jawetz *et al.*, 1982).

2.2.1.2 The measurement of growth

Microbial growth can be measured in terms of cell concentration (the number of cells per unit volume of culture) or cell density (dry weight of cells per unit volume of culture):

- Cell concentration

  The viable cell count is usually considered to be a measure of cell concentration. The general practice, however, is to measure the light absorption or light scattering of a culture by photoelectric means and to relate viable counts to optical measurements in the form of a standard curve. By means of the standard curve all further readings can be converted to cell concentration. Cell concentration determinations are normally used in studies on microbial inactivation.

- Cell density

  To determine cell density it is usually necessary to perform a large number of dry weight measurements which may be difficult technically. For this reason these measurements are normally performed by indirect methods such as nitrogen determination and photoelectric measurements. Cell density determinations are used primarily in studies of microbial biochemistry and nutrition.

2.2.1.3 Exponential growth

- The growth constant

  Since the two new cells produced by the growth and division of a single cell are each capable of growing at the same rate as the parent cell, the number of cells in a culture increases exponentially. The rate of growth of a culture at a given moment is directly proportional to the number of cells present at that moment. This relationship is expressed in the following equation:

$$\frac{dN}{dt} = kN \qquad (c.7)$$

Integration of equation c.7 gives :

$$N = N_0^{ekt} \qquad (c.8)$$

N_0 = number of cells at time zero

N = number of cells at any later time t

In equation c.8 above, k is the growth constant.

Solving the equation for k gives:

$$k = \frac{\ln(N/N_0)}{t} \qquad (c.9)$$

k represents the rate at which the natural logarithm of cell number increases with time and can be determined from the slope of a curve where log cell number is plotted against time.

- The generation

It is general practice to express the growth rate of a microbial culture in terms of generations per hour. For organisms which reproduce by binary fission a generation is defined as the doubling of cell number. The number of generations per hour is usually determined by plotting cell number against time on a semilogarithmic scale and reading directly from the plot the time required for the number to double. Alternatively the generation time can be calculated from the following equation:

$$g = \frac{\log N - \log N_0}{\log 2} \qquad (c.10)$$

g = generations

$\log N_0$ = log of number of cells at time 0

log N = log of number of cells at any later time t

2.2.1.4 The growth curve

If a liquid medium is inoculated with microbial cells taken from a culture that has previously been grown to saturation and the number of viable cells per milliliter determined periodically and plotted, a curve of the type shown in Figure C.2 is usually obtained. The curve can be divided into 6 phases with the four most important being:

- The lag phase (A)

The lag phase represents a period during which the cells, depleted of enzymes, as a result of unfavourable conditions at the end of their previous culture history, adapt to their new environment. Enzymes and intermediates are formed and accumulate until they are present in concentrations sufficient for growth to resume. If the cells

are taken from an entirely different medium, it often happens that they are genetically incapable of growth in a new medium. In such cases a longer lag may occur representing the period necessary for a few mutants in the inoculum to multiply sufficiently for a net increase in cell number to become apparent.

- The exponential phase (C)

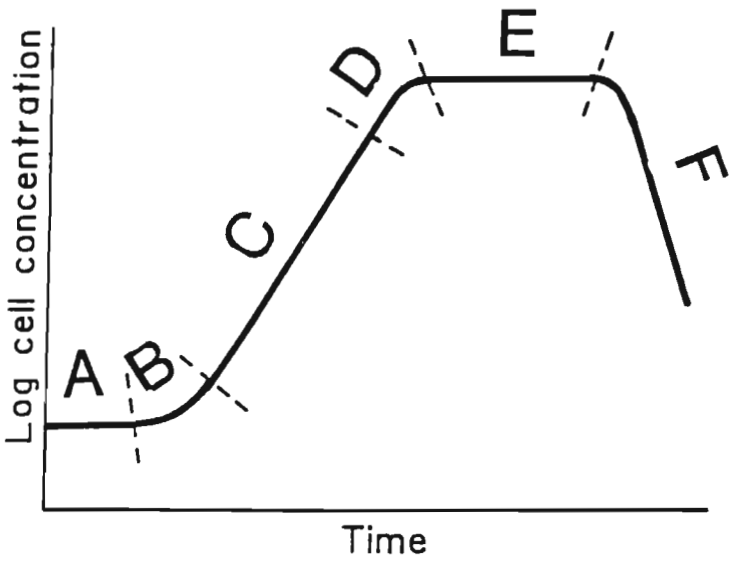
During the exponential phase the cells are at steady state. New cell material is being synthesized at a constant rate, but the new material is itself catalytic and the mass increases in an exponential manner. As this continues either one or more nutrients in the medium become depleted, or toxic metabolic products which inhibit growth accumulate. For aerobic organisms, the commodity that becomes limiting is usually oxygen. As a result, growth rate decreases when the cell concentration exceeds $1x10^7/m\ell$. At 4 to $5x10^9/m\ell$, the rate of oxygen diffusion can no longer meet the demand even in an aerated medium and the growth becomes progressively slower.

- The maximum stationary phase (E)

In the stationary phase the nutrients are exhausted and the accumulation of toxic products causes growth to cease almost completely. In most cases, however, some cell turnover takes place in the stationary phase. There is a slow loss of cells through death, which is just balanced by the formation of new cells through growth and division. When this occurs the total cell count increases slowly although the viable count stays constant.

- The phase of decline (death phase, F)

After a period of time in the stationary phase, which depends on the organism and on the culture conditions, the death rate increases until it reaches a steady state where cell death and cell growth are constant. Frequently, after the majority of cells have died,the death rate decreases drastically, so that a small number of survivors may persist for months or even years (Jawetz *et al.*, 1982).



Section of curve	Phase	Growth Rate
A	Lag	Zero
B	Acceleration	Increasing
C	Exponential	Constant
D	Retardation	Decreasing
E	Maximum stationary	Zero
F	Decline	Negative (death)

Fig C.2 Phases of the microbial growth curve

(Jawetz et al., 1982)

2.2.2 Bacterial death

2.2.2.1 Definition

For a microbial cell, death means irreversible loss of the ability to reproduce (grow and divide).

2.2.2.2 Measurement of death

When dealing with microorganisms, one does not customarily measure the death of an individual cell but the death of a population. The number of cells dying during each time interval is a function of the number of survivors present so that death of a population proceeds as an exponential process according to the following equation:

$$S = S_0 e^{-kt} \qquad \text{(c.11)}$$

S_0 = number of microorganisms at time 0
S = number of survivors at any later time t
k = rate of exponential death

2.2.2.3 Effect of drug concentration

When antimicrobial drugs are used to inactivate microbial cells, it is commonly observed that the concentration of drug present is often related to the time required to kill a given fraction of the population as expressed by the following equation:

$$k = C^n \cdot t \qquad \text{(c.12)}$$

C = drug concentration
t = time required to kill a given fraction of cells
N and k are constants.

For example, if n = 5 (as it is for phenol), then doubling the concentration of the drug will reduce the time required to achieve the same extent of inactivation 32-fold. This can be determined by measuring the slope of the line that results when log t is plotted against log C (Jawetz *et al.*, 1982).

2.3 APPROACHES TO RELATE THE GROWTH OR DEATH OF MICROORGANISMS ISOLATED FROM PATIENTS TO ANTIMICROBIAL DRUG CONCENTRATION

2.3.1 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) can be defined as the lowest concentration of an antibiotic that visibly inhibits growth of the initial inoculum *in vitro*. The MIC is determined by a serial twofold dilution of the antibiotic incubated for 24 hours with an inoculum of the organism isolated from a particular patient. The different samples are inspected after 24 hours for visible growth and the MIC is then taken as the lowest concentration of the antibiotic where no visible growth is noted (Ellner and Neu, 1981).

For minimum bactericidal concentration (MBC) determinations an additional incubation step is required. Samples of the dilutions from which MIC were determined are plated onto agar plates and incubated overnight. The antibiotic concentration in the sample from which no growth (99.9% kill) is obtained is taken as the MBC (Schentag *et al.*, 1984).

The MIC value was the first parameter used for the determination of the susceptibility of organisms to a specific antibiotic. Assumptions of susceptibility in relation to concentration soon led to the suggestion that antibiotic concentrations should be maintained at plasma levels exceeding the MIC in order to be effective (Schentag *et al.*, 1984).

At present, efficacy and spectrum of antibiotics are determined by comparing the MIC values for different organisms. The main disadvantage of both the MIC and MBC values is that they are obtained at fixed antibiotic concentrations. These values as such do not allow for the variability in plasma concentrations among different patients. When individualizing a dose for a particular patient according to MIC or MBC values the pharmacokinetic profile of the antibiotic in a particular patient should thus be incorporated in some way.

2.3.2 Serum bactericidal activity (SBA) and serum bacteristatic activity (SBC)

The serum bactericidal activity (SBA) is defined as the greatest dilution (lowest concentration) of a serum sample, obtained while the patient is receiving antibiotic treatment, that kills > 99.9% of an inoculum of the infecting pathogen *in vitro* over 18-24 hours. The serum bacteristatic activity (SBC) is determined in a similar way but inhibition of visible growth is taken as the end point. The results are expressed as dilution titers eg. 1 : 8 or 1 : 16 (Wolfson and Swartz, 1985).

The SBC and SBA values differ from the MIC and MBC values in that the former incorporates the patients antibiotic plasma concentration in the measurement. Measurement of SBA has a number of advantages and disadvantages for monitoring antibiotic therapy:

Advantages

- The test takes into account not only the susceptibility of the infecting organism to the antibiotic but at the same time the patients ability to absorb, distribute, and eliminate the antibiotic
- The test allows for the determination of *in vivo* synergism of simultaneously administered antibiotics
- In patients who are immunocompromised or in patients with infective endocarditis, in which the immuno-phagocytic system is unlikely to exert its full influence, the test may offer an approximation of the contribution of an antibiotic to therapy
- SBA determinations do not require unusual or expensive laboratory equipment.

Disadvantages

- SBA identifies an incorrectly treated patient, but provides no guidance on how to treat correctly. The actual drug concentration is unknown
- A myriad of technical variables can influence measurements (see Wolfson and Swartz, 1985)
- The test does not account for the contribution of the immune system in combating infection (the plasma is inactivated during the test)

- Timing of SBA titer measurements (peak, trough or random) is a matter of controversy.

Various serum antibacterial tests were already in use in the pre-antibiotic era (1912 and 1917) to evaluate the efficacy of quinine derivatives in the treatment of pneumonia. Current use dates from 1947 when SBA was successfully used as a therapeutic guide in the treatment of two patients with infective endocarditis (Wolfson and Swartz, 1985; Drake *et al.*, 1983).

The possible correlation between SBA and the outcome of infection has been investigated by a number of researchers with contradictory results. Wolfson and Swartz (1985) were unable to show any correlation while Mortino *et al.* (1985) and Schieler and Klastersky (1984) reported positive correlations. In the Schieler study the correlation between outcome and SBA in patients with gram negative bacteremia was highly significant ($P < 0.0001$) (Schieler and Klastersky, 1984). A peak SBA of higher than 1:8 for granulocytopenic and higher than 1:16 for immunocompetent patients was associated with a favourable outcome. The results of the Mortino study were similar but indicated that an even higher SBA value of 1:4 may be required in certain granulocytopenic patients.

Determination of the SBA can be seen as a relatively easy method for the prediction of the outcome of infection. A major drawback of SBA estimations is the fact that SBA is a relative value. Neither the actual MIC nor the actual antibiotic plasma concentration is known.

2.3.3 Peak to MIC ratio

The peak to MIC ratio is determined by dividing the peak plasma concentration of the antibiotic by the MIC value (Blaser *et al.*, 1987). According to the literature this value appears to be one of the most useful indicators of successful clinical outcome of infections reported in the literature. Moore *et al.* (1984(a)) showed a 78% success rate for both tobramycin and gentamicin in patients with pneumonia when the peak concentrations were higher than 7μg/mℓ ($p < 0.006$) such peaks were obviously associated with a high peak to MIC ratio. They also showed that the duration of therapy could be shortened if the peak concentration was maintained relatively high. Multivariate regression analysis of their data revealed that peak to MIC ratio was the most significant factor predicting outcome of an infection ($p = 0.01$) followed by age ($p = 0.06$) and underlying disease ($p = 0.09$) (Moore *et al.*, 1984(b)).

A further study by Moore *et al.* (1987) verified the significance of aminoglycoside peak to MIC ratios in predicting successful outcome in patients with gram negative bacteremia.

2.3.4 Serum bactericidal rate (BRT)

Serum bactericidal rate (BRT) refers to the rate of bacterial killing by an antibiotic as measured in a serum medium. Serum allows for protein binding and is a better reflection of the *in vivo* situation due to the fact that organisms also grow slower in a serum than broth medium. Tisdale *et al.* (1989) were the first to advocate BRT as a parameter to distinguish between antibiotics. They derived BRT values from the slope of different killing curves performed at different antibiotic concentrations. These values were then compared to differentiate between the effect of drug concentrations, drug combinations and different antibiotics.

2.3.5 Dynamic response concentration (DRC)

The reason for the slow evolution of dual individualization in antibiotic therapy has been the time lag required to determine quantitative susceptibility. Recently an automated susceptibility testing device the MS-2 Research System was developed (Schentag *et al.*, 1984). The MS-2 Research System generates bacterial profiles in the presence of varying antibiotic concentrations within a relatively short time (6 - 8 hours). A computer interfaced with this system assimilates the data and provides analysis of antibiotic- dependent bacterial growth with reference to the kinetics of uninhibited growth. The antibiotic susceptibility index derived in this way is designated the dynamic response concentration (DRC). Schentag *et al.* (1984) proposed a fundamental model in antibiotic therapy. The steps involved in their proposal for dual individualization are schematically presented in Figure C.3 and can be described as follows:

- The causative organism (blood culture, throat swab, etc) is isolated from each patient and plasma levels are obtained to measure antibiotic levels

- Growth curves of the causative organism in the presence of varying concentrations of antibiotic are then generated by the MS-2 research system and the DRC is calculated

- The pharmacokinetic plasma concentration profile of the specific drug in the patient is calculated

- The DRC and plasma concentration are then combined in a model to calculate the "ideal" dosage regime for the individual (Schentag *et al.*, 1984).

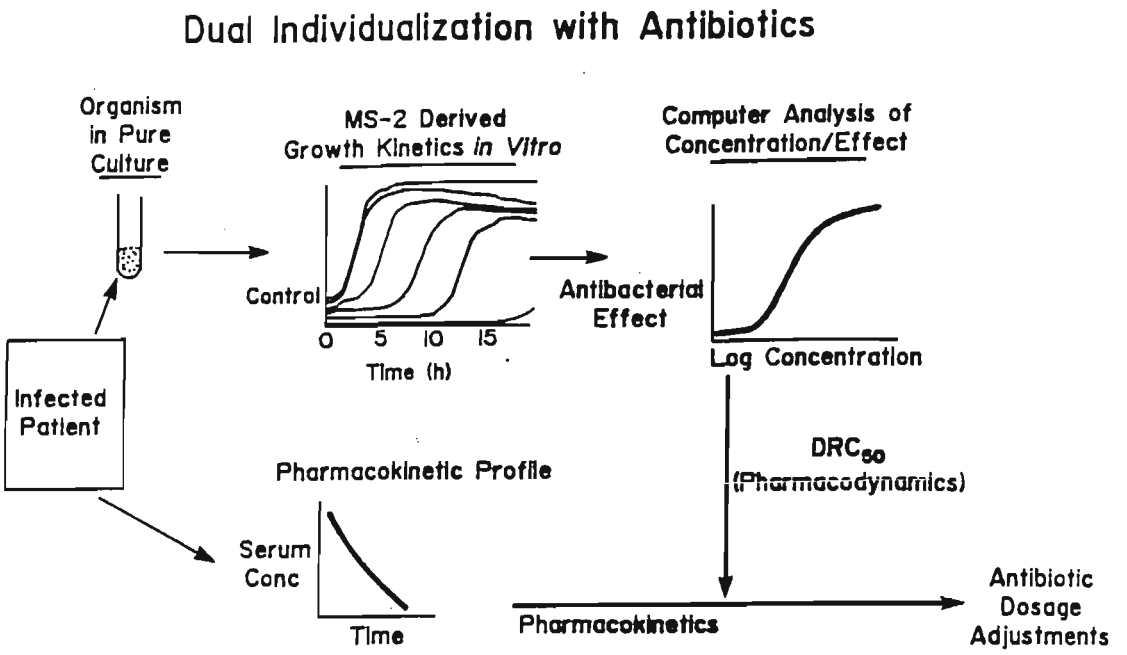


Fig C.3 The steps involved in dual individualization

(Schentag, 1986)

The development and validation of the dual individualization method were performed in two stages. The first stage involved the derivation of a treatment algorithm. Patients with pneumonia who were treated with a fixed dose of cefmenoxime were studied. A series of cefmenoxime blood levels were determined for each patient. Endotracheal aspirates were cultured daily to identify the day of bacterial eradication. The bacterium was inoculated into the MS-2 Research System and a DRC value was calculated. The clinical response, DRC, pharmacokinetic profile and factors influencing the response were then documented and analyzed. By multivariate regression analysis the relationship between DRC and other pharmacokinetic parameters was evaluated. It was shown that

the area under the curve (AUC) above the DRC was a good predictor of the days to bacterial eradication (clinical outcome) ($r = 0.71$).

In the second stage a prospective study was initiated to test the clinical effectiveness of a dosage regimen designed prospectively to achieve a target AUC over DRC (AUC/DRC). The clinical purpose of the prospective study was to eradicate bacteria causing pneumonia within 4 days by adjusting the cefmenoxime dose to produce an AUC/DRC of 140μg.hr.mℓ^{-1} (Schentag *et al.*, 1984). Using this approach it was possible to cluster the eradication time to 4-6 days (5 ± 2.1 days) whereas the average eradication time in the first uncontrolled study was 9 ± 4 days. The difference was statistically significant ($p < 0.05$). Treatment time was significantly shortened ($p < 0.05$) and the correlation between AUC/DRC and eradication time improved over the first study ($r = 0.89$ $p < 0.007$).

For the testing and evaluation of this approach to dual individualization Schentag *et al.* (1984) laid down certain criteria :

- The infection had to be monomicrobial to minimize variances introduced by host defence factors

- Patients had to be on only one antibiotic in order to evaluate the effect of that antibiotic alone. The disadvantage of this criterion is that most neutropenic patients are excluded because they usually receive a number of antibiotics

- In order to exclude another variable drugs without a post-antibiotic effect had to be used

- Samples of the organism had to be readily obtained as for example from tracheal aspirates

- There had to be a wide variety of susceptibility of the organisms to ensure a difference in DRC values for comparisons and calculations.

In their review on dual individualization Schentag *et al.* (1984) stated the need for more research on the subject, particularly with other drugs and other types of infection. They also advocated in depth research on post-antibiotic effect which may be an important factor in the whole process (Schentag *et al.*, 1985).

2.4 PROPOSALS FOR NOVEL APPROACHES TO INVESTIGATE THE PHARMACOKINETIC-PHARMACODYNAMIC LINKS WHICH CAN BE USED FOR DUAL INDIVIDUALIZATION

Utilizing the MS-2 Research System to determine dynamic response concentrations Schentag *et al.* (1984) provided a highly effective approach to dual individualization of antibiotic therapy. A major advantage of this approach is that the actual effect of a specific antibiotic concentration on growth rate is determined. The MS-2 Research System is not available in South Africa. Introduction of the MS-2 Research System into the Baragwanath Hospital complex will be costly. At present it is not a feasible proposition because of a shift in financial priorities towards other more urgently required facilities to improve medical care in a partially Third World situation. In this context the present study aimed to devise alternative methods for the validation of dual individualization. These include the following:

2.4.1 Bactericidal rate (BR)

The concept of bactericidal rate as a measure of killing rate is proposed and is defined as the time required for the bacterial population to decline by 90% or 1 log cycle at the minimum bacteridical drug concentration (MBC). The formulation of the concept bactericidal rate is based upon the following :

- **Decimal reduction time** (Hurwitz and McCartny, 1985; Fung *et al.*, 1988)
  The parameter of decimal reduction time is widely used in the pharmaceutical field for the comparison of the effectiveness of different preservatives and disinfectants. Decimal reduction time is defined as the time required for a particular concentration of a preservative or disinfectant in a particular medium at a specific pH and temperature to cause a 90% reduction in viable organisms.

  The rationale for applying the principle of decimal reduction time to the dual individualization approach is the assumption that each organism is killed at a rate specific for that organism and that this killing rate will influence the dosing regimen for the individual patient.

- **Serum bactericidal rate (BRT)** (Tisdale *et al.*, 1989)

The parameter of BRT has already been discussed in 2.3.4. It refers to the time required by the colony form units to decline by one logarithmic cycle in the serum medium. BRT is calculated from the slope of the regression line of a killing curve performed at a specific antibiotic concentration over a period of time. For the purpose of the present study the killing curve was performed in a broth instead of a serum medium and the BRT determined at the MBC concentration. The MBC was selected because it represents the lowest concentration of an antibiotic which displays killing action *in vitro*.

2.4.2 Concentration at 50% effect

For DRC determinations Schentag *et al.*(1984) used growth rates. An alternative measure of dynamic response which could be considered in dual individualization is the killing rate. The killing rate is obtained by plotting killing curves (fig C.4) for different concentrations of the antibiotic over a specified time period. The EC_{50} value can be defined as that concentration of the drug at which 50% of the effect occurs. An example of killing curves for a specific patient's causative organism at different gentamicin concentrations as performed in the present study can be seen in fig C.4. The EC_{50} values are calculated from a plot of concentration versus % suppression of growth, measured one hour after the lag phase. This time period is chosen to ensure that growth is still in the exponential phase. The suppression of growth for the different drug concentrations (curves B, C and D) relative to the control curve, A, (when no drug was present) is then calculated. The different drug concentrations with their corresponding effects (suppression of growth) are then fitted according to Sigmoid Emax (Hill) or Emax (Langmuir) models. (see 2.1.1). The EC_{50} and Emax values were then derived by non-linear least square regression using the STATIS 2 software (Clyde Soft) (see fig.C.5) (Kelman, 1988).

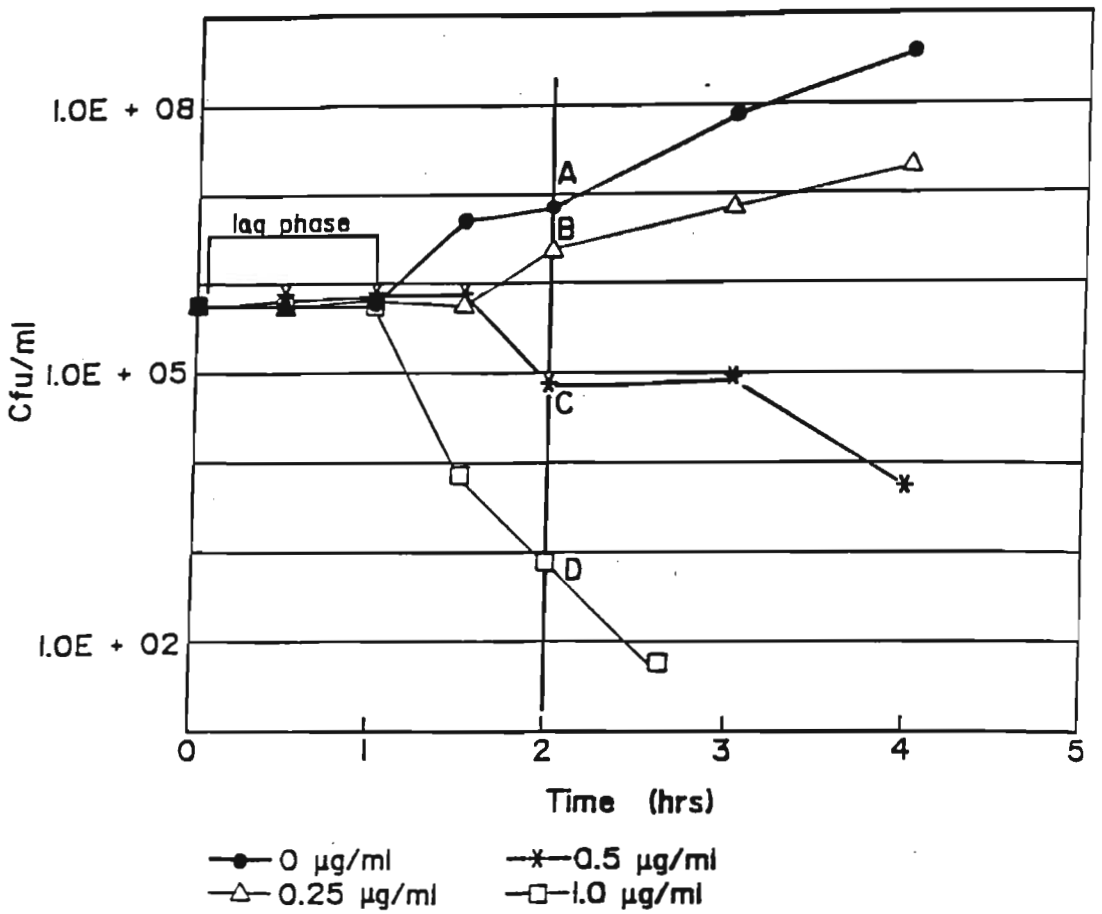


Fig C.4 A typical killing curve from an organism at different drug concentrations over a 4 hour time period

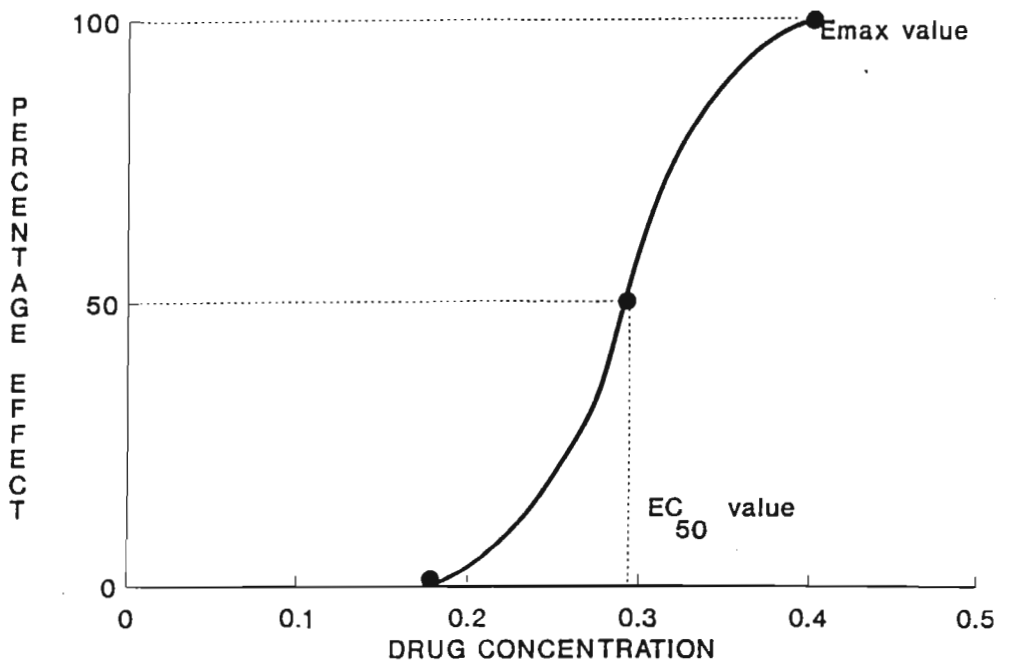


Fig C.5 An example of a drug response curve derived from the killing curve data

2.5 POST-ANTIBIOTIC EFFECT AND LEUCOCYTE ENHANCEMENT

2.5.1 Definitions

Post-antibiotic effect (PAE) is the phenomenon of suppression of bacterial growth that persists after a short exposure of bacteria to certain antimicrobials (Isaaksson, 1988).

It needs to be emphasized that it is the persistent suppression of growth and not an action of exposure to persisting subinhibitory concentrations for a long period of time. Post-antibiotic leucocyte enhancement (PALE) is the enhanced susceptibility of organisms in the PAE phase to the antimicrobial activity of human leucocytes.

2.5.2 History

Bigger *et al.*(1944) were the first to document a delay in turbidity (growth) when penicillinase was added to a culture of streptococci previously exposed to penicillin G. A delay of 1 to 3 hours in normal growth of staphylococci previously exposed to penicillin G was demonstrated by Parker and Mash (1948). The same phenomenon was also documented for gram negative bacteria and antibiotics developed after penicillin (Vogelman and Craig, 1985).

2.5.3 Mechanisms of post-antibiotic effect

(Bundtzen et al., 1981; Vogelman and Craig, 1985)

The precise mechanisms by which antimicrobials induce post-antibiotic suppression of growth are unknown. The observed differences in the PAE of various antibiotics against different organisms suggest that more than one mechanism may be involved. The growth curves of the test and control cultures are however parallel in the post-antibiotic phase which implies that the effect is not due to a population shift to slower growing variants (Vogelman and Craig, 1985).

Two major hypotheses have been proposed to explain the PAE:

- non- lethal damage produced by the antimicrobial or

- limited persistence of the drug at a bacterial binding site.

For antibiotics which inhibit protein or RNA synthesis the PAE represents a period of resynthesis of proteins necessary for intermediary metabolism and unhampered growth of the microorganisms. These antibiotics include erythromycin, tetracyclines and chloramphenicol which are reversibly bound to subunits on ribosomes and the aminoglycosides which are lethally and irreversibly attached to ribosomes. In contrast the β-lactam antibiotics bind to multiple penicillin-binding proteins some of which are enzymes involved in cell wall synthesis. It has been shown that the covalently bound penicillin-enzyme complex can break down thus regenerating active enzyme molecules which may become involved once again in cell wall synthesis. The rate of this process differs from organism to organism. The differences in the PAE of various β-lactam antibiotics can therefore be explained by differences in the rates of antibiotic release and of regeneration of active enzyme molecules after the removal from the drug milieu (Gerber and Craig, 1981).

2.5.4 Quantification of PAE

(Bundtzen *et al.*, 1981)

To determine the PAE, organisms are allowed to grow in a control medium (no drug added) and a test medium containing the antibiotic. After exposure to the antibiotic for a fixed period of time, the drug effect is terminated by a 1000 times dilution. Thereafter the control and test cultures are subjected to identical procedures. Both cultures are allowed to grow and the PAE can then be quantified by the following equation:

$$PAE = T - C \qquad \text{(c.13)}$$

T = time required for the colony formed units (CFU) in the test medium to increase by one log cycle above the count observed immediately after drug removal by dilution.

C = Time required for the CFU in the untreated control culture to increase by 1 log cycle above the count observed immediately after dilution.

2.5.5 Biological significance of PAE

The PAE has a number of biological consequences which may be important in the clinical setting:

- Post- antibiotic leucocyte enhancement (PALE)

  McDonald *et al.* (1981) and Pruul *et al.* (1981) demonstrated that organisms are more susceptible to the antimicrobial activity of human leucocytes in the PAE phase. *S. aureus* and *S. pyogenes* were however more susceptible than *E. coli* to the antimicrobial effect of leucocytes in the PAE phase (McDonald *et al.*, 1981; Pruul *et al.*, 1981) suggesting that gram positive organisms are affected to a larger extent than gram negative organisms.

  The PALE effect was also investigated in the mouse thigh model (Gerber *et al.*, 1983). Organisms pretreated with antibiotics, and therefore in the PAE phase when injected into the thighs of mice were killed faster than untreated organisms injected in the same way. When the same pretreated organisms were injected into neutropenic mice they were not killed and regrowth started as soon as the PAE wore off. In the neutropenic mice the PALE effect, therefore did not manifest itself. By means of the mouse thigh model *in vivo* manifestations of PAE and PALE effects were demonstrated suggesting that they may have clinical implications with respect to the selection of continuous or intermittent dosing regimens of antibiotics (Van der Auwera and Klastersky, 1987).

- Decreased bactericidal activity

  Data are limited but it appears that organisms are less susceptible to the bactericidal activity of the antimicrobial drug during the PAE phase while the susceptibility to leucocyte antimicrobial effects increases. This implies that inhibition of bactericidal activity occurs during the PAE phase. The degree of inhibition of bactericidal activity in the PAE phase is dependent on both the organism and the antimicrobial. In this respect it was shown that gram negative bacteria are more affected than *S.aureus* and that the antibiotics, trimethoprim and β-lactams are subjected to greater inhibition of bactericidal activity than the aminoglycosides (Craig and Gudmundsson, 1986).

  Theoretically this phenomenon may be clinically important. It should be considered when dosage intervals of antibiotics are determined particularly when combinations of antibiotics are used. Further investigation in this field is however still necessary.

- Implications for dosage regimens

  More recent data on PAE for a larger number or antimicrobials and microorganisms suggest that the presence or absence of a PAE may influence the choice of dosing regimens (Craig and Gudmundsson, 1986). From these results of *in vitro* studies it

was postulated that an intermittent dosing regimen may be justified for drugs with a prolonged PAE and a continuous dosing regimen for drugs which lack a PAE. Verification of this postulate was provided in the thigh model of neutropenic and control mice (Craig and Gudmundsson, 1986; Bakker-Woudenberg *et al.*, 1984). In this model the action of drugs with a pronounced PAE (erythromycin and gentamicin) and β-lactams (ampicillin, penicillin and ticarcillin) which lack a PAE against gram negative organisms were investigated against staphyloccus. For erythromycin and gentamicin no major differences were observed between continuous and intermittent dosing regimens in nonneutropenic mice. In contrast for the β-lactam antibiotics the continuous dosing regimen was significantly superior in nonneutropenic mice. In neutropenic mice, however, a continuous dosing regimen was always superior regardless of whether the antibiotic displayed a PAE or not. The role of host defence mechanisms in the eradication of microorganisms was emphasized in these studies (Gerber and Craig, 1981; Gerber *et al.*, 1983).

Data on the possible applications of the PAE concept in human clinical trials are limited. All investigators however, agree that PAE may play an important role in the determination of an "ideal" dosing regimen and particularly in the determination of dosing intervals (Kirby and Craig, 1981; Grafford and Nilsson, 1981). McCormack and Schentag (1987) suggested the following equation for the determination of the ideal dosing interval:

$$\text{Ideal dosing interval} = M + P + T \qquad \text{(c.14)}$$

M = Time from the previous dose during which the plasma concentration was above the MIC or MBC

P = Duration of PAE from previous dose

T = Time required for organism to enter a susceptible log phase.

This equation was derived after studying methicillin treated rabbits with staphylococcal endocarditis. Methicillin displayed a PAE against staphylococci *in vivo* which is in keeping with the finding that the β-lactam antibiotics do produce a PAE with gram positive but not with gram negative organisms. All rabbits received the same dose of methicillin per 24 hours but it was administered according to different dosage regimens namely: 20 mg/kg 4 hourly, 40 mg/kg 8 hourly, 60 mg/kg 12 hourly or continuous infusion. The pharmacokinetic profile of methicillin in each rabbit was monitored. Time above MIC during the dosing interval, peak to MIC ratio, AUC above MIC, AUC above MBC, PAE (duration of the time the serum concentration dropped below MIC

until the beginning of bacterial growth) and log growth time (LGT) were also determined.

The 4 hourly and 8 hourly dosing regimes achieved a significantly higher cure rate than the 12 hourly and continuous dosing regimens ($P < 0.05$). The 12 hourly regimen was the least effective. The finding that the continuous infusion regimen was less effective than the 4 and 8 hourly regimen, stresses the role of a higher susceptibility of organisms to the leucocytes in a PAE period and the validation of PAE as a factor to be considered when dosage regimens are determined.

Considerations of the significance of the various parameters investigated, led to the formulation of the ideal dosing interval equation (C.14).

McCormack and Schentag (1987) extrapolated these data to human dosing regimes for aminoglycosides. The situation that an antibiotic concentration can fall below MIC values is highly unlikely in an *in vivo* situation due to dosing patterns. The PAE effect *in vivo* is partly dependent on host defence mechanisms to increase phagocytosis by leucocytes. They therefore assumed that PAE and LGT are related *in vivo* and that LGT is dependent on host defence mechanisms. They therefore concluded that LGT and PAE can be expressed as one concept to describe PAE *in vivo*. With above assumptions in mind they formulate the following:

$$C\ max = \frac{Cmin}{e^{-keA}} \qquad (c.15)$$

$$C\ min = MIC \cdot [e^{-ke}T] \qquad (c.16)$$

$$LD = Vd \cdot Cmax \qquad (c.17)$$

$$MD = (Cmax - Cmin) \cdot Vd \qquad (c.18)$$

LD = loading dose
MD = maintenance close
A = time above MIC
T = time of PAE

These equations represent a method of applying dual individualization in the calculation of aminoglycoside dosages. McCormack and Schentag performed a number of simulations to look at different MIC's, serum creatinine values, post-antibiotic effects and dosages with the following observations:

- Organisms highly susceptible (MIC's of 1 or smaller) required lower dosages of aminoglycosides

- Organisms with MIC's > 4 μg/mℓ should be considered resistant to aminoglycosides

- In patients with renal impairment low doses of the aminoglycosides every 4 hours represent continuous infusions and that may be nephrotoxic and with the PAE kept in mind, less effective (McCormack and Schentag, 1987).

3 METHODOLOGY

A prospective investigation of *in vitro* gentamicin pharmacodynamic parameters (bacteriological data) and *in vivo* gentamicin pharmacokinetics (pharmacokinetic data) was performed in patients with gram negative bacteremia.

3.1 LOCATION OF STUDY

The study was performed in the medical and paediatric wards of Baragwanath Hospital during 1987 and 1988.

3.2 APPROVAL OF THE STUDY

The study was approved by the Ethics Committees of both Baragwanath Hospital and the University of Durban Westville.

3.3 INFORMED CONSENT

The patients and parents of patients gave informed consent

3.4 STUDY DESIGN

3.4.1 Inclusion criteria

- **Diagnosis**
  Patients with a monomicrobial*E. coli* or *K. pneumoniae* bacteremia confirmed by a positive blood culture were included in the study. Blood cultures were performed by SAIMR and the origin of the gram negative bacteremia was identified by the physician in charge.

- **Antibiotic susceptibility**
  Only strains of *E. coli* and *K. pneumoniae* which were susceptible to gentamicin and resistant to ampicillin were included. This was necessary in order to investigate the relationship between gentamicin and the causative organism.

- **Body temperature**

Only patients with a body temperature of 37.5°C or higher on the first day of gentamicin administration were included in the study. The diagnosis of gram negative bacteremia was confirmed by the physician in charge.

- **Sex**
  Males and females were included in the study.

- **Age**
  No restrictions were placed on age.

3.4.2 DEFINITIONS OF DATA DERIVED FROM MEDICAL RECORDS

- **Route of entry**
  The origin of the infection as documented by the physician.

- **Severity of underlying disease**
  The physician in charge classified patients according to their underlying disease as described by McCabe and Jackson (1962(a)):

  i. Rapidly fatal-disease: This group included patients with leukemia and other forms of cancer with a poor prognosis

  ii. Ultimately fatal - disease: This group included patients with an underlying disease which could possibly become fatal within four years. Aplastic anemia, chronic leukemia and metastatic carcinoma are some of the diseases in this group

  iii. Non-fatal - disease: This group consisted of patients with diabetes mellitus and genitourinary, gastrointestinal or obstetrical diseases.

- **Study populations**
  Two study populations namely those infected with *E. coli* and *K. pneumoniae* were differentiated according to the blood cultures.

- **Normalization of body temperature**
  The time (days) taken for body temperature to normalize to 37°C or less after the initiation of gentamicin therapy was taken as a clinical indication of eradication of the causative organism. Body temperature although a "soft " indication of successful

outcome of therapy was the only parameter which was readily available in this study. White blood cell counts (WBC) have also been documented to reflect the successful outcome of therapy (Moore *et al.*, 1984(b)) but in the present study WBC counts were performed on the day of admission only.

Body temperature was taken at eight o'clock every morning from the day of initiation of gentamicin therapy until the temperature was back to normal ($< 37°C$).

- **Community-acquired and nosocomial infections**
  When the patient was admitted with an infection (blood culture positive within the first 72 hours of admission) the infection was recorded as community acquired.

  Nosocomial infections were all those contracted in hospital.

3.4.3 INVESTIGATIONS ROUTINELY PERFORMED ON ADMISSION

- Full blood count (including platelet count and differential white cell count)
- Blood urea and electrolyte levels
- Blood cultures
- Serum creatinine levels.

Blood samples were collected on the day of admission for all the above examinations except gentamicin plasma levels and serum creatinine levels which were performed on the day of initiation of gentamicin therapy.

3.4.5 GENTAMICIN ADMINISTRATION AND SAMPLING

Blood samples for the determination of gentamicin concentration levels were only collected at the request of the attending physician usually when toxicity was suspected. In most instances both trough and peak level samples were collected. The samples (5 ml of blood from adults and 2 ml from neonates) were only collected after steady state blood levels had apparently been achieved i.e after four or more doses of gentamicin had been administered.

3.5. ASSAYS

The blood samples collected from individual patients with suspected bacteremia were routinely sent to the Microbiology Laboratory of the SAIMR for organism identification and sensitivity testing.

Organisms isolated from the first positive blood culture of each patient were inoculated into a semi-solid Mueller-Hinton broth,incubated for 24 hours and stored (3 to 6 months) for further investigations. **All further microbial assays were performed by the investigator.** (See Appendix B).

The following assays were performed:

- Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)
- Serum bacteristatic activity (SBC) and serum bactericidal activity (SBA)
- Time kill curves
- Post antibiotic effect (PAE).

3.5.1 Gentamicin serum levels

The Gentamicin serum levels were determined by the EMIT method discussed in section B.

3.6 STATISTICAL ANALYSES

3.6.1 Student-t test

The student-t test of the BMDP programme was used to determine if any difference existed between the means of PAE, BR and EC_{50} values in the two study populations namely patients with *E. coli* and *K. pneumoniae* bacteremia, respectively (Hill, 1987).

3.6.2 Log linear regression

The STATIS 2 program (Clyde Soft, 1987) was used to perform log linear regressions on the decline data of the MBC concentration killing curve to determine the bactericidal rate constants (Kelman, 1988).

3.6.3 Multiple linear regression

Multiple linear regression was performed using the BMDP programme supplied by the statistical department of the Potchefstroom University (Hill, 1987).

3.6.4 Pharmacodynamic modeling

The STATIS 2 (nonlinear regression model) program (Clyde Soft, 1987), was used to analyse the data. The data derived from the killing curves were fitted to the Hill and Langmuir sigmoid E_{max} model and E_{max} model respectively (Kelman, 1988).

3.6.5 Calculation of the area under concentration versus time curve (AUC) for gentamicin

A model independent method for the calculation of the AUC for aminoglycosides when a few data points are available was used in the present study (Gibaldi and Perrier, 1982):

$$AUC = \left(\frac{C_1 + C_2}{2}\right)\Delta t + \left(\frac{C_2 - C_3}{2}\right) te \qquad (c.19)$$

C_1 and C_3 = trough concentrations at steady state

C_2 = peak concentration

Δt = time of infusion

te = time from peak to next trough

4. RESULTS

4.1 CLINICAL PROFILES

The clinical profiles of the patients admitted to the study are summarized in table C.1.

4.1.1 Number of patients, age and sex

Fifteen patients were included in the study, eight females and seven males, of which four (3 males and 1 female) were infants under the age of one year. The average age of the adults was 43.6 ± 18.2 years with the youngest and the eldest being respectively 21 and 70 years old.

4.1.2 Type of bacteremia, route of entry and severity of underlying disease

All the patients had a gram negative bacteremia confirmed by a positive blood culture. *K. pneumoniae* and *E. coli* were the causative pathogens in respectively eight and seven of the patients admitted.

Four females and four males had *K. pneumoniae* bacteremia. The *K. pneumoniae* group consisted of 5 patients with pneumonia, an infant with diarrhea and a scalp abscess and two adults with hepatitis and carcinoma of the prostate respectively. One of the adults and three of the infants contracted the klebsiella bacteremia in hospital. None of the patients in the *E. coli* bacteremia group had a nosocomial infection. The *E. coli* group consisted of an infant with diarrhea, four adults with genitourinary tract infections, one adult with a septic abortion and one diabetic patient. Only one patient in the study group had a disease classified as rapidly fatal according to the McCabe and Jackson classification (3.4.2).

4.1.3 Days to bacterial eradication

Normalization of body temperature was taken as an indication of bacterial eradication in the present study. The average body temperature on the day of initiation of gentamicin therapy was 38.7 ± 0.5°C. (Range 37.9 - 40.0°C.) Eradication of the infection was assumed to occur on the day when temperature had returned to 37.0°C or less. The average days to eradication was 2.67 ± 0.9 days (Range 1 - 4 days.).

TABLE C.1

Clinical profiles from patients in study population

ID	Route of infection	Diagnosis	Severity of underlying disease	Bacterium	Age (years)	Sex	C/N	Na^+ Meq/ℓ	K^+ Meq/ℓ	Cl^- Meq/ℓ	Urea μmol/ℓ	Scr μmol/ℓ	WBC 10^3cells mm^2	Temp °C
1	lung	Pneumonia	NF	K. pneum.	32	M	C	125	3.5	87	7.3	105	18.1	37.9
2	GUT	Prostatic carcinoma	RF	K. pneum.	63	M	N	129	4.4	92	9.6	93	19.1	38.9
3	GIT	Scalp abscess Diarrhea	NF	K. pneum.	0.42	M	N	124	2.7	98	6.9	88	18.2	38.5
4	GIT	Hepatitis	NF	K. pneum.	57	F	C	141	2.8	108	4.0	79	14	38.4
5	lung	Pneumonia	NF	K. pneum.	0.33	F	N	135	5.5	109	6.0	73	12	38.3
6	lung	Pneumonia	NF	K. pneum.	31	F	C	132	4.2	104	8.3	187	14.8	39.2
7	GIT lung	Diarrhea Pneumonia	NF	K. pneum.	0.75	M	N	133	4.5	106	4.1	60	17.1	38.3
8	lung	Pneumonia	NF	K. pneum.	29	F	C	132	2.8	96	2.9	103	11.2	39.0
9	GIT	Diarrhea	NF	E. coli	0.67	M	C	125	4.9	94	1.7	51	19.9	38.1
10	GUT	Urinary tract infection	NF	E. coli	21	F	C	128	3.4	92	12.2	95	13.6	38.0
11	GUT	Urinary tract infection	NF	E. coli	45	M	C	141	3.2	104	7.6	116	15.0	39.2
12	GIT	Abortion	NF	E. coli	44	F	C	135	3.4	110	3.9	115	20.7	38.7
13	GUT	Urinary tract infection	NF	E. coli	70	F	C	140	2.2	98	5.7	105	8.2	40.0
14	GUT	Urinary tract infection	NF	E. coli	21	M	C	126	4.3	96	3.3	60	10.4	39.1
15	GIT	Diabetic	NF	E. coli	67	F	C	135	3.8	97	3.8	72	6.9	38.6

GUT	Genitourinary tract	GIT	Gastrointestinal tract
NF	Non-fatal	RF	Rapidly fatal
M	Male	F	Female
C	Community acquired	N	Nosocomial
Na	Sodium	K	Potassium
Cl	Chloride	Scr	Serum creatinine value

4.2 GENTAMICIN PHARMACOKINETIC DATA

The pharmacokinetic data collected for the study population are presented in table C.2. Dosages and routes of administration of gentamicin varied for individual patients and can be summarized as follows:

- The four infants received intravenous (IV) dosages ranging from 12.5-25 mg gentamicin 8 hourly. The average trough and peak gentamicin levels in this group were respectively 1.2 μg/mℓ and 6.9 μg/mℓ respectively.

- Eight adults received intramuscular (IM) dosages of 80 mg gentamicin 8 hourly resulting in an average trough level of 0.6 μg/mℓ and and average peak level of 5.3 μg/ℓ.

- Two adults received intramuscular (IM) dosages of 60 and 80 mg gentamicin 12 hourly and one a dose of 120mg 8 hourly.

- The average AUC for the 15 patients was 22.71 μg.hr.mℓ^{-1} $\pm$ 12.26 (Range 11.59 - 58.96 μg.hr.mℓ^{-1}

TABLE C.2

Gentamicin pharmacokinetic data collected from study population

Patients	Dose (mg)	Dosing interval (hrs)	Route of administration (IM/IV)	Gentamicin conc. ($\mu g/m\ell$)		AUC $\mu g.hr.\ m\ell^{-1}$
				Trough	Peak	
1	80	8	IM	0.4	6.4	18.6
2	80	8	IM	0.9	3.5	15.0
3	12.5	8	IV	0.5	5.7	18.0
4	80	8	IM	0.2	4.3	11.59
5	20	8	IV	1.6	10.6	39.43
6	60	12	IM	0.1	4.4	14.86
7	15	8	IV	1.4	5.5	24.71
8	120	8	IM	1.1	3.7	15.88
9	25	8	IV	1.4	5.8	25.60
10	80	8	IM	0.4	4.5	14.30
11	80	8	IM	0.9	6.3	23.02
12	80	8	IM	0.4	5.1	15.66
13	80	12	IM	2.4	8.5	58.96
14	80	8	IM	0.4	6.0	17.67
15	80	8	IM	1.5	6.2	27.35

IM Intramuscular
conc Concentration
IV Intravenous
AUC Area under curve

4.3 MIC, MBC, SBC and SBA

The MIC, MBC, SBC and SBA values obtained for organisms isolated from the individual patients are summarized in table C.3. The MBC values were identical for all organisms isolated (1 μg/mℓ) and the MIC values were either 0.5 or 1 μg/mℓ. The SBC values varied from 1:4 to 1:64 with 1:8 being the most common. SBA values varied from 1:4 to 1:32 with 1:8 once again the most common.

TABLE C.3

Minumum inhibitory concentrations (MIC), minimum bacterial concentrations (MBC), serum bacteristatic activity (SBC) and serum bactericidal activity (SBA) in the study population

ID	Organism	MIC (μg/mℓ	MBC (μg/mℓ)	SBC	SBA
1	K. pneum.	0.5	1	1:64	1:32
2	K. pneum.	0.5	1	1:4	1:4
3	K. pneum.	1	1	1:8	1:8
4	K. pneum.	0.5	1	1:8	1:8
5	K. pneum.	0.5	1	1:64	1:32
6	K. pneum.	1	1	1:4	1:4
7	K. pneum.	0.5	1	1:16	1:16
8	K. pneum.	1	1	1:8	1:8
9	E. coli	0.5	1	1:16	1:16
10	E. coli	1	1	1:8	1:8
11	E. coli	1	1	1:8	1:8
12	E. coli	1	1	1:8	1:4
13	E. coli	0.5	1	1:16	1:16
14	E. coli	0.5	1	1:16	1:8
15	E. coli	0.5	1	1:16	1:16

4.4 EC_{50} VALUES

The data and graphs from which the EC_{50} values were constructed are presented in Appendix C. The different models used to calculate individual EC_{50} values, and corresponding r^2 values are displayed in table C.4. EC_{50} values for the *K. pneumoniae* (average 0.334± 0.1) and *E. coli* group (0.369 ±0.15) did not differ significantly (p = 0.593). Using the statistical experimental design programme of the STATIS package, a type 1 error of 0.05 and power of 0.8, it was calculated that at least 37 patients in each study group will be required to detect a 20% statistical difference.

TABLE C.4

EC_{50} values from the study population. (MODELS: 1 = Hill 2 = Langmuir)

Patients	Drug concentration (μg/mℓ)	Suppression of growth (%)		r^2	EC_{50} (μg/mℓ)	Number of model
		Curve 1	Curve 2			
1	0.25 0.5 1	24 78 99	11 59 99	0.96	0.40	1
2	0.25 0.5 1	66 99 99	65 99 99	0.82	0.20	2
3	0.25 0.5 1	32 90 99	17 88 99	0.98	0.30	1
4	0.25 0.5 1	59 92 99	73 93 99	0.98	0.32	1
5	0.25 0.5 1 1.5	23 66 98 99	55 75 95 99	0.98	0.34	1
6	0.25 0.5 1	13 58 99	22 42 99	0.86	0.53	2
7	0.25 0.5 0.75 1	38 88 96 99	44 92 98 99	0.99	0.27	1
8	0.25 0.5 1	66 99 99	77 99 99	0.79	0.31	2
9	0.5 0.75 1	83 99 99	68 90 99	0.77	0.23	2
10	0.5 1 1.5	21 98 99	19 99 99	0.99	0.64	1
11	0.5 0.75 1	79 95 99	89 98 99	0.83	0.33	1
12	0.5 1 1.5	61 72 99	64 76 99	0.87	0.46	2
13	0.5 0.75 1	96 99 99	90 99 99	0.70	0.29	1
14	0.5 0.75 1	90 95 99	89 92 99	0.76	0.4	2
15	0.5 0.75 1	84 99 99	68 90 99	0.97	0.23	1

4.5 BACTERICIDAL RATE

The data from which the BR values were derived, the r^2values of the regression lines and the BR values are summarized in table C.5. BR values varied from 0.27 to 1.8 hr with no statistical difference between the *K. pneumoniae* (0.47±0.22) and *E. coli* (0.76±0.49) groups (P = 0.16).

TABLE C.5 Bactericidal rate (BR) data from study population

Patients	DATA VALUES			r^2	BR (hrs)
	Time	Sample 1	Sample 2		
1	60 90 120	5.59 2.40 2.18	5.59 2.93 2.30	0.93	0.27
2	60 90 120	4.53 3.62 2.73	4.60 3.71 2.70	0.99	0.54
3	60 90 120	5.82 5.70 3.18	5.85 5.60 3.30	0.99	0.39
4	60 90 120	5.18 2.88 2.92	5.60 3.04 2.30	0.82	0.29
5	60 90 120	5.18 4.15 3.04	5.56 4.30 3.44	0.97	0.46
6	90 120 180 240	6.13 5.08 4.15 3.26	6.00 4.95 4.00 3.54	0.94	0.91
7	60 90 120	5.62 4.74 2.60	5.62 3.48 2.4	0.93	0.30
8	60 90 120 180	5.65 4.48 4.16 2.5	5.69 4.56 4.07 2.3	0.98	0.62
9	60 120 180	4.7 3.4 2.0	4.65 3.48 2.18	0.99	0.77
10	120 180 240	5.33 4.60 3.24	5.30 3.68 2.78	0.92	0.80
11	90 120 180 240	4.05 3.51 2.95 2.66	4.00 3.48 2.90 2.70	0.93	1.8
12	120 180 240	6.00 3.83 2.71	6.00 4.52 3.14	0.95	0.62
13	60 90 120	4.60 3.40 2.10	4.48 3.48 2.30	0.99	0.42
14	90 120 150 180	4.74 4.34 3.50 2.60	4.85 4.44 3.48 2.63	0.93	0.53
15	60 90 120 150	5.40 4.86 2.45 1.6?	5.18 4.85 2.30 1.78	0.91	0.35

4.6 POST-ANTIBIOTIC EFFECT

The data and graphs from which the duration of post-antibiotic effect was calculated are included in Appendix D and summarized in table C.6.

The duration of the PAE for *E. coli* and for *K. pneumoniae* strains did not differ significantly. After one hour's exposure to gentamicin the duration of PAE for *K. pneumoniae* was 0.64 ±0.21 hr and for *E. coli* 0.82 ±0.27 hr (p = 0.8). The same held for the two hour exposure PAE with values for *K. pneumoniae* being 1.25 ± 0.43 hr and *E. coli* 1.18 ± 0.29 hr (p = 0.773) respectively.

For each organism however a longer exposure time was associated with a significant difference in PAE. For *K. pneumoniae* the PAE after one hour's exposure to gentamicin (0.64 ± 0.21 hr) differed significantly (p = 0.0028) from the PAE after two hours exposure (1.25 ± 0.43 hours). The same held for *E. coli* where the PAE after one hour's exposure (0.82 ± 0.27 hr) was significantly shorter (p = 0.0337) than the PAE after two hour's exposure (1.18 ± 0.29 hr).

TABLE C.6

The post-antibiotic effect data from the study population

Patients	Organism	Post antibiotic effect (hr)	
		1 hr exposure	2 hr exposure
1	K. pneum.	0.75	0.83
2	K. pneum.	0.61	1.48
3	K. pneum.	0.93	1.48
4	K. pneum.	0.46	1.65
5	K. pneum.	0.53	0.95
6	K. pneum.	0.50	1.28
7	K. pneum.	0.38	0.55
8	K. pneum.	0.93	1.77
9	E. coli	0.48	0.75
10	E. coli	0.91	1.20
11	E. coli	1.10	1.35
12	E. coli	0.63	1.31
13	E. coli	1.00	0.85
14	E. coli	0.51	1.60
15	E. coli	1.1	1.21

4.7 CLINICAL OUTCOME

The parameters (variables) investigated in the present study regressed against days to normalization of temperature (independent variable) as an indication of clinical outcome are summarized in table C.7(A) and C.7(B). The results of the multiple linear regression analyses on the data are shown in table C.8.

When only one variable was introduced SBA gave the best correlation (r^2 = 0.54 ; p = 0.002) followed by AUC/EC_{50} (r^2 = 0.39; p = 0.02) and peak drug concentration (r^2 = 0.38; p = 0.02). When a second variable was introduced the combination of SBA and EC_{50} gave the best correlation (r^2 = 0.78 ; p = 0.0001) followed by SBA and AUC/ EC_{50} (r^2 = 0.66 ; p = 0.0024) and finally SBC and EC_{50} (r^2 = 0.68; p = 0.001). A regression of SBA and EC_{50} with a third variable, either BR or PAE gave a r^2 = 0.85 and r^2 = 0.84 respectively. When more than four variables were regressed no further improvement of the p value occurred: SBA + EC_{50} + BR + PAE (r^2 = 0.90 and P = 0.001).

TABLE C.7(B)

Different variables regressed against time to normalization in body temperature

Patients	PAE_1 (hr.)	PAE_2 (hr.)	EC_{50} ($\mu g/m\ell$)	SBC	SBA	AUC/EC_{50} ($\mu g.hr.m\ell^{-1}$ / $\mu g/m\ell$)	Peak/MIC ($\mu g/m\ell$ / $\mu g/m\ell$)	AUC/MIC ($\mu g.hr.m\ell^{-1}$ / $\mu g/m\ell$)	AUC/MBC ($\mu g.hr.m\ell^{-1}$ / $\mu g/m\ell$)
1	0.75	0.83	0.4	1:64	1:32	18.6	12.8	17.79	13.69
2	0.61	1.48	0.2	1:4	1:4	21.9	7.0	19.56	12.18
3	0.93	1.48	0.3	1:8	1:8	19.63	5.7	14.27	14.27
4	0.46	1.65	0.32	1:8	1:8	10.53	8.6	9.34	6.07
5	0.53	0.95	0.34	1:64	1:32	50.13	9.40	48.72	44.35
6	0.50	1.28	0.53	1:4	1:4	11.68	4.40	8.60	8.60
7	0.38	0.55	0.27	1:16	1:16	34.88	11.2	32.68	28.03
8	0.93	1.77	0.31	1:8	1:8	23.26	3.2	16.68	16.68
9	0.48	0.75	0.23	1:16	1:16	36.88	11.6	33.56	29.00
10	0.91	1.2	0.64	1:8	1:8	12.43	4.5	9.86	9.86
11	1.10	1.35	0.33	1:8	1:8	27.84	6.3	22.13	22.13
12	0.63	1.31	0.46	1:8	1:4	14.87	5.1	11.22	11.22
13	1.0	0.85	0.29	1:16	1:16	78.64	17.0	77.28	71.94
14	0.51	1.60	0.4	1:16	1:8	17.67	12.0	16.89	13.17
15	1.1	1.21	0.23	1:16	1:16	38.59	12.4	36.20	31.58

PAE_1 Post antibiotic effect after 1 hour

PAE_2 Post antibiotic effect after 2 hours

SBC Serum bacteristatic concentration

SBA Serum bactericidal activity

AUC/EC_{50} Area under concentration curve **above** Concentration at 50% effect

Peak/MIC Peak **divided by** MIC

AUC/MIC Area under concentration curve **above** Minimum inhibitory concentration

AUC/MBC Area under concentration curve **above** Minimum bactericidal concentration

TABLE C.8

Summary of variables and r^2 values obtained with multiple regression

Dependent variable	r^2	P
SBA	0.54	0.002
AUC/EC_{50}	0.39	0.02
Peak gentamicin concentration	0.38	0.02
SBA + EC_{50}	0.78	0.0001
AUC/EC_{50} + SBA	0.66	0.0024
SBC + EC_{50}	0.68	0.001
SBA + EC_{50} + BR	0.85	0.0001
SBA + EC_{50} + PAE	0.84	0.0001
SBA + EC_{50} + MBC	0.83	0.0002
SBA + EC_{50} + BR + PAE	0.90	0.0001
SBA + EC_{50} + BR + MBC	0.86	0.0003

5. DISCUSSION

When comparing the clinical results of this small group of 8 patients, with *K. pneumoniae* and 7 patients with an *E. coli* bacteremia with those reported in the literature and in Section A (another group of patients from Baragwanath Hospital), the following similarities and differences were noted:

- In the small group studied in this section (15 patients), the aim was to investigate differences in microbial parameters relating to *E. coli* and *K. pneumoniae* bacteremia. Patients were selected such that approximately equal numbers with *E. coli* and *K. pneumoniae* were entered into the study and as a result these numbers do not reflect the true incidence of bacteremias associated with the two respective causative pathogens. From the literature and the results reported in Section A it would appear that *E. coli* is the most common cause of gram negative bacteremia and *K. pneumoniae* is the second most common causative organism.

- Four (50%) of the patients with *K. pneumoniae* bacteremias acquired their infections while in hospital, in five of them (63%) the infection originated in the lungs. These findings are consistent with literature reports of *K. pneumoniae* as a common cause of hospital acquired bacterial pneumonia (Montgomery, 1979).

- The present investigation confirms reports (Haddy *et al.*, 1987) that the genitourinary tract is the most common source of *E. coli* bacteremia (four out of seven patients in this study) and that *E. coli* infections are usually community acquired (all seven patients).

- No deaths were associated with gram negative bacteremia in the present study compared with the mortality rate of 32% reported in section A. Possible reasons are the following:

    - According to the criteria of McCabe and Jackson (1962(a)), only one patient namely the patient with cancer of the prostate could be considered as having a rapidly fatal underlying disease. Such patients often have an impaired immune system or multiple infections making them a high fatality risk group. The present study was designed in such a way that patients with multiple infections were excluded.

- Patients were only included in the study if they were still alive when the first positive blood culture results were obtained. A blood culture took 24 to 72 hours to be performed, the patients who died within the first 24 hours of onset of bacteremia were automatically excluded. Kreger *et al.*, (1980(a) and 1980(b)) reported that 40 to 50% of patients with ultimately fatal or non-fatal diseases died, usually from shock within the first 24 hours of contracting gram negative bacteremia.

- In the present study only three patients were older than 50 years. In patients with gram negative bacteremia higher mortality rates appear to be associated with older age. Mortality rates of 62% and 38% have been reported in patients respectively older than 50 years and younger (McCue, 1987; Kreger *et al.*, 1980(b)).

The objective of this study was to determine whether a correlation existed between clinical outcome, pharmacokinetic and microbiological variables. The time for body temperature to normalize was taken as a reflection of the eradication of the causative organism and thus the outcome of gram negative bacteremia. The reliance in the present study on the normalization of body temperature as the only indicator of bacterial eradication is not entirely satisfactory. However, the present study was performed on routinely collected data and body temperature was the only indicator that was regularly recorded. Other indicators which may be considered in future studies are decreases in WBC counts, eradication of bacteria from the site of origin and eradication of bacteria from the blood stream (Schentag *et al.*, 1984 ; Moore *et al,*. 1987 ; Nix *et al.*, 1987). A correlation appears to exist between the pharmacokinetic parameters (peak serum gentamicin concentration and AUC) and normalization of body temperature. In the patient with the highest peak gentamicin level (10.6 μg/mℓ and largest AUC (47.0 μg.hr.mℓ^{-1}) body temperature normalized after 1 day of therapy. In the three patients in whom body temperature took longer than 4 days to normalize peak gentamicin levels were lower than 5.5 μg/mℓ and the AUC values less than 20 μg.hr.mℓ^{-1} In two of these patients gentamicin therapy was changed to other antibiotics. Peak gentamicin levels of 6μg/mℓ are advocated for the treatment of gram negative bacteremia (Moore *et al.*, 1984(a)). It would therefore appear that the three patients in whom body temperature took longer than four days to normalize, were underdosed. Dosage adjustments in these patients might therefore have obviated the need to change to other antibiotics.

A peak level of 6 μg/mℓ or higher is advocated for the best results in treating gram negative bacterial infections (Moore *et al.*, 1987). Moore *et al.*, (1987) reported a highly

significant correlation ($p = 0.00001$) between a high peak gentamicin level/MIC ratio and clinical response.

With the varied dosing regimens in the present study, peak levels of gentamicin ranged from 3.2 μg/mℓ to 10.6 μg/mℓ and only 6 out of 15 patients had peak gentamicin levels exceeding 6 μg/mℓ. Only one patient had a trough level of higher than 2 μg/mℓ which is normally associated with toxicity. In view of favourable clinical responses and the lack of toxic manifestations the clinicians decided to maintain the dosage schedules after the results of gentamicin plasma levels became available. Dosages were not adjusted to increase the peak plasma levels.

From the investigations of the sensitivity of causative organisms to gentamicin (MIC, MBC, SBC and SBA determinations) it would appear that the individual strains did not differ significantly. In all instances MBC values of 1 μg/mℓ and MIC values were either 0.5 or 1 μg/mℓ were obtained.

SBC and SBA are both dimensionless values that incorporate the peak gentamicin concentration and MIC or MBC value. These values displayed a more marked variation than the MIC or MBC values. Where all the MIC and MBC values were either 1 or 0.5 μg/.ℓ the SBA values varied from 1 : 4 to 1 : 32. A SBA equal to 1:8 or higher has been reported to be associated with a better outcome of infections (Scielier and Klastersky, 1984; Mortino *et al.*, 1985). Only three patients in this study had SBA values of less than 1:8. Although the peak gentamicin concentration in this study was generally lower than that recommended for the treatment of gram negative bacterial infections, the SBA values were mostly within the range associated with a favourable outcome of infections. These findings accentuate the role of multiple factors contributing to the successful outcome of antibiotic therapy. Furthermore it would appear that if the organisms are highly susceptible to the drug a smaller dose of the drug may be used.

EC_{50} and BR values reflecting the rate of killing of organisms, did not differ significantly for *E. coli* and *K. pneumoniae* strains. The lack of variation in the susceptibility of these organisms to gentamicin may account for this finding. When investigating parameters which may be used in dual individualization, significance more readily manifests if a large variability in the susceptibility of organisms and pharmacokinetics of the drug is present in the test population (Schentag *et al.*, 1984).

The observation that the BR values varied from 0.27 - 1.8 hr in the study population is, however, interesting. The BR values were calculated from the MBC regression lines at

constant MBC values of 1μg/mℓ. This observation confirms the suggestion (Schentag *et al,*. 1984) that individual organisms when exposed to an antibiotic can display different growth or killing rates which are not reflected in the more static MIC and MBC values, (which depend on concentration only).

The differences in PAE of *K. pneumoniae* and *E. coli* after both 1 and 2 hours of exposure to the MIC of gentamicin were not significant. For the same organism, however, the PAE was significantly longer after a 2 hour than after a 1 hour exposure ($p = 0.0337$ and $p = 0.0028$ respectively for *E. coli* and *K. pneumoniae*). It is difficult to compare the PAE values obtained in the present study to PAE values reported in the literature. PAE values reported in the literature for gram negative organisms were usually obtained from animal studies and were determined at antibiotic concentrations of 4 to 5 times the MIC. Based upon research in experimental animals McCormack and Schentag(1987) reported a PAE for aminoglycosides of 2 to 4 hours in *E. coli* strains. In another study of *E. coli* strains a PAE of 1.8 hours was reported for gentamicin at a concentration of 2 μg/mℓ (Bundtzen *et al.*, 1981). The average PAE of gentamicin at the MIC of 0.5 μg/mℓ in *E. coli* strains (0.82 $\pm$ 0.27 hrs) in the present study therefore appears to be in agreement with the results reported by these investigators. The present study where the PAE was determined at the MIC of gentamicin may furthermore be more representative of the clinical setting.

It has also been reported that the PAE increases with prolongation of antibiotic exposure time until a maximum of 4 to 5 hours (McCormack and Schentag, 1987). This observation was confirmed in this study with the statistically significant difference observed between the 1 and 2 hour exposure times for both *K. pneumoniae* and *E. coli.* The increase in the duration of the PAE after 2 hour exposure was greater for *K. pneumoniae* than *E. coli* although it did not reach statistical significance. This would imply that in the treatment of *K. pneumoniae* infections it may be possible to administered gentamicin at longer dosing intervals than with *E. coli* infections.

Furthermore it has been reported that the PAE increases with increasing concentrations of the antibiotic up to a concentration of 6-8 times the MIC (Craig and Gudmundsson, 1986). The effect of different concentrations of gentamicin on the PAE was, however not investigated in the present study.

The objective of this study was to devise dynamic pharmacodynamic parameters which can possibly be incorporated with pharmacokinetic parameters in the dual individualization approach to gentamicin therapy in patients with gram negative

bacteremia. For the validation of these parameters, they had to be tested against the clinical response to gentamicin in patients with gram negative bacteremia. Clinical response was measured as the time to normalization of body temperature. Multiple linear regression was performed on the data. The analyses were carried out in a stepwise logistic way, introducing one variable at a time (see table C.8) When only one variable was regressed the use of SBA gave the best correlation followed by AUC/EC_{50} value The role of SBA as a predictor of outcome is well documented. Both Wolfson and Swartz (1987) and Mortino *et al.*, (1985) associated a SBA of more than 1:8 with a favourable outcome in patients with a normal and a SBA of more than 1:16 in patients with an impaired immune system. In the present study no mortalities were reported, all the patients had a normal immune system and in only three patients was the SBA lower than 1:8.These findings provide support for the role of SBA as an indicator of clinical outcome.

In a study by Schentag *et al.* (1984) on dual individualization the AUC/DRC provided the best correlation ($r^2 = 0.50$) in the prediction of outcome. In the present study the paramcter that closely resembles the DRC value was the EC_{50} value which also refers to killing rate. The AUC/EC_{50} parameter in the present study however only gave the second best correlation (table C.8). The only explanation for the inability of EC_{50} values to perform better may be the lack of variation in susceptibility of the organisms in the present study. Furthermore Schentag *et al.*,(1984) included patients with renal impairment which accounted for a wide variation in AUC/EC_{50} values.

The peak to MIC ratio in the present study failed to produce a good correlation with outcome ($p = 0.02$). In the literature, however , a good correlation between peak/MIC ratio and outcome has been documented ($p = 0.0001$) (Moore *et al.* 1987). A possible explanation for the mediocre correlation in the present study is the lack in variation of MIC values (Blaser *et al.*, 1987).

The correlation improved markedly when a second variable was introduced.The EC_{50} value was the most prominent variable and featured in 3 of the best correlations with two variables: SBA and EC_{50} ; SBC and EC_{50} and SBA and AUC/EC_{50}). These findings accentuate the importance of the introduction of a dynamic measurement of bacterial killing (EC_{50}) in the prediction of the clinical outcome of gram negative bacteremia. The AUC/EC_{50} and SBA parameter ($p = 0.0024$) did not perform better than EC_{50} and SBA parameter alone ($p = 0.0001$). AUC has been advocated as a good indicator of outcome in a number of literature reports (Moore *et al,*. 1987 ; Schentag *et al,*. 1984). The discrepancy between these findings and the results of the present study can once again

be explained by the homogenous nature of the MIC of gentamicin and of the renal status in the population investigated. (Only one patient show a degree of renal failure.)

With the introduction of a third variable SBA and EC_{50} remained important and both BR and PAE (1hr) improved the fit markedly ($p = 0.0001$ in both the equations). BR is a measurement of killing rate and PAE gives an indication of the time period during which killing persists. The importance of including killing rate can once more be seen. When a fourth variable was regressed the combination of EC_{50} + SBA + BR + PAE performed the best. These 4 variables can therefore be regarded as optimal because further inclusion of variables did not improve the fit further (With 5 variables the p value increased to 0.0003). The following equation can be derived from the 4 variables in the optimum fit and be used to predict the outcome of bacteremia most accurately in the population studied:

Days to eradication = 2.91148 - 0.507014 BR - 0.810509 PAE (1hr) + 4.2964 $9EC_{50}$ - 0.0641010 SBA (c.20)

The importance of a dynamic indication of bacterial killing becomes apparent if it is taken into consideration that 3 of these 4 variables refer to bacterial killing rate over time (BR, PAE (1hr) and EC_{50}). Another interesting observation with these four variables is the similarities between them and the parameters used for the calculation of the ideal dosing interval proposed by McCormack and Schentag (1987).

Ideal dosing interval = Time above MBC + PAE + LGT. (c.14)

LGT is an indication of log growth over a period of time whereas BR is log kill over a period of time. The EC_{50} and SBA parameters used in this investigation represent the MBC. The EC_{50} value only refer to a more dynamic MBC value whereas the SBA parameter incorporates both the drug level and the MBC. The SBA parameter (already incorporating a plasma level) will be excluded from any equation for dosing intervals. It would therefore appear from this study that the ideal dosing interval formula proposed by McCormack and Schentag (1987) included the most relevant parameters for the prediction of the outcome of an infection.

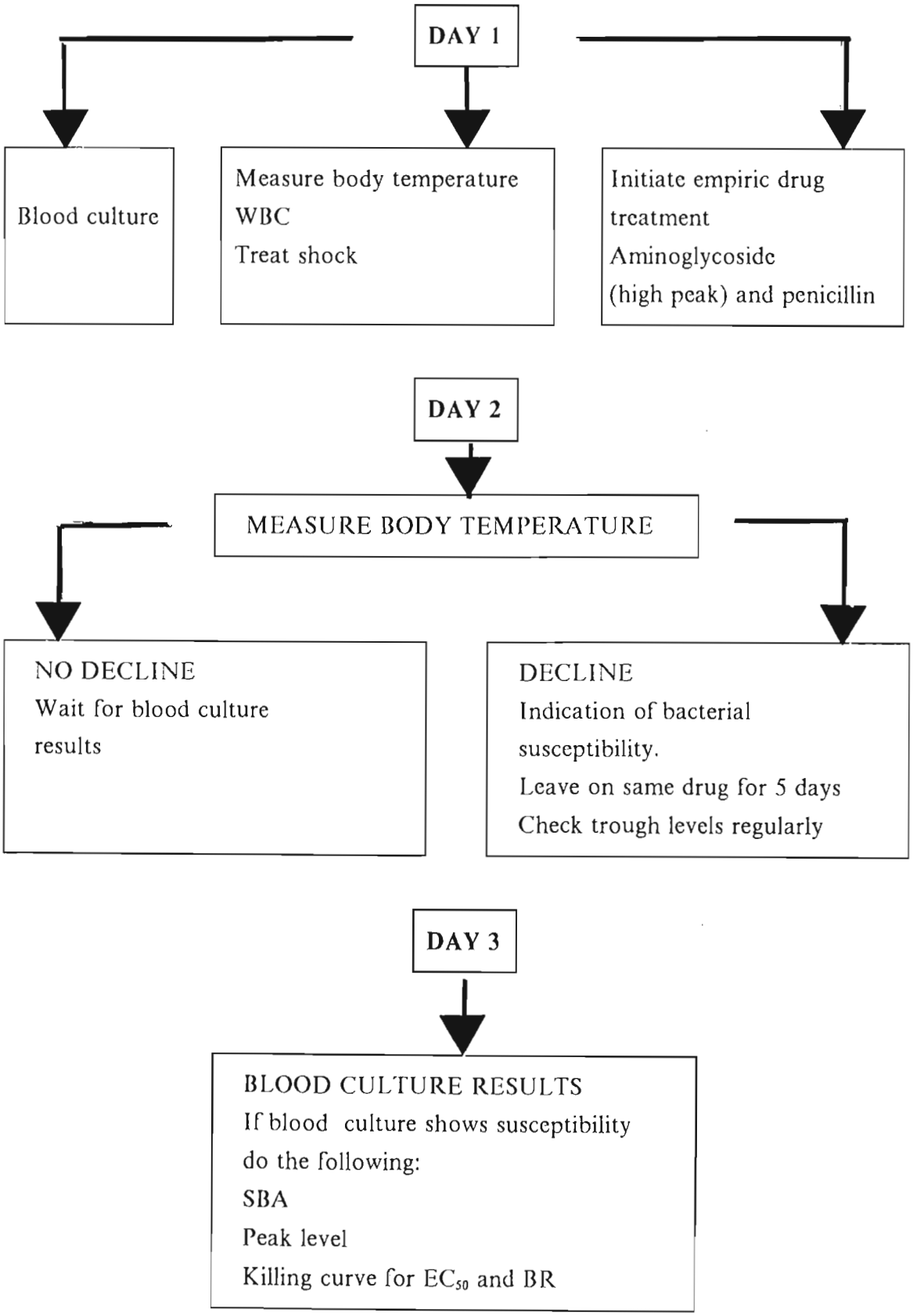
6. CONCLUSIONS

The relatively short average time to normalization of body temperature (2.6 days) in this study was associated with an absence of mortalities. A single kinetic parameter i.e. peak plasma antibiotic concentration is insufficient to predict the outcome of treatment in patients with gram negative bacteremia (table C.8). The derived pharmacodynamic parameters which measure the actual killing rate of the causative organism at specific drug concentrations (BRT and EC_{50}) were good predictors of outcome. It is therefore suggested that these parameters be included in the determinations of antibiotic sensitivity.

The equation derived from BR, PAE (1hr), EC_{50} and SBA (c.20) for the determination of days to eradication provides information on the adequacy or inadequacy of antibiotic treatment in specific patients. It appears that treatment will be adequate if the equation predicts 2.6 or fewer days to eradication. If the equation predicts more than 2.6 days to eradication, treatment is probably inadequate and needs reassessment. The equation further accentuates the importance of inclusion of non static bacterial parameters in sensitivity determinations. Treatment may be inadequate because the organisms are not killed at a satisfactory rate. This possibility is frequently overlooked in the clinical situation. PAE as an important determinant of a dosing interval featured strongly in the present study (table C.8). It also became apparent that longer exposure of the organism to the antibiotic played a more important role with *K. pneumoniae* than with *E. coli* (table C.6) ($p = 0.0028$ for *K. pneumoniae* and $p = 0.0337$ for *E. coli*).

The minimum time required for the determination of the pharmacodynamic parameters (EC_{50}, PAE, BRT and SBA) after initial isolation is approximately 2 days. It is therefore not practical to routinely determine the parameters in patients with gram negative bacteremia. However the clinical advantages of dual individualization in antibiotic therapy have been accentuated in this study. The development of automated systems for the determination of non static bacteriological parameters seems to be crucial for improved antibiotic therapy in patients with gram negative bacteremia. The following flow chart is a proposal for the improved treatment of gram-negative bacteremia

PROPOSED FLOW CHART FOR TREATMENT OF GRAM NEGATIVE BACTEREMIA BASED UPON PRESENT STUDY



DAY 4

Work out days to eradication based upon following equation:
2.91 - 0.51 BR - 0.81 PAE(1hr) + 4.30 EC_{50} - 0.06 SBA
If the predicted days to eradication exceed 2.6 days it is an indication of a more **resistant organism** or **an inadequate dose** for a particular organism.
In both cases a dosing adjustment is justified.

The following approach to dosing adjustment is recommended taking into consideration the killing rate as measured by the EC_{50} and the PAE.

(1) Estimate C max and C min for a convenient dosing interval :

$$Cmax = \frac{EC_{50}}{e^{-ke(\tau - T)}}$$

$$Cmin = EC_{50} \cdot (e^{-keT})$$

(2) Maintenance dose for an infusion,

$$MD = \frac{t_i \cdot Cmax \cdot Cl\ (1 - e^{-k\tau})}{(1 - e^{-kt_i})}$$

(3) Maintenance dose for a bolus,

$$MD = (Cmax - C\,min) \cdot Vd$$

C max = Maximum drug concentration
C min = Minimum drug concentration
T = Time of PAE
τ = Dosing interval
t_i = Time of infusion

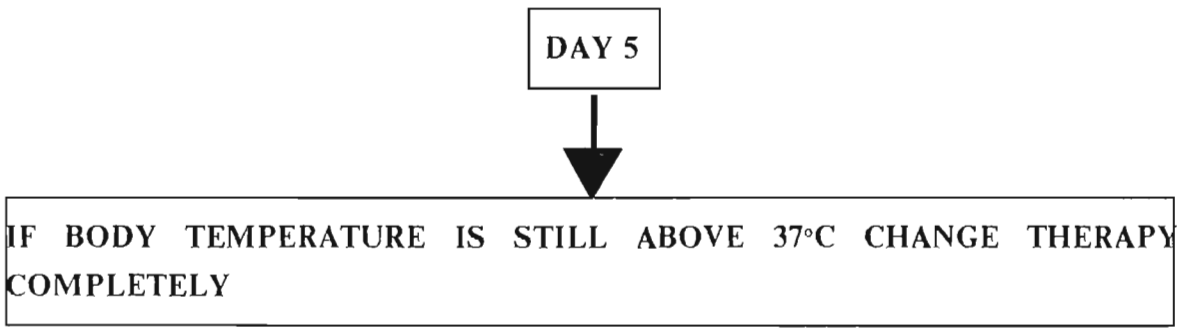
For example :

For a 60 kg male , Cl = 3.528 ℓ/h , Vd = 12.5ℓ, EC_{50} = 0.64 , PAE = 0.91hr. and τ = 8 hours the following can be expected:

C max = 4.7 μg/mℓ

C min = 0.5 μg/mℓ

MD = 56.9 mg 8 hourly



SUMMARY

SECTION A

A retrospective epidemiological study to determine the nature of gram-negative bacteremia in an adult and neonatal population at Baragwanath Hospital was performed. The incidence of bacteremia in the adult population was 5.8/1000 admissions. This was associated with a mortality rate of 32% of which 67% of deaths occurred within the first 72 hours after admission. *E. coli* was the organism most commonly isolated (53%), followed by klebsiella (23%), proteus (9%) and pseudomonas (8%). Other organisms accounted for 5% or fewer of the bacteremias. The genitourinary tract was the most common route of infection (34%), followed by the lungs (24%), skin (14%) and gastrointestinal tract (6%). In 16% of patients the route of infection was not identifiable.

A correlation existed between age and mortality: 38%(9) of patients younger than 50 years and 63%(15) older than 50 years died. Patients with a rapidly fatal disease had a mortality rate of 67% in comparison with 48% in patients with ultimately fatal, 38% with non-fatal and 8% with no underlying disease.

All the isolated organisms were highly resistant to ampicillin. *E.coli* and klebsiella were susceptible to amikacin only, whereas pseudomonas was resistant to all the aminoglycosides.

E. coli and klebsiella were susceptible to all the third generation cephalosporins, and pseudomonas was resistant to cefotaxime and ceftriaxone.

The incidence of gram negative bacteremia in the neonatal population was 66/1000 admissions. The mortality rate in this population was 66%.

Pseudomonas was the organism most commonly isolated (44%) followed by klebsiella and carynobacter (19% each), *E. coli* (9%) and enterobacter (3%), 72% of the infants with a birth weight lower than 2,5 kg died. None of the infants with a birth weight higher than 2,5 kg died. The same number of male and female infants contracted bacteremia. However, 75% of the males and 56% of the females died.

There was also a correlation between younger gestational age and late onset of bacteremia and deaths. All the organisms, tested, were resistant to ampicillin. *E. coli* was susceptible to all the aminoglycosides and third generation cephalosporins tested. Klebsiella was resistant to all three aminoglycosides and ceftazidime and pseudomonas to all aminoglycosides and third generation cephalosporins tested.

SECTION B

Population parameter values for amikacin and cefotaxime in an neonatal and gentamicin in an adult population with gram-negative bacteremia were determined by using the NONMEM programme.

Adjusting Cl and Vd for weight or BSA significantly decrease the OBF for amikacin. The inclusion of serum creatinine concentrations in addition to weight did not decrease the OBF significantly. From these results it appears that Cl and Vd for amikacin can be calculated as follows:

$$Cl(\ell/h/kg) = 0.0683 \cdot WT$$
$$Vd\ (\ell/kg) = 0.238 \cdot WT$$

Adjusting Cl and Vd simultaneously for weight or BSA significantly decrease the OBF for cefotaxime. The inclusion of serum creatinine in addition to weight did not decreased the OBF significantly. From these results it appears that Cl and Vd of cefotaxime can be calculated as follows:

$$Cl(\ell/h/kg) = 0.0919 \cdot WT$$
$$Vd\ (\ell/kg) = 0.312 \cdot WT$$

Adjusting Cl alone or both Cl and Vd for weight significantly decreased the OBF for gentamicin. Adjustment of Vd only however increased the OBF. The inclusion of serum creatinine concentration in addition to weight did not decrease the OBF significantly.

From these results it appears Cl and Vd of gentamicin can be calculated as follows:

$$Cl(\ell/h/kg) = 0.588 \cdot WT$$
$$Vd\ (\ell) = 12.5$$

SECTION C

The influence of selected clinical, pharmacokinetic and microbial parameters on the outcome of gram negative bacteremia was investigated. These parameters as well as the

influence of PAE were regressed against the time to normalization of body temperature as an indication of cure. When only 1 variable was regressed SBA performed the best (r^2 = 0.54), with 2 variables SBA + EC_{50} (r^2 = 0.78) and SBA + AUC/EC_{50} (r^2 = 0.66) respectively were the best performers.

With 3 variables both BR and PAE gave good r^2 values. SBA + EC_{50} + BR (r^2 = 0.85) and SBA + EC_{50} + PAE (r^2 = 0.84).

The optimum fit was obtained with 4 variables: SBA + EC_{50} + BR + PAE (r^2 = 0.90). When more than 4 variables were regressed the p value started to increase. The number of days to normalization of body temperature as an indication of cure can therefore be predicted with the following equation:

Days to eradication = 2.91 - 0.51 BR - 0.81 PAE(1hr) + 4.30 EC_{50} - 0.06 SBA

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No man can reveal to you aught but that which already lies half asleep in the dawning of your knowledge.

The teacher who walks in the shadow of the temple, among his followers, gives not of his wisdom but rather of his faith and his lovingness. If he is indeed wise he does not bid you enter the house of his wisdom, but rather leads you to the threshold of your own mind.

(Kahlil Gibran)

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APPENDIX A

PREPARATION OF STANDARDS USED FOR CALIBRATION AND CONTROLS DURING ANALYSIS OF CEFOTAXIME.

Commercially available cefotaxime, 500 mg base as sodium salt per vial, (Claforan^{R}) for injection was used (lot No.: 0788PPF, Exp. dat: 07-90), to prepare a lmg-lml stock solution. Drug free serum, Q-Pak Lot No.: 3831N001AA was used and samples were spiked separately with the cefotaxime stock solution. The analysis was done within 60 minutes, as the stability of cefotaxime in plasma is limited and therefore all standards had to be prepared separately. HPLC grade water was used to compensate for the volume deficit.

Standard solution ($\mu\ell$)	Stock solution ($\mu\ell$)	Water addition ($\mu\ell$)	A.U.C. x 10^{-4}
Blank	0.0	200.00	no peaks recorded
10.0	10.0	190.0	11.9891
25.00	25.00	175.00	32.3470
50.0	50.0	150.00	66.9986
75.0	75.0	125.00	102.1200
100.0	100.0	100.0	132.6200
125.0	125.0	75.0	163.2600
150.0	150.0	50.0	198.9600
200.0	200.0	0.0	268.4500

The best standard calibration line was generated by a 2nd degree polynomial, r = 0.99984419. The concentrations of all the unknown samples as well as the at random included standards were calculated from the above mentioned standard curve.

An example of a characteristic HPLC chromatogram for a cefotaxime and desacetyl-cefotaxime sample can be seen on page 170.

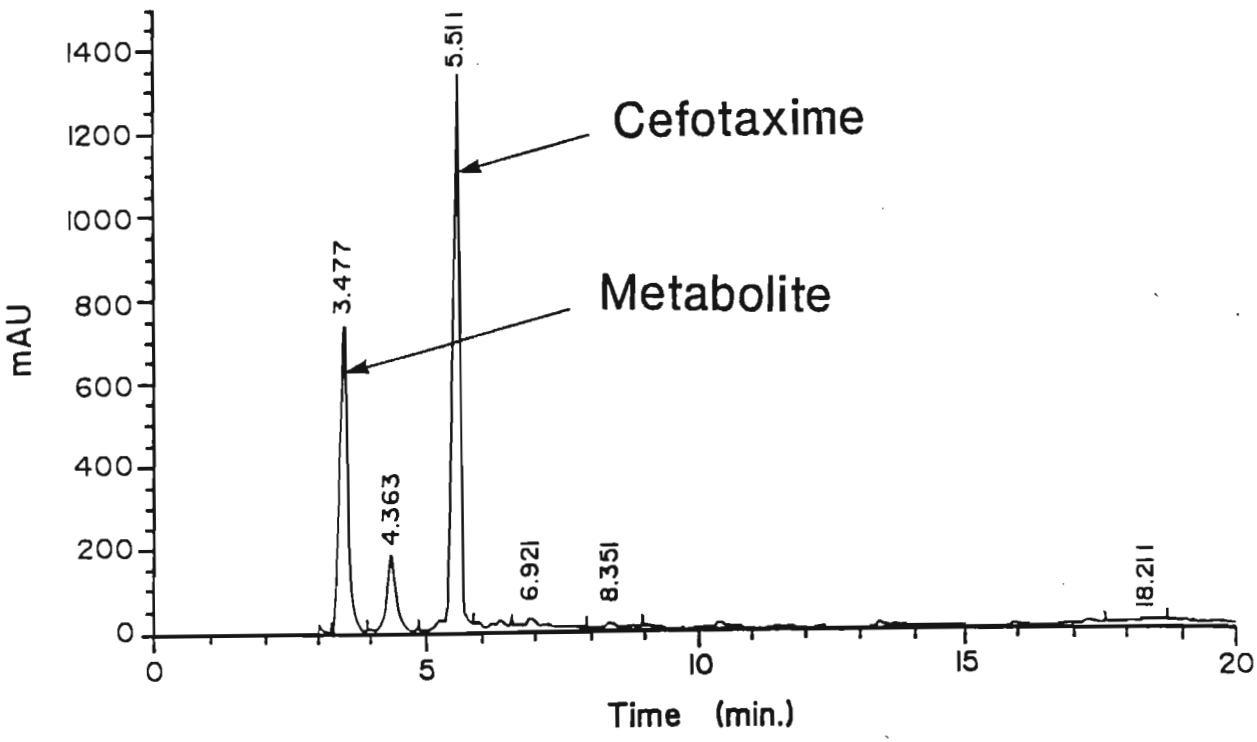


Fig B.1 A characteristic HPLC chromatogram of a cefotaxime and desacetyl-cefotaxime sample

(Seifart, 1989)

APPENDIX B

APPENDIX B

MICROBIOLOGICAL METHODS

1 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Materials

- Gentamicin was supplied by the Scherag corporation.
- Mueller-Hinton broth supplemented with Ca^{2+} (50 mg/ℓ) and Mg^{2+}. (25 mg/ℓ) ions.
- Mueller-Hinton agar plates
- Microtiter plates
- Reference strain *E. coli* ATCC 25922
- Organisms cultured from the first positive blood culture of each patient henceforth referred to as patient's strain.

Method

1. Each microtiter plate well was filled with 100 μℓ Mueller-Hinton broth.
2. 256 μg gentamicin diluted in 100 μℓ broth (final concentration 128μg/mℓ) was added to the first well of 100 μℓ
3. A serial twofold dilution of gentamicin was performed up to the lowest concentration of 0.25 μg/mℓ.
4. The last well in each series acted as a control (i.e. no gentamicin was added).
5. The strains were prepared as follows. A few colonies from a stock solution of the patient's and reference strains were inoculated into two separate Mueller-Hinton broth flasks and allowed to grow for approximately 3 hours to ensure log-phase

growth. The concentration of the solutions was then validated with a Mac Farland standard to assure that a concentration of 5 x 10^5 cfu/ml was added to each well.

6. 5 $\mu\ell$ of the patients, or 5$\mu\ell$ of the reference strain was added to the wells after which the microtiter plates were incubated at 37°C for 18-24 hours.

7. After incubation for 18-24 hours the microtiter plates were inspected under a magnifying glass and lamp. The well with the lowest concentration of antibiotic where no visible growth was observed, was noted. The concentration of the antibiotic in that well was then taken as the MIC value.

8. The patient's and reference strains were performed in duplicate. The MIC value for the patients strain was only included if the MIC value of the control reference strain was 0.25-1 μg/mℓ.

9. Subculturing was then performed on the patient's strain to determine the MBC value. Aliquots of organisms obtained from the wells containing the MIC, the four serial concentrations above the MIC and the control were then plated on Mueller-Hinton agar plates and allowed to grow for a further 18-24 hours.

10. Following a period of growth for 18-24 hours the agar plates were inspected and the concentration of the antibiotic at which a > 99.9% kill had occurred, was taken as the MBC. MBC is defined as the lowest concentration at which a 99.9% reduction of the initial inoculum (control) occurs.

2 Serum bacteristatic activity (SBC) and serum bactericidal activity (SBA)

Materials

1. Mueller-Hinton serum diluent i.e. 50% Mueller-Hinton broth with 50% pooled inactivated human serum supplemented with Ca^{2+} (50 mg/ℓ) and Mg^{2+} (25 mg/ℓ) ions

2. Mueller-Hinton agar plates

3. Microtiter plates.

Method

1. The microtiter plate wells were each filled with 100 $\mu\ell$ Mueller-Hinton serum diluent.

2. 100 $\mu\ell$ of the patient's serum sample containing the peak level of gentamicin was then added to the first well. Serial twofold dilutions of the patient's serum were added to other wells.

3. The patient's strain was allowed to grow to log phase and then tested against the MacFarland standard to ensure an end concentration of 5×10^5 cfu/ mℓ.

4. A 5 $\mu\ell$ aliquot of a solution containing the patient's strain was then added to each well and incubated for 18-24 hours.

5. After incubation the wells were inspected under a magnifying lamp and mirror and the greatest dilution at which no viable growth had occurred was taken as the SBC value.

   It is clear that SBC is determined in a similar fashion to MIC except that for the determination of the MIC known antibiotic concentrations are used whereas for the determination of SBC serial dilutions of the patient's peak antibiotic serum concentration are used.

6. The sample where no visible growth was observed the four serial samples above and the control sample were next subcultured onto Mueller-Hinton antibiotic free agar plates and incubated for another 18-24 hours for the determination of SBA.

3 Time kill curves, killing curves or time kill plots

(Drake,1983)

In order to calculate the EC_{50} and bactericidal rate constants, time killing curves were performed.

Materials

- Mueller-Hinton broth supplemented with $Ca^{2}+$ (50 μg/ℓ) and $Mg^{2}+$ (25 μg/mℓ) ions

- Bacteria isolated from patient

- Gentamicin
- Mueller-Hinton agar plates
- Disposable pipettes, glassware, broth and all other instruments were sterilized before use
- Shakers with adjustable speed rates and temperature maintained at 37°C.

Method

1. The concentration of the patient's strain of organisms for the initial inoculum was standardized at 5 x 10^5 and 6 x 10^5 cfu/mℓ

2. The equipment consisted of four Erlenmeyer flasks to which Mueller-Hinton broth, organisms and various concentrations of gentamicin were added. The composition of each flask was as follows:

   - Control: 9 mℓ Mueller Hinton broth and 1 mℓ organism solution.
   - Samples: 8 mℓ Mueller Hinton broth, 1 mℓ organism solution and 1 mℓ antibiotic solution with concentrations of ½; ¾ or 2 x the MIC.

   Every experiment was performed in duplicate and if the two results differed by more than 1 log cycle a third experiment was performed.

3. Immediately after the contents of the control flask were mixed a sample was taken, plated as 20 ul/ml dots onto agar plates and incubated for 24 hours after which a colony count was performed to provide the organism concentration at time 0. This procedure was repeated at fixed intervals for each of the flasks investigated.

   Colony formed units per ml(cfu/ml) were calculated from the colony counts per 20 $\mu\ell$ dot.
   For example:

   25 colonies / 20 $\mu\ell$
   = 125 colonies / 100 $\mu\ell$
   = 1250 colonies / 1000 $\mu\ell$
   = 1.25 x 10^3 cfu/ml

4. Immediately after the addition of the organisms the flasks were placed on a rotator at 37°C and agitated at a constant speed for the duration of the experiment.

5. Sampling for the determination of colony counts was performed at 0, 0.5, 1, 1.5, 2 and 3 hours. The samples were transferred onto agar plates and were incubated overnight.

6. All the colony counts were performed the next morning and the results plotted on semilogarithmic graph paper as cfu/mℓ versus time.

7. The EC_{50} and bactericidal rate were then calculated. (see figures C.4 and C.5 on page 114)

4 Post-antibiotic effect (PAE)

The PAE was determined according to the method of Craig and Gudmundsson, 1986).

Materials

- Mueller-Hinton broth supplemented with Mg^{2+} (25 mg/ℓ) and Ca^{2+} (40 mg/ℓ ions.
- Gentamicin
- Mueller-Hinton agar plates
- Sterilized disposable pipette tips, glassware and instruments.

Method

1. In order to determine the PAE two prerequisites have to be met (Craig and Gudmundsson, 1986). Firstly the organisms must be in the log growth phase. Secondly the concentration of the initial inoculum must be high enough to ensure that some organisms are left after dilution. In order to fulfil these requirements an initial inoculum concentration higher than those required for the execution of killing curves , MIC or SBA determinations was selected and allowed to grow for 3 hours (ensures log phase growth) before gentamicin was added.

2. The initial microbial concentration was then taken as the concentration determined at 3 hours immediately prior to the addition of the antibiotic. This concentration was standardized to 10^6-10^7 cfu/mℓ. The antibiotic concentration added was the

MIC of gentamicin. The MIC concentration was selected because Schentag and McCormick (1987) defined PAE as the time required for regrowth to occur after the antibiotic concentration had fallen below the MIC.

3. The apparatus for each experiment, consisted as in the case of killing curve experiments, of 4 flasks, 2 control flasks (i.e. no antibiotic added) and 2 flasks to which the antibiotic was added. Each flask contained 8 mℓ broth, 1 mℓ organisms and 1 mℓ antibiotic with the control flasks containing just 9 mℓ broth and 1 mℓ organisms.

4. The flasks were placed on the rotator at 37°C and immediately exposure to the antibiotic was initiated. Two pairs of flasks (each comprising 1 control and 1 experimental flask) were incubated for 1 and 2 hours respectively. Colony counts were performed at times 0, 1 and 2 hours for the control and at 1 and 2 hours for each experiment.

5. At the end of the exposure time the antibiotic effect was terminated by dilution. A 10^{-3} dilution (10 $\mu\ell$) from both the control and experimental flask was then added to 10 mℓ of prewarmed antibiotic free Meullen-Hinton broth.

6. Sampling for colony counts was performed at times 0 (immediately after dilution) and 0.5, 1, 1.5, 2 and 3 hours after transferred to the antibiotic free Muellen-Hinton broth. These samples were transferred to agar plates and incubated over night.

7. During each experiment, additional controls were prepared by adding the test drug at a concentration of 1/1000 of the drug concentration present during the exposure time. These controls were included to ensure that residual drug present after dilution did not affect the rate of growth.

8. The agar plates were incubated over night and colony counts were performed the next morning on the same basis as for killing curves (cfu/ml)

9. The results were plotted on semilogarithmic graph paper as cfu/mℓ versus time.

10. PAE was calculated using the following equation which was discussed in section 2.5.4

$$PAE = T - C$$

T = Time required for the CFU count in the test culture to increase by one logarithmic cycle above the count observed immediately after drug removal.

C = time required for the count of CFU in the control culture to increase by 1 log cycle above the count observed immediately after termination of antibiotic action.

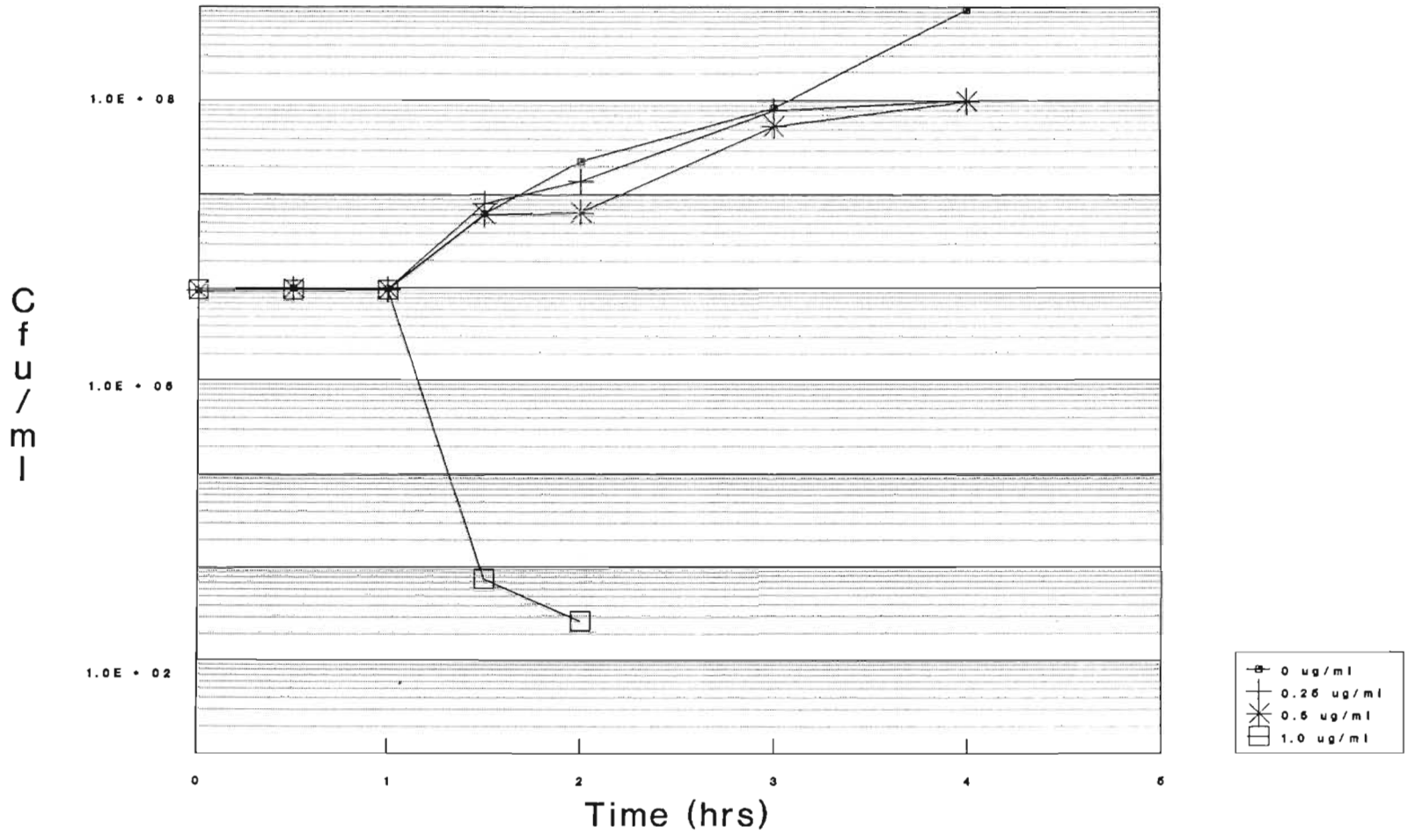
11. Each experiment was performed in duplicate and if the results differed by more than 1 log cycle a third experiment was performed.

APPENDIX C

ID 1

ORGANISM K.PNEUMONIAE

Drug concentration (μg/mℓ)	Time (hr)	Sample 1	Sample 2	Average
0	0	9.5×10^5	7.9×10^5	8.7×10^5
	0.5	9.5×10^5	1.0×10^6	9.8×10^5
	1	8.9×10^5	1.2×10^6	1.0×10^6
	1.5	7.2×10^6	1.1×10^6	4.2×10^6
	2	2.5×10^7	7.9×10^6	1.6×10^7
	3	8.5×10^7	5.0×10^7	6.8×10^7
	4	9.5×10^8	7.6×10^8	8.6×10^8
0.25	0.5	9.7×10^5	8.9×10^5	9.3×10^5
	1	8.9×10^5	9.5×10^5	9.2×10^5
	1.5	7.1×10^6	8.0×10^6	7.6×10^6
	2	1.9×10^7	8.3×10^6	1.4×10^7
	3	6.3×10^7	6.0×10^7	6.2×10^7
	4	1.4×10^8	1.0×10^8	1.2×10^8
0.5	0.5	9.5×10^5	8.9×10^5	9.2×10^5
	1	9.2×10^5	8.6×10^5	8.9×10^5
	1.5	4.3×10^6	3.8×10^6	4.1×10^6
	2	5.4×10^6	3.7×10^6	4.6×10^6
	3	4.0×10^7	3.0×10^6	2.2×10^7
	4	1.0×10^8	8.9×10^7	9.5×10^7
1.0	0.5	8.2×10^5	9.5×10^5	8.9×10^5
	1	8.6×10^5	9.2×10^5	8.9×10^5
	1.5	2.5×10^2	8.5×10^2	5.5×10^2
	2	1.5×10^2	2.0×10^2	1.8×10^2

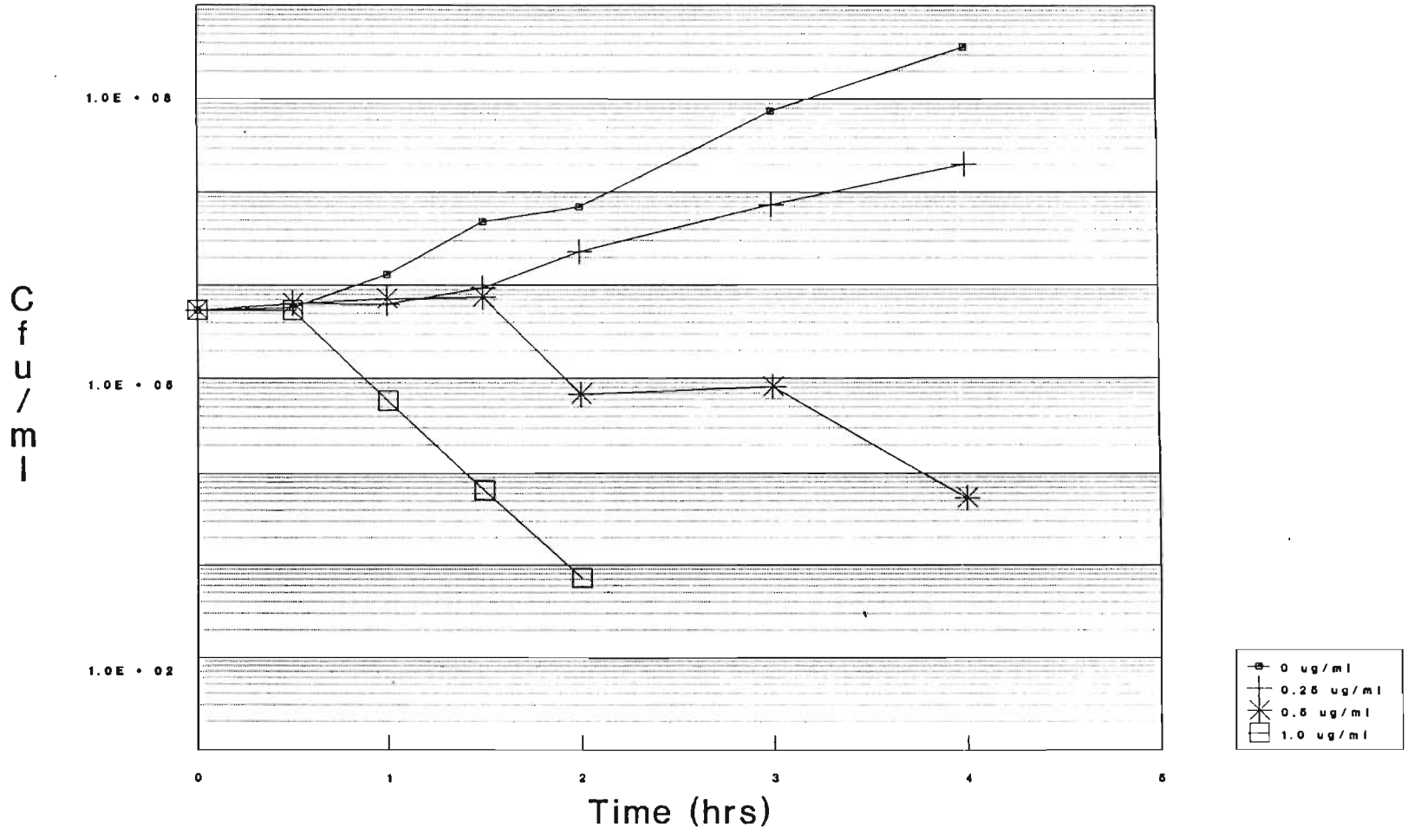


ID.1 Killing curve

ID 2

ORGANISM K.PNEUMONIAE

Drug concentration ($\mu g/m\ell$)	Time (hr)	Sample 1	Sample 2	Average
0	0	3.5×10^5	4.0×10^5	3.8×10^5
	0.5	3.4×10^5	4.2×10^5	3.8×10^5
	1	4.6×10^5	5.7×10^6	3.1×10^6
	1.5	1.5×10^6	1.2×10^6	1.4×10^6
	2	4.8×10^6	5.0×10^7	2.7×10^7
	3	5.1×10^7	5.7×10^7	5.4×10^7
	4	3.0×10^8	1.5×10^8	2.3×10^8
0.25	0.5	4.0×10^5	4.5×10^5	4.3×10^5
	1	4.1×10^5	5.2×10^5	4.7×10^5
	1.5	8.5×10^5	8.8×10^5	8.7×10^5
	2	1.6×10^6	1.8×10^6	1.7×10^6
	3	5.0×10^6	6.0×10^6	5.5×10^6
	4	1.8×10^7	1.4×10^7	1.6×10^7
0.5	0.5	4.1×10^5	4.5×10^5	4.3×10^5
	1	5.1×10^5	5.0×10^5	5.1×10^5
	1.5	6.5×10^5	5.0×10^5	5.8×10^5
	2	5.6×10^4	4.0×10^4	4.8×10^4
	3	7.0×10^4	6.0×10^4	6.5×10^4
	4	3.0×10^3	4.0×10^3	3.5×10^3
1.0	0.5	3.4×10^5	4.0×10^5	3.7×10^5
	1	3.4×10^4	4.0×10^4	3.7×10^4
	1.5	4.2×10^3	5.0×10^3	4.6×10^3
	2	5.4×10^2	5.0×10^2	5.2×10^2

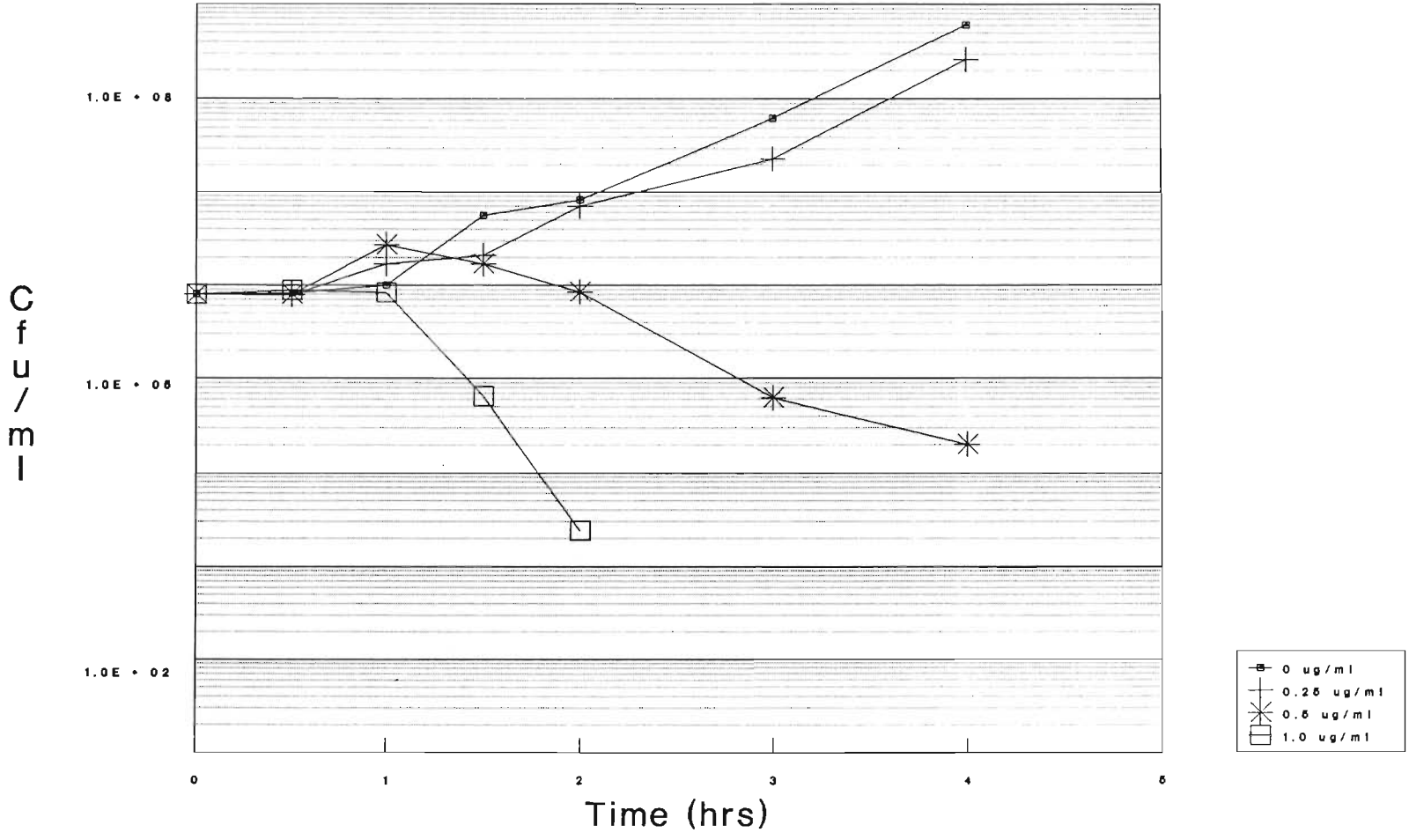


ID.2 Killing curve

ID 3

ORGANISM K.PNEUMONIAE

Drug concentration (μg/mℓ)	Time (hr)	Sample 1	Sample 2	Average
0	0	5.7×10^5	6.9×10^5	6.3×10^5
	0.5	6.0×10^5	7.0×10^5	6.5×10^5
	1	1.1×10^6	1.2×10^6	1.2×10^6
	1.5	3.5×10^6	3.7×10^6	3.6×10^6
	2	7.3×10^6	6.0×10^6	6.7×10^6
	3	3.8×10^7	4.5×10^7	4.2×10^7
	4	3.7×10^8	4.3×10^8	4.0×10^8
0.25	0.5	6.0×10^5	6.3×10^5	6.2×10^5
	1	1.1×10^6	2.0×10^6	1.6×10^6
	1.5	1.7×10^6	2.5×10^6	2.1×10^6
	2	4.8×10^6	5.2×10^6	5.0×10^6
	3	1.2×10^7	2.5×10^7	1.9×10^7
	4	1.2×10^8	3.0×10^8	2.1×10^8
0.5	0.5	6.0×10^5	6.5×10^5	6.3×10^5
	1	1.1×10^6	3.0×10^6	2.1×10^6
	1.5	1.1×10^6	2.0×10^6	1.6×10^6
	2	6.8×10^5	7.2×10^5	7.0×10^5
	3	4.4×10^4	4.0×10^4	4.2×10^4
	4	1.3×10^4	2.0×10^4	1.7×10^4
1.0	0.5	7.7×10^5	8.0×10^5	7.9×10^5
	1	6.6×10^5	7.0×10^5	6.8×10^5
	1.5	5.0×10^4	4.0×10^4	4.5×10^4
	2	1.5×10^3	2.0×10^3	1.8×10^3

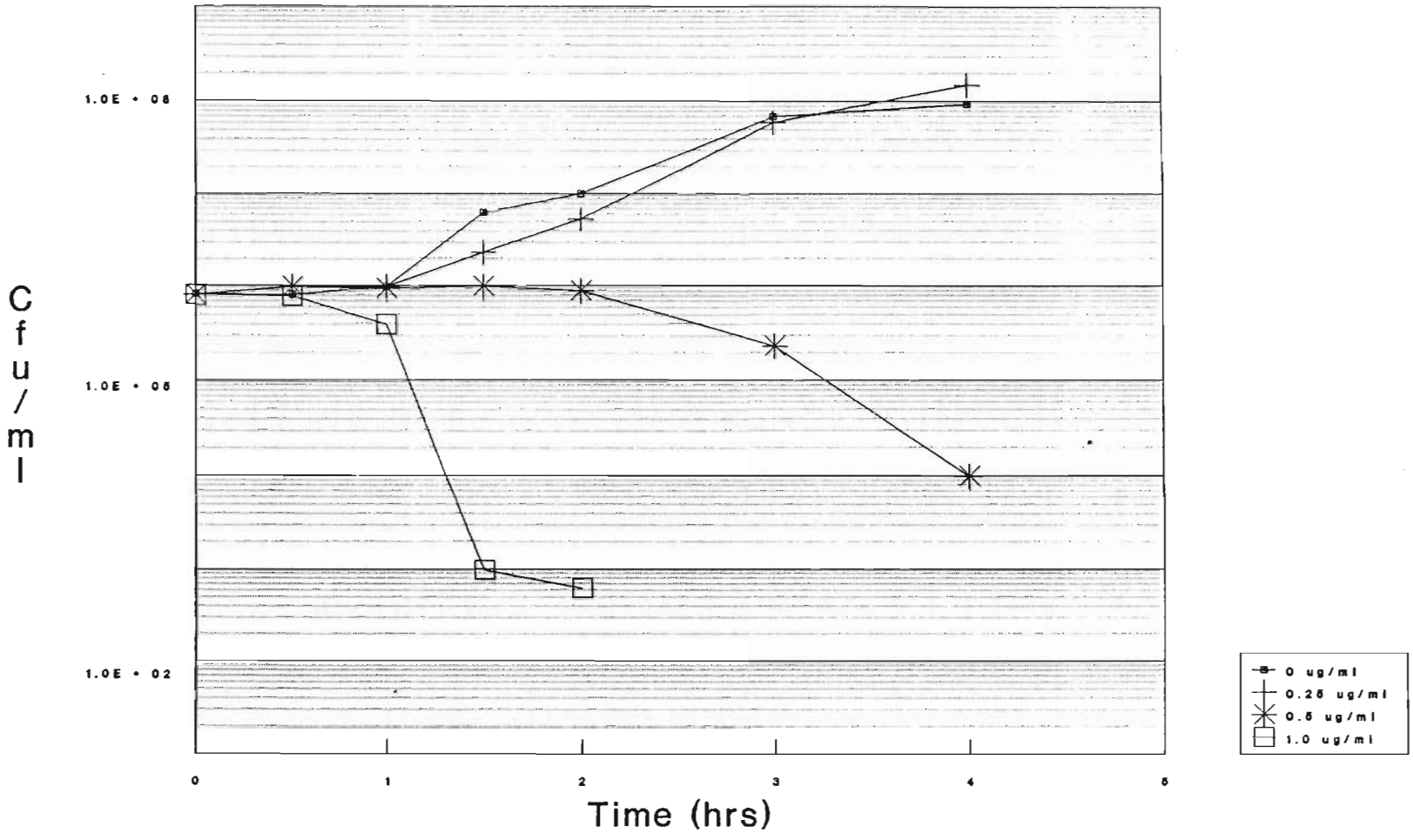


ID.3 Killing curve

ID 4

ORGANISM K.PNEUMONIAE

Drug concentration ($\mu g/m\ell$)	Time (hr)	Sample 1	Sample 2	Average
0	0	4.7×10^5	9.0×10^5	6.9×10^5
	0.5	6.5×10^5	6.0×10^5	6.3×10^5
	1	9.6×10^5	8.9×10^5	9.3×10^5
	1.5	3.3×10^6	4.0×10^6	3.7×10^6
	2	9.2×10^6	1.1×10^7	1.0×10^7
	3	4.3×10^7	5.0×10^7	4.7×10^7
	4	7.9×10^7	9.6×10^7	8.8×10^7
0.25	0.5	6.5×10^5	6.0×10^5	6.3×10^5
	1	1.0×10^6	9.0×10^5	9.5×10^5
	1.5	1.4×10^6	2.0×10^6	1.7×10^6
	2	3.8×10^6	3.0×10^6	3.4×10^6
	3	3.7×10^7	4.0×10^7	3.9×10^7
	4	1.0×10^8	2.0×10^8	1.5×10^8
0.5	0.5	6.3×10^5	5.0×10^5	5.7×10^5
	1	9.5×10^5	8.0×10^5	8.8×10^5
	1.5	1.2×10^6	8.0×10^5	1.0×10^6
	2	7.0×10^5	8.0×10^5	7.5×10^5
	3	1.4×10^5	1.2×10^5	1.3×10^5
	4	1.0×10^4	9.0×10^3	9.5×10^3
1.0	0.5	6.5×10^5	5.5×10^5	6.0×10^5
	1	1.5×10^5	4.0×10^5	2.8×10^5
	1.5	7.5×10^2	1.1×10^3	9.3×10^2
	2	8.4×10^2	2.0×10^2	5.2×10^2

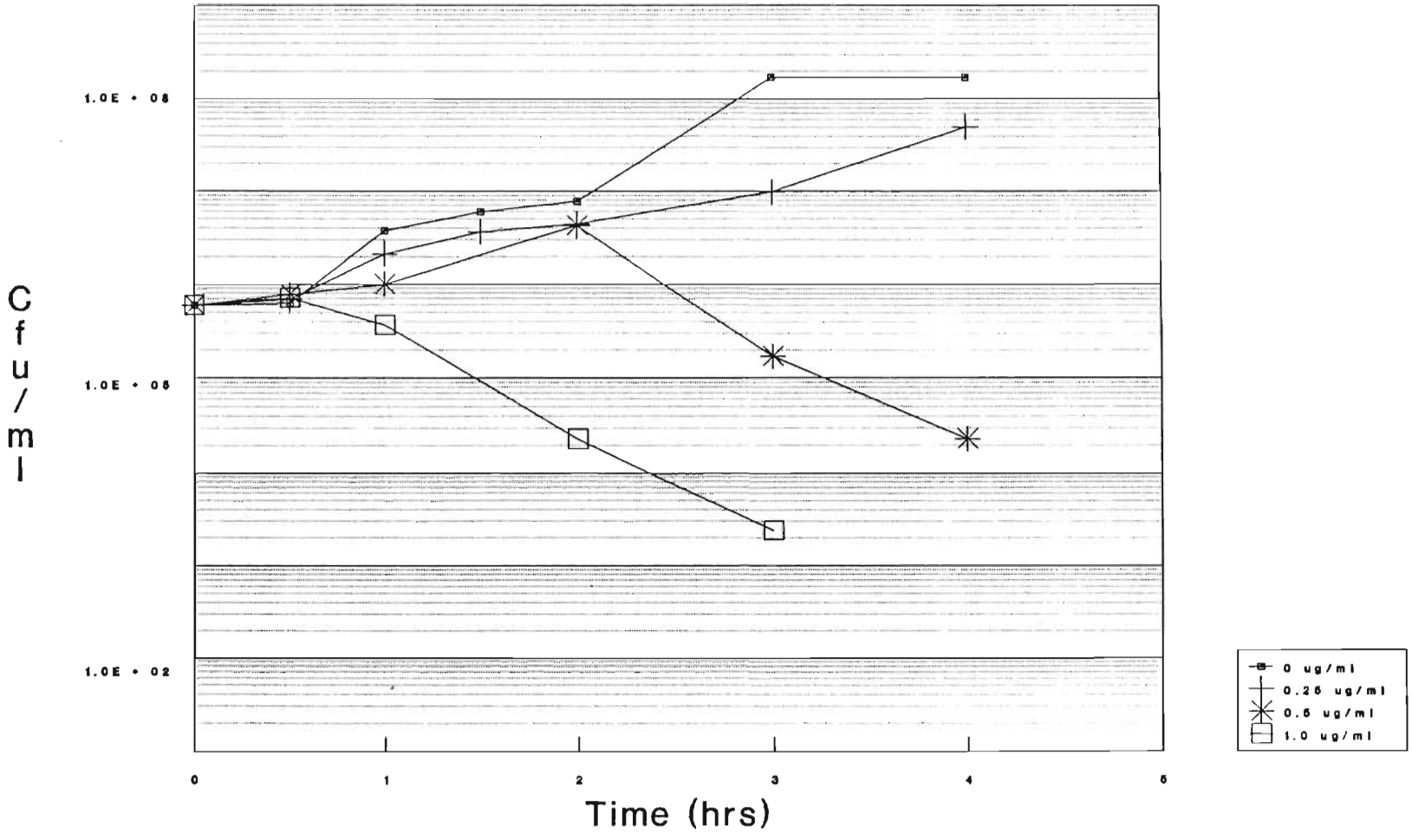


ID.4 Killing curve

ID 5

ORGANISM K.PNEUMONIAE

Drug concentration (μg/mℓ)	Time (hr)	Sample 1	Sample 2	Average
0	0	5.2×10^5	3.0×10^5	4.1×10^5
	0.5	5.6×10^5	3.4×10^5	4.5×10^5
	1	2.7×10^6	2.0×10^6	2.4×10^6
	2	4.6×10^6	7.8×10^6	6.2×10^6
	3	1.1×10^8	2.0×10^8	1.6×10^8
	4	4.6×10^8	4.8×10^8	4.7×10^8
0.25	0.5	4.8×10^5	5.2×10^5	5.0×10^5
	1	1.5×10^6	1.8×10^6	1.7×10^6
	2	4.2×10^6	1.8×10^6	3.0×10^6
	3	2.0×10^7	4.7×10^6	1.2×10^7
	4	8.5×10^7	1.2×10^7	4.9×10^7
0.5	0.5	7.0×10^5	5.3×10^5	6.2×10^5
	1	1.0×10^6	1.5×10^6	1.3×10^6
	2	1.5×10^6	4.5×10^5	9.8×10^5
	3	1.1×10^5	2.0×10^5	1.6×10^5
	4	1.8×10^4	1.6×10^4	1.7×10^4
1.0	0.5	5.0×10^5	5.3×10^5	5.2×10^5
	1	1.5×10^5	3.7×10^5	2.6×10^5
	2	1.4×10^4	2.0×10^4	1.7×10^4
	3	1.1×10^3	2.8×10^3	2.0×10^3

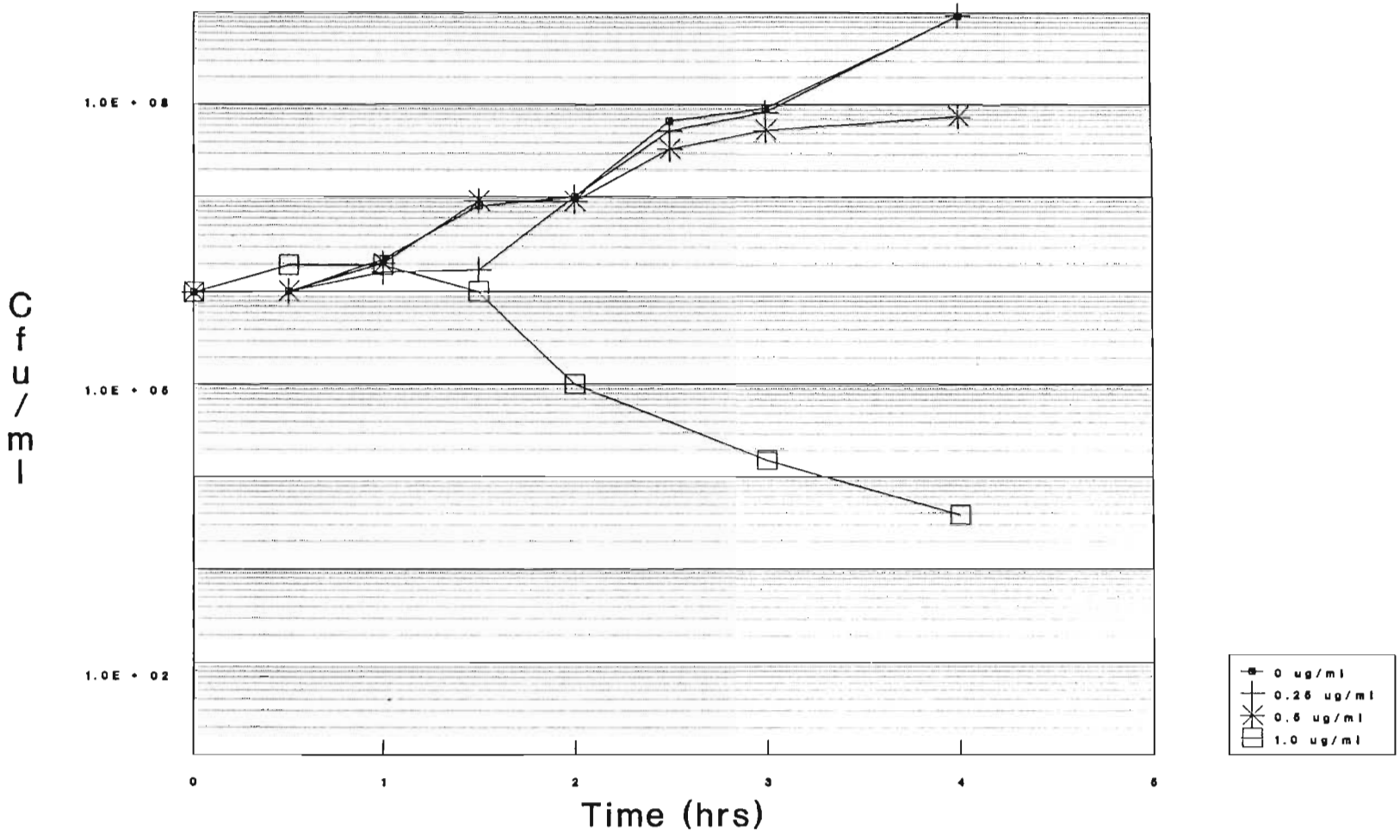


ID.5 Killing curve

ID 6

ORGANISM K.PNEUMONIAE

Drug concentration ($\mu g/m\ell$)	Time (hr)	Sample 1	Sample 2	Average
0	0	7.5×10^5	1.4×10^6	1.1×10^6
	0.5	1.4×10^6	6.0×10^5	1.0×10^6
	1	2.5×10^6	8.1×10^5	1.7×10^6
	1.5	1.2×10^7	1.1×10^6	6.6×10^6
	2	7.3×10^6	1.6×10^7	1.2×10^7
	3	8.0×10^7	8.3×10^7	8.2×10^7
	4	8.0×10^8	8.5×10^8	8.3×10^8
0.25	0.5	8.2×10^5	1.4×10^6	1.1×10^6
	1	7.7×10^5	2.2×10^6	1.5×10^6
	1.5	1.4×10^6	1.5×10^7	8.2×10^6
	2	5.3×10^6	1.6×10^7	1.1×10^7
	3	7.0×10^7	6.0×10^7	6.5×10^7
	4	8.0×10^8	9.0×10^8	8.5×10^8
0.5	0.5	7.9×10^5	1.5×10^6	1.1×10^6
	1	7.9×10^5	2.4×10^6	1.6×10^6
	1.5	1.6×10^6	1.3×10^6	1.5×10^6
	2	9.8×10^6	1.8×10^7	1.4×10^7
	3	1.8×10^7	2.9×10^7	2.4×10^7
	4	1.5×10^7	9.5×10^7	5.5×10^7
1.0	0.5	2.2×10^6	9.0×10^5	1.6×10^6
	1	2.4×10^6	1.1×10^6	1.8×10^6
	1.5	1.4×10^6	1.0×10^6	1.2×10^6
	2	1.2×10^5	9.0×10^4	1.1×10^5
	3	1.8×10^4	1.0×10^4	1.4×10^4
	4	1.8×10^3	3.0×10^3	2.4×10^3

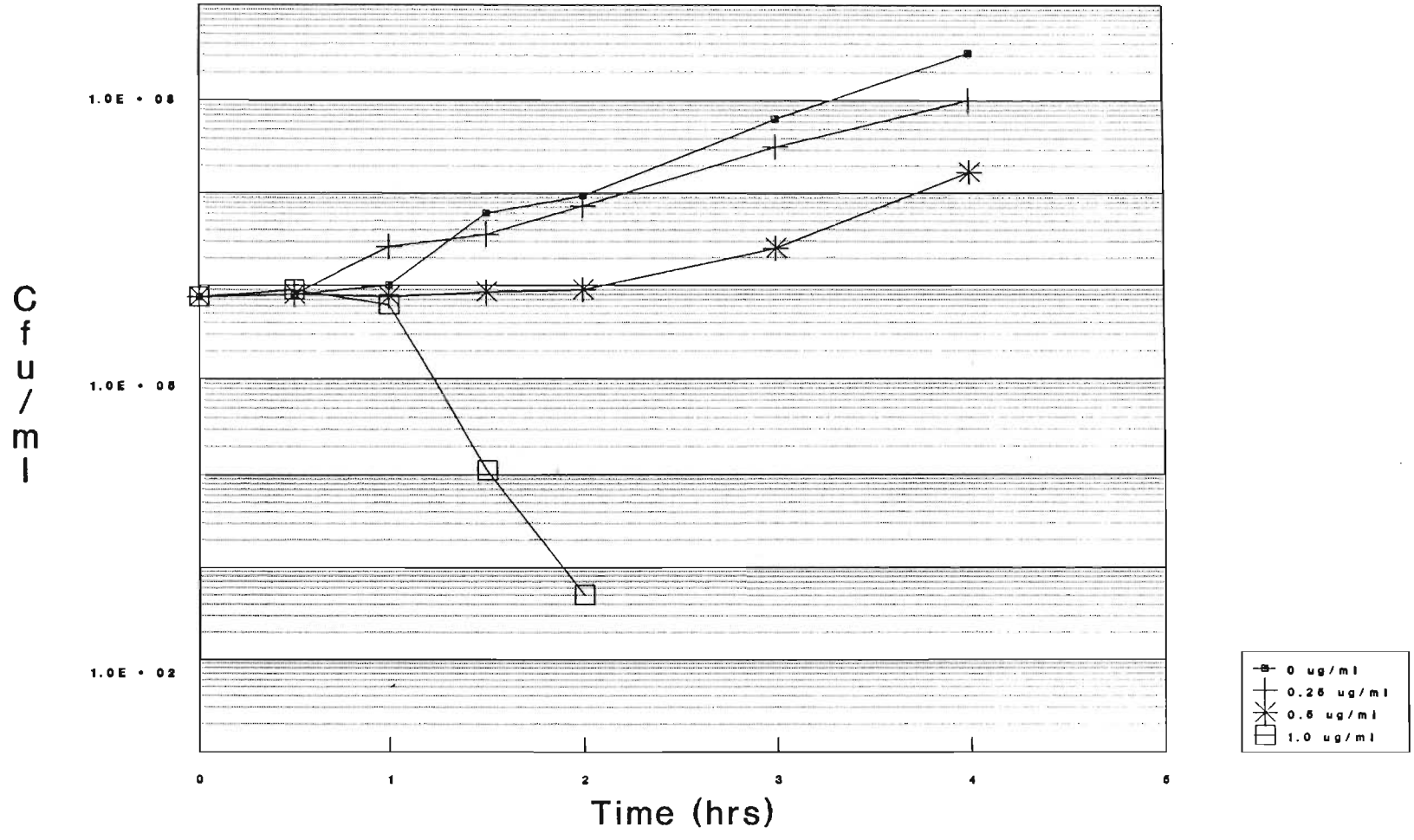


ID.6 Killing curve

ID 7

ORGANISM K.PNEUMONIAE

Drug concentration ($\mu g/m\ell$)	Time (hr)	Sample 1	Sample 2	Average
0	0	5.5×10^5	5.6×10^5	5.6×10^5
	0.5	6.3×10^5	6.2×10^6	6.3×10^5
	1	1.1×10^6	1.5×10^6	1.3×10^6
	1.5	3.8×10^6	4.2×10^6	4.0×10^6
	2	8.5×10^6	8.9×10^6	8.7×10^6
	3	4.8×10^7	4.0×10^7	4.4×10^7
	4	1.4×10^8	2.9×10^8	2.2×10^8
0.25	1	1.3×10^6	2.5×10^6	1.9×10^6
	1.5	2.5×10^6	2.0×10^6	2.3×10^6
	2	5.2×10^6	5.0×10^6	5.1×10^6
	3	1.4×10^7	3.0×10^7	2.2×10^7
	4	1.0×10^8	1.5×10^8	1.3×10^8
0.5	1	6.5×10^5	5.0×10^5	5.8×10^5
	1.5	8.9×10^5	5.5×10^5	7.2×10^5
	2	1.0×10^6	6.3×10^5	8.2×10^5
	3	1.0×10^6	3.0×10^6	2.0×10^6
	4	1.1×10^7	2.0×10^7	1.6×10^7
1.0	0.5	8.3×10^5	7.6×10^5	8.0×10^5
	1	4.3×10^5	4.1×10^5	4.2×10^5
	1.5	5.5×10^4	3.0×10^3	2.9×10^4
	2	4.0×10^2	2.5×10^2	3.3×10^2

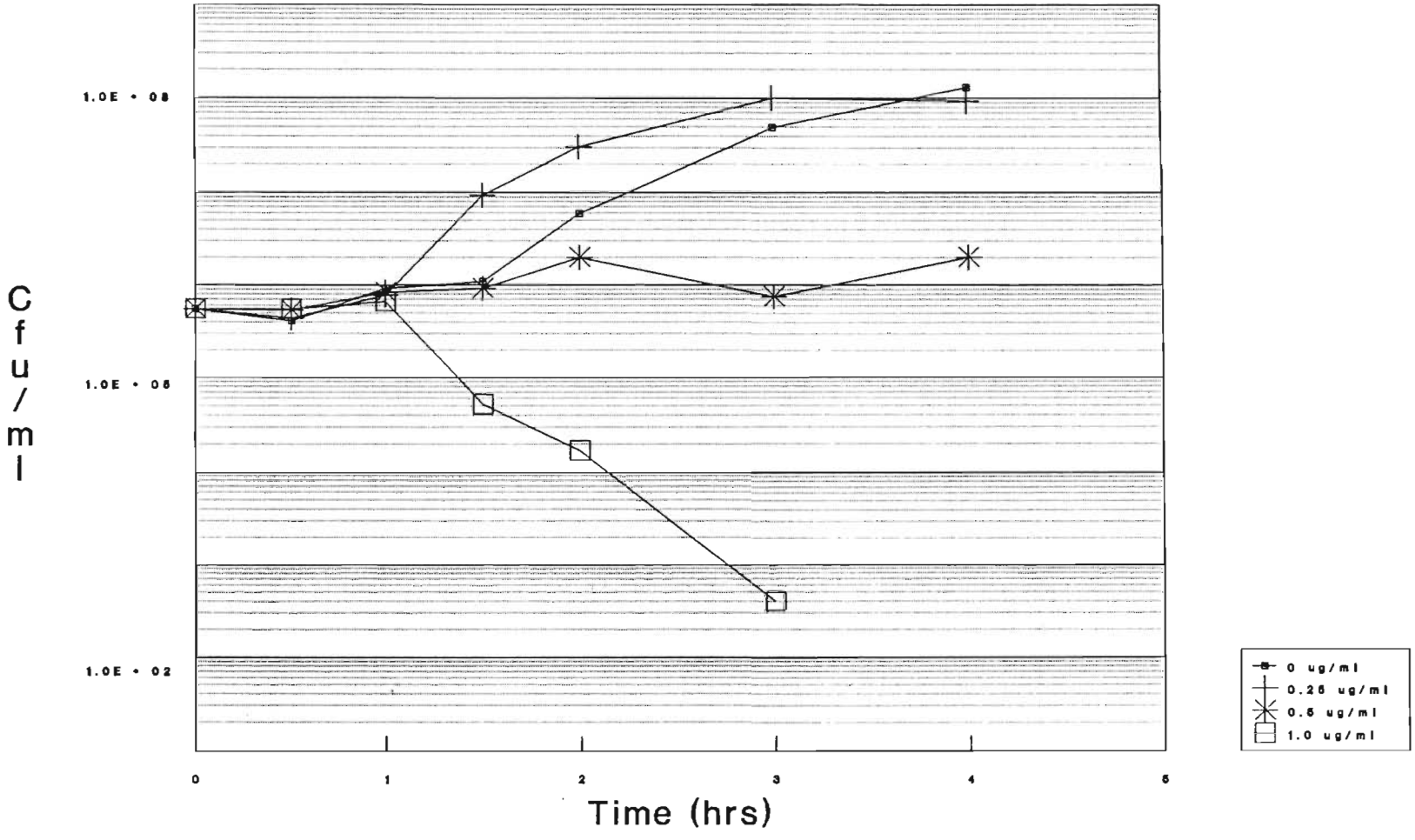


ID.7 Killing curve

ID 8

ORGANISM K.PNEUMONIAE

Drug concentration ($\mu g/m\ell$)	Time (hr)	Sample 1	Sample 2	Average
0	0	2.0×10^5	6.3×10^5	4.2×10^5
	0.5	3.3×10^5	2.0×10^5	2.7×10^5
	1	8.0×10^5	8.5×10^5	8.3×10^5
	1.5	1.2×10^6	1.3×10^6	1.3×10^6
	2	3.5×10^6	4.3×10^6	3.9×10^6
	3	1.6×10^7	6.0×10^7	3.8×10^7
	4	6.8×10^7	2.6×10^8	1.6×10^8
0.25	0.5	3.0×10^5	2.5×10^5	2.8×10^5
	1	5.2×10^5	7.0×10^5	6.1×10^5
	1.5	8.5×10^5	9.0×10^5	8.8×10^5
	2	2.9×10^6	1.5×10^6	2.2×10^6
	3	1.9×10^7	9.0×10^6	1.4×10^7
	4	8.2×10^7	9.0×10^7	8.6×10^7
0.5	0.5	4.0×10^5	3.0×10^5	3.5×10^5
	1	8.0×10^5	5.9×10^5	7.0×10^5
	1.5	9.9×10^5	7.0×10^5	8.5×10^5
	2	1.2×10^6	1.0×10^6	1.1×10^6
	3	3.5×10^5	8.8×10^5	6.2×10^5
	4	8.5×10^5	3.0×10^6	1.9×10^6
1.0	1	4.5×10^5	5.0×10^5	4.8×10^5
	1.5	3.0×10^4	3.7×10^4	3.4×10^4
	2	1.5×10^4	1.2×10^4	1.4×10^4
	3	3.0×10^2	2.0×10^2	2.5×10^2

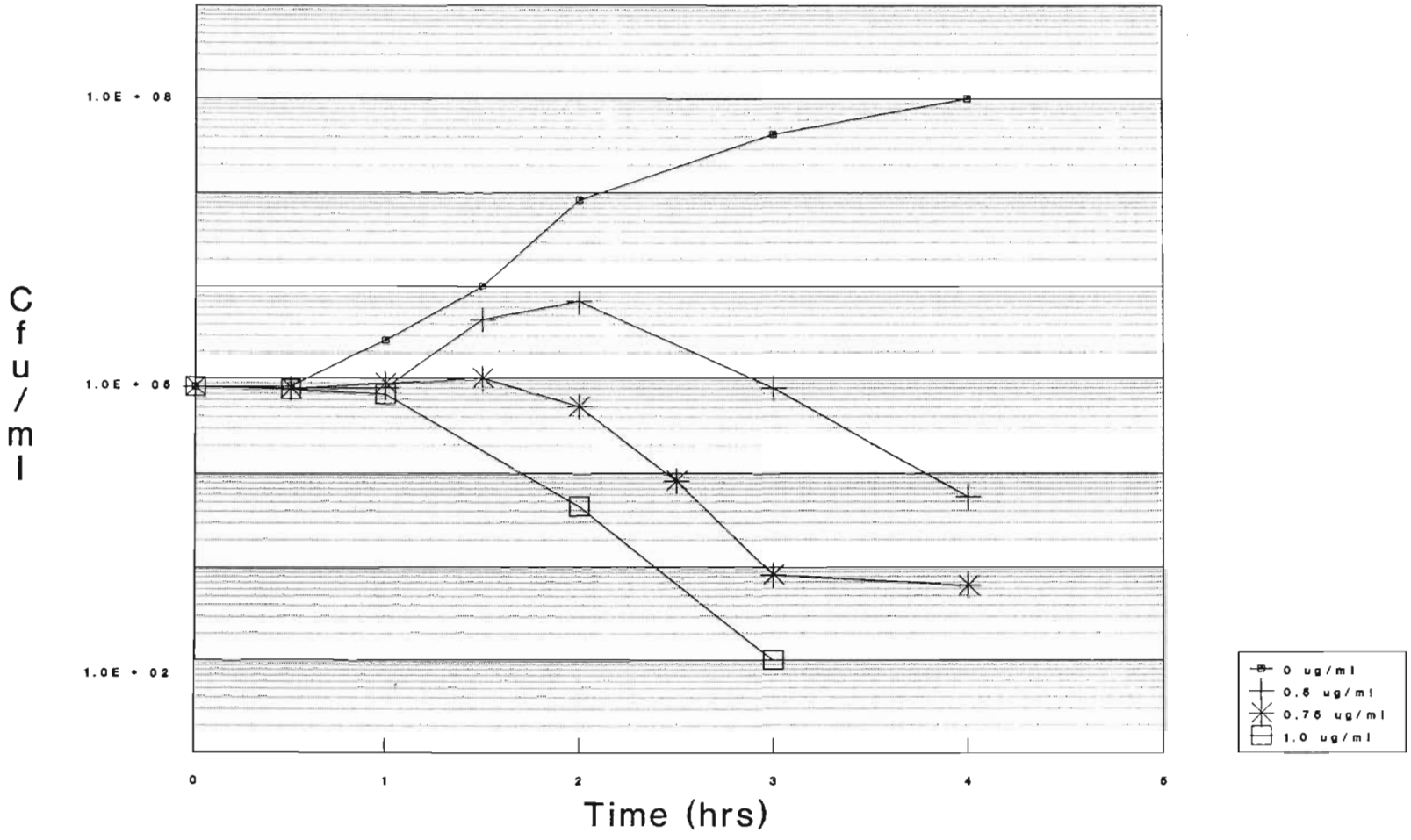


ID.8 Killing curve

ID 9

ORGANISM E.COLI

Drug concentration ($\mu g/m\ell$)	Time (hr)	Sample 1	Sample 2	Average
0	0	6.9×10^4	6.0×10^4	6.5×10^4
	0.5	6.8×10^4	6.2×10^4	6.5×10^4
	1	1.7×10^5	2.0×10^5	1.9×10^5
	1.5	1.5×10^6	9.3×10^5	1.2×10^6
	2	6.3×10^6	7.1×10^6	6.7×10^6
	3	1.3×10^7	4.0×10^7	2.7×10^7
	4	1.0×10^8	1.5×10^8	1.3×10^8
0.25	0.5	6.0×10^4	6.2×10^4	6.1×10^4
	1	6.3×10^4	6.0×10^4	6.2×10^4
	1.5	2.5×10^5	3.0×10^5	2.8×10^5
	2	4.7×10^5	5.0×10^5	4.9×10^5
	2.5	2.3×10^5	3.1×10^5	2.7×10^5
	3	5.8×10^4	6.7×10^4	6.3×10^4
	4	4.3×10^3	3.0×10^3	3.7×10^3
0.5	0.5	6.2×10^4	5.8×10^4	6.0×10^4
	1	7.2×10^4	8.0×10^4	7.6×10^4
	1.5	1.5×10^5	9.0×10^4	1.2×10^5
	2	3.3×10^4	3.1×10^4	3.2×10^4
	2.5	6.3×10^3	7.0×10^3	6.7×10^3
	3	7.6×10^2	6.0×10^2	6.8×10^2
	4	5.7×10^2	3.0×10^2	4.4×10^2
1.0	0.5	5.5×10^4	6.0×10^4	5.8×10^4
	1	5.0×10^4	4.5×10^4	4.8×10^4
	2	3.4×10^3	3.0×10^3	3.2×10^3
	3	1.0×10^2	1.5×10^2	1.3×10^2

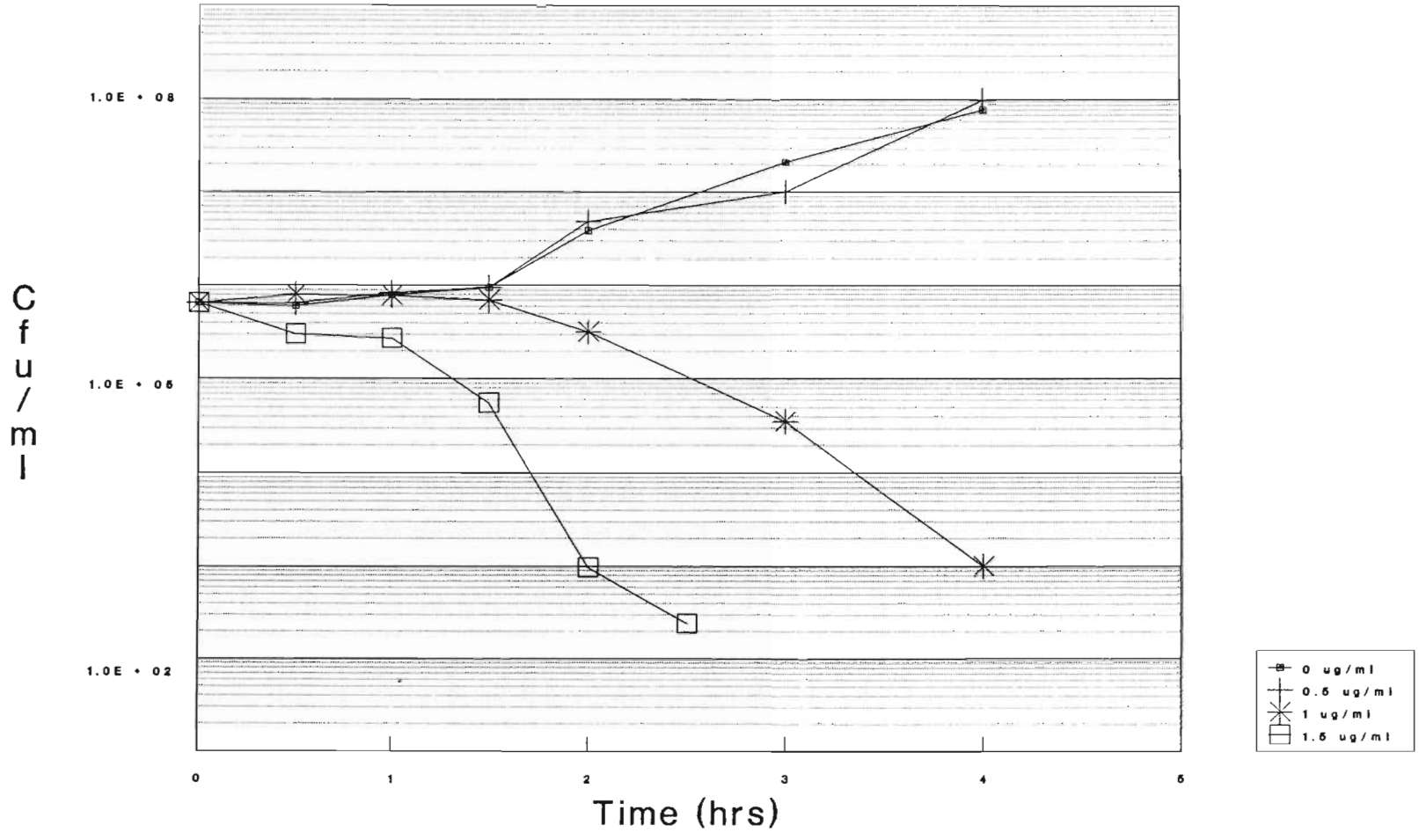


ID.9 Killing curve

ID 10

ORGANISM E.COLI

Drug concentration (μg/mℓ)	Time (hr)	Sample 1	Sample 2	Average
0	0	4.0×10^5	5.0×10^5	4.5×10^5
	0.5	4.2×10^5	3.8×10^5	4.0×10^5
	1	5.1×10^5	7.3×10^5	6.2×10^5
	1.5	8.8×10^5	9.0×10^5	8.9×10^5
	2	1.3×10^6	3.5×10^6	2.4×10^6
	3	1.5×10^7	1.8×10^7	1.7×10^7
	4	1.8×10^8	8.3×10^7	1.3×10^8
0.5	0.5	4.2×10^5	5.0×10^5	4.6×10^5
	1	6.5×10^5	7.0×10^5	6.8×10^5
	1.5	9.0×10^5	9.2×10^5	9.1×10^5
	2	2.8×10^6	3.0×10^6	2.9×10^6
	3	1.0×10^7	1.4×10^7	1.2×10^7
	4	9.8×10^7	9.6×10^7	9.7×10^7
1.0	0.5	2.0×10^5	1.8×10^5	1.9×10^5
	1	7.0×10^5	5.2×10^5	6.1×10^5
	1.5	5.8×10^5	4.0×10^5	4.9×10^5
	2	2.1×10^5	2.0×10^5	2.1×10^5
	3	4.0×10^4	4.8×10^3	2.2×10^4
	4	1.8×10^3	6.0×10^2	1.2×10^3
1.5	0.5	2.0×10^5	2.4×10^5	2.2×10^5
	1	1.7×10^5	2.0×10^5	1.9×10^5
	1.5	3.0×10^4	4.2×10^4	3.6×10^4
	2	1.2×10^3	7.0×10^2	9.5×10^2
	3.5	2.0×10^2	1.5×10^2	1.8×10^2

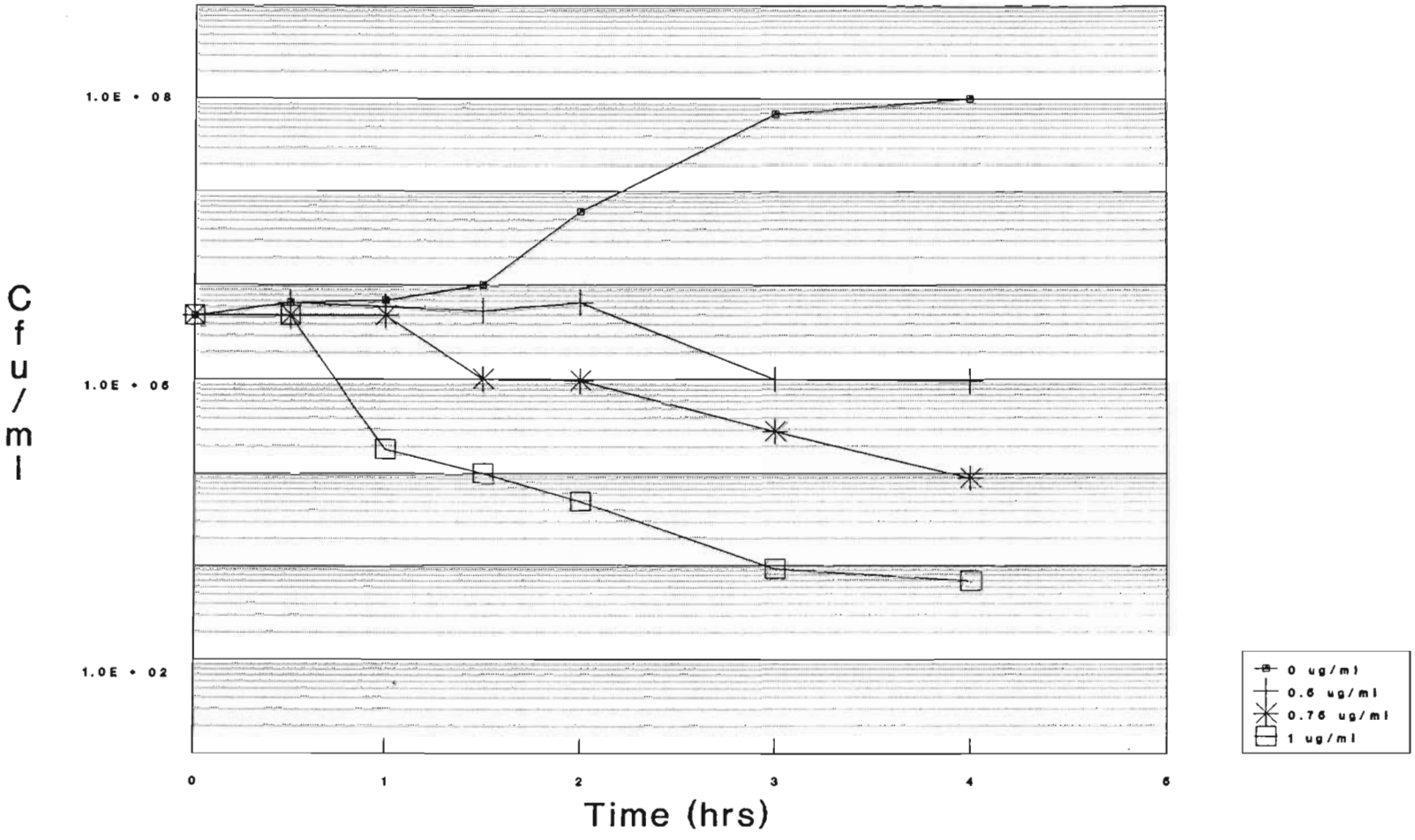


ID.10 Killing curve

ID 11

ORGANISM E.COLI

Drug concentration ($\mu g/m\ell$)	Time (hr)	Sample 1	Sample 2	Average
0	0	2.3×10^5	3.8×10^5	3.1×10^5
	0.5	2.5×10^5	6.6×10^5	4.6×10^5
	1	2.8×10^5	7.0×10^5	4.9×10^5
	1.5	1.2×10^6	1.1×10^6	1.2×10^6
	2	1.8×10^6	5.0×10^6	3.4×10^6
	3	6.0×10^7	3.5×10^7	4.8×10^7
	4	1.5×10^8	1.1×10^8	1.3×10^8
0.25	1	3.8×10^5	4.0×10^5	3.9×10^5
	1.5	3.4×10^5	3.6×10^5	3.5×10^5
	2	3.7×10^5	5.5×10^5	4.6×10^5
	3	1.0×10^5	1.2×10^5	1.1×10^5
	4	9.8×10^4	9.0×10^4	9.4×10^4
0.75	1	2.9×10^5	3.1×10^5	3.0×10^5
	1.5	1.4×10^5	1.0×10^5	1.2×10^5
	2	9.3×10^4	8.0×10^4	8.7×10^4
	3	1.8×10^4	2.0×10^4	1.9×10^4
	4	7.5×10^3	8.0×10^3	7.8×10^3
1.0	1	4.7×10^4	1.5×10^4	3.1×10^4
	1.5	1.1×10^4	1.0×10^4	1.1×10^4
	2	3.3×10^3	3.0×10^3	3.2×10^3
	3	9.0×10^2	8.0×10^2	8.5×10^2
	4	4.6×10^2	5.0×10^2	4.8×10^2

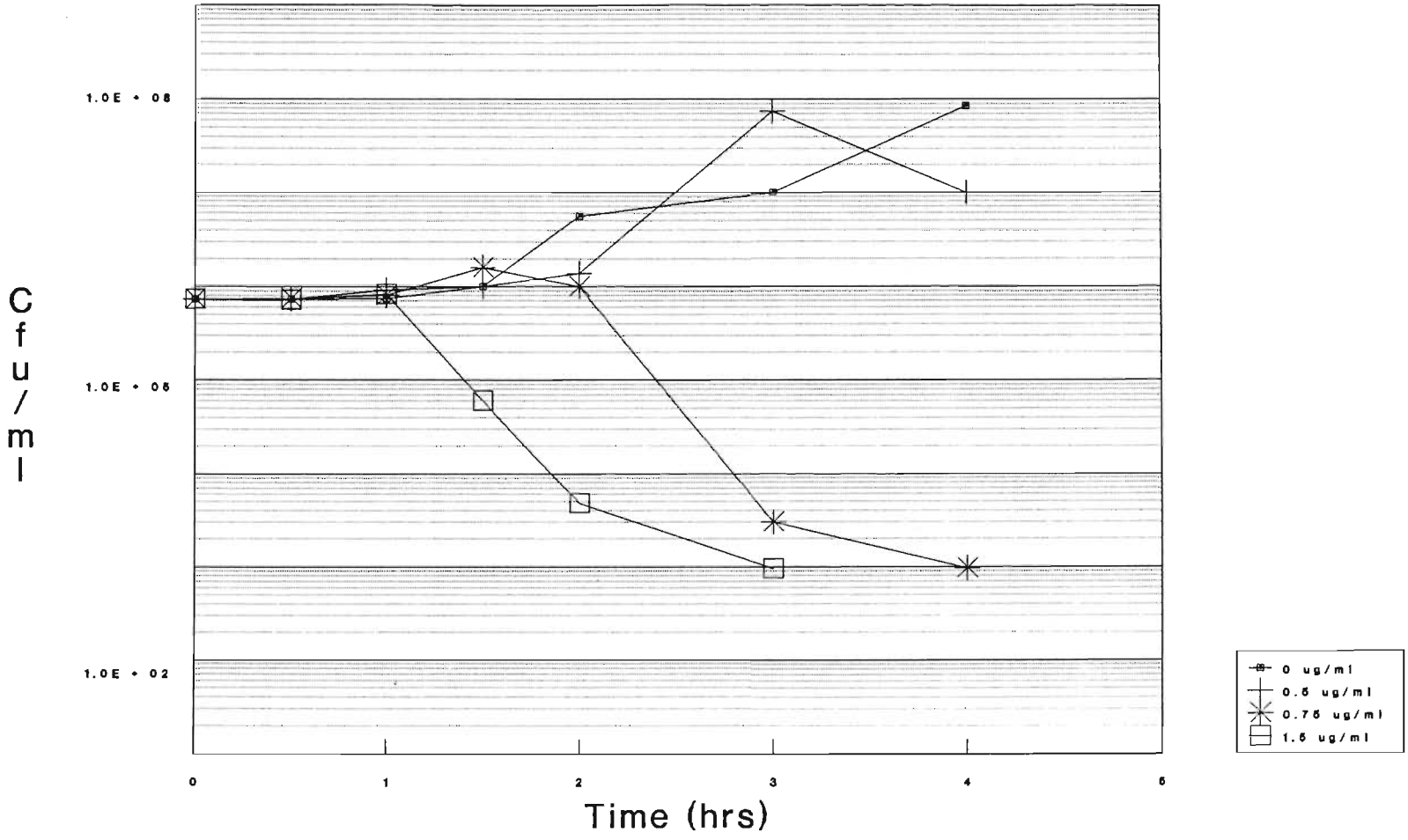


ID.11 Killing curve

ID 12

ORGANISM E.COLI

Drug concentration ($\mu g/m\ell$)	Time (hr)	Sample 1	Sample 2	Average
0	0	5.2×10^5	5.0×10^5	5.1×10^5
	0.5	5.2×10^5	5.5×10^5	5.4×10^5
	1	5.6×10^5	5.8×10^5	5.7×10^5
	1.5	1.1×10^6	2.0×10^6	1.6×10^6
	2	3.6×10^6	4.2×10^6	3.9×10^6
	3	1.1×10^7	1.0×10^7	1.1×10^7
	4	6.3×10^7	8.0×10^7	7.2×10^7
0.25	0.5	5.5×10^5	5.0×10^5	5.3×10^5
	1	8.3×10^5	7.0×10^5	7.7×10^5
	1.5	1.3×10^6	1.0×10^6	1.2×10^6
	2	1.4×10^6	1.5×10^6	1.5×10^6
	3	5.5×10^6	6.0×10^6	5.8×10^6
	4	1.2×10^7	1.0×10^7	1.1×10^7
1	0.5	5.5×10^5	5.7×10^5	5.6×10^5
	1	7.3×10^5	5.5×10^5	6.4×10^5
	1.5	1.6×10^6	1.2×10^6	1.4×10^6
	2	1.5×10^6	1.0×10^6	1.3×10^6
	3	6.7×10^3	3.3×10^4	2.0×10^4
	4	5.1×10^2	1.4×10^3	9.6×10^2
1.5	0	5.5×10^5	5.0×10^5	5.3×10^5
	0.5	5.4×10^5	6.0×10^5	5.7×10^5
	1	7.7×10^5	7.0×10^5	7.4×10^5
	1.5	2.0×10^4	6.0×10^4	4.0×10^4
	2	2.2×10^3	3.0×10^3	2.6×10^3
	3	9.0×10^2	8.0×10^2	8.5×10^2

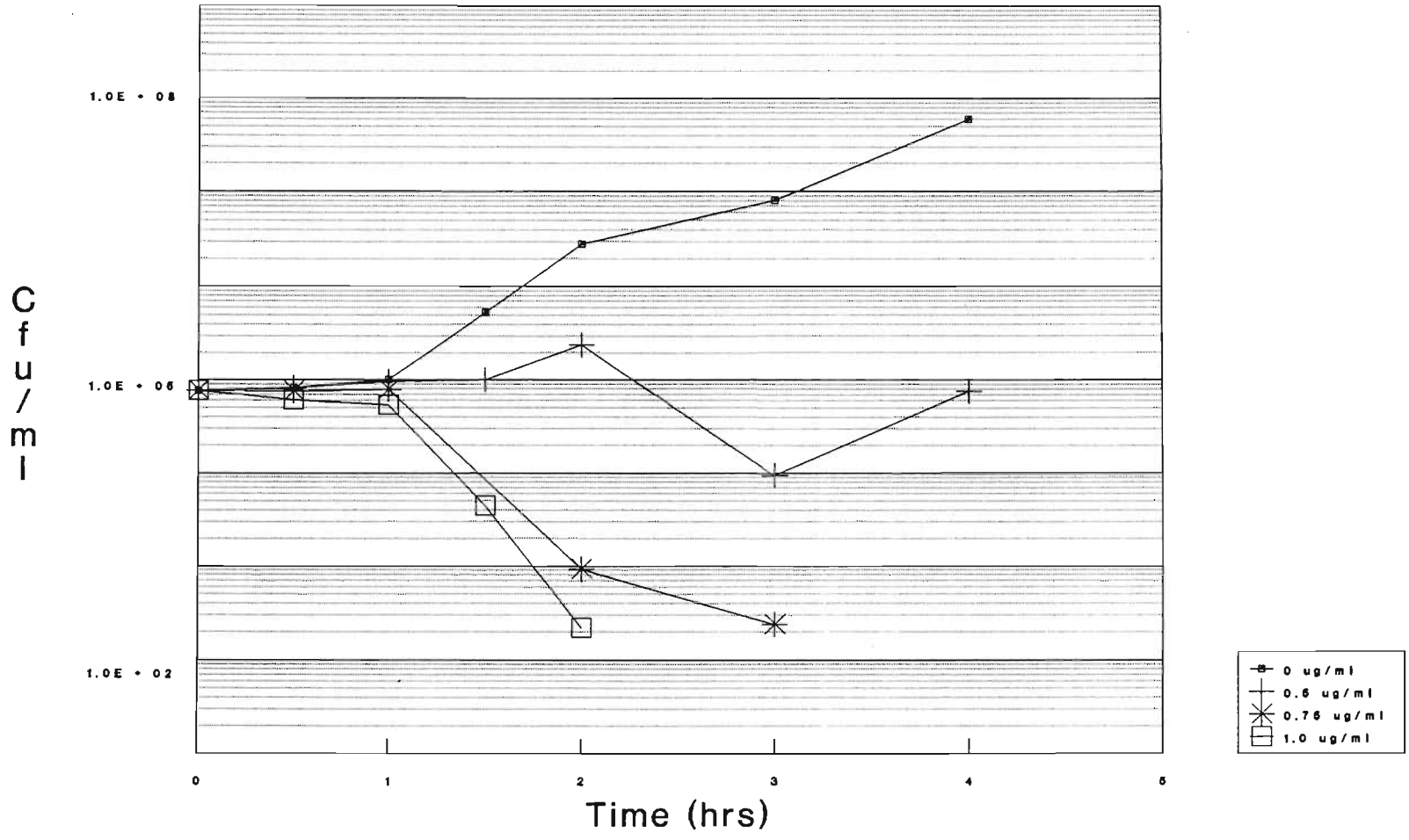


ID.12 Killing curve

ID 13

ORGANISM E.COLI

Drug concen = tration (μg/mℓ)	Time (hr)	Sample 1	Sample 2	Average
0	0	4.7×10^4	7.2×10^4	6.0×10^4
	0.5	5.5×10^4	8.0×10^4	6.8×10^4
	1	9.0×10^4	1.2×10^5	1.1×10^5
	1.5	3.9×10^5	2.8×10^5	3.4×10^5
	2	2.5×10^6	1.3×10^6	1.9×10^6
	3	5.0×10^6	7.8×10^6	6.4×10^6
	4	2.7×10^7	5.2×10^7	4.0×10^7
0.5	1	8.5×10^4	1.0×10^5	9.3×10^4
	1.5	8.3×10^4	1.2×10^5	1.0×10^5
	2	9.5×10^4	1.3×10^5	1.1×10^5
	2.5	5.2×10^4	7.8×10^4	6.5×10^4
	3	6.8×10^3	1.1×10^4	8.9×10^3
	4	4.0×10^3	9.0×10^3	6.5×10^3
0.75	0.5	4.6×10^4	7.0×10^4	5.8×10^4
	1	6.3×10^4	6.0×10^4	6.2×10^4
	2	8.0×10^2	9.0×10^2	8.5×10^2
	3	1.5×10^2	2.0×10^2	1.8×10^2
1.0	0.5	4.2×10^4	4.0×10^4	4.1×10^4
	1	4.0×10^4	3.0×10^4	3.5×10^4
	1.5	2.5×10^3	3.0×10^3	2.8×10^3
	2	1.3×10^2	2.0×10^2	1.7×10^2

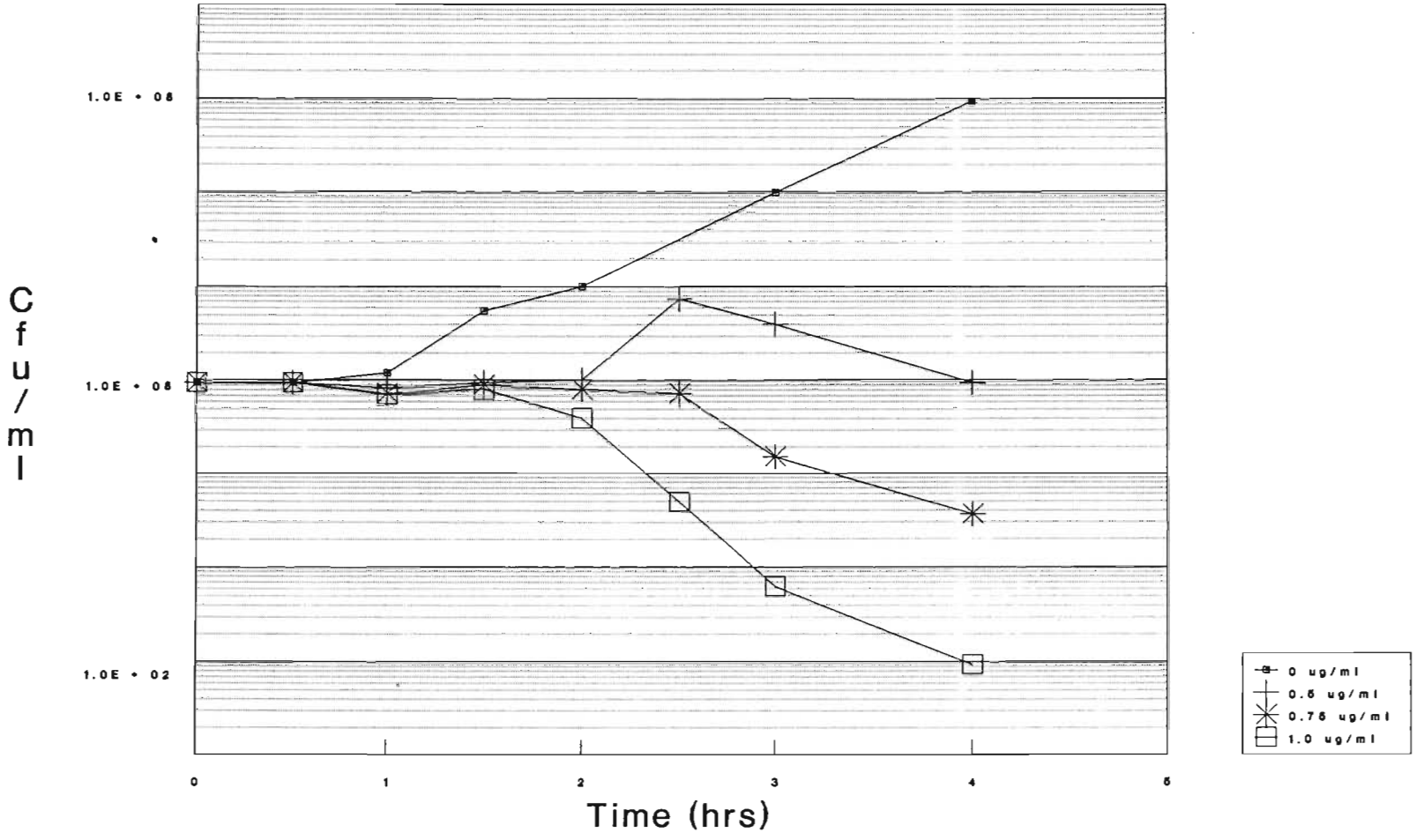


ID.13 Killing curve

ID 14

ORGANISM E.COLI

Drug concentration ($\mu g/m\ell$)	Time (hr)	Sample 1	Sample 2	Average
0	0	9.5×10^4	8.0×10^4	8.8×10^4
	0.5	9.7×10^4	8.8×10^4	9.3×10^4
	1	1.6×10^5	1.0×10^5	1.3×10^5
	1.5	4.5×10^5	2.5×10^5	3.5×10^5
	2	1.2×10^6	8.0×10^5	1.0×10^6
	3	1.0×10^7	9.0×10^6	9.5×10^6
	4	9.0×10^7	8.0×10^7	8.5×10^7
0.5	1	6.5×10^4	7.0×10^4	6.8×10^4
	1.5	9.0×10^4	8.5×10^4	8.8×10^4
	2	1.2×10^5	9.0×10^4	1.1×10^5
	2.5	6.0×10^5	5.0×10^5	5.5×10^5
	3	3.0×10^5	2.0×10^5	2.5×10^5
	4	9.5×10^4	8.0×10^4	8.8×10^4
0.75	1	6.0×10^4	5.5×10^4	5.8×10^4
	1.5	8.0×10^4	7.0×10^4	7.5×10^4
	2	6.0×10^4	6.3×10^4	6.2×10^4
	2.5	5.5×10^4	5.0×10^4	5.3×10^4
	3	2.0×10^4	1.0×10^4	1.5×10^4
	4	1.6×10^3	3.0×10^3	2.3×10^3
1	1	4.7×10^4	5.3×10^4	5.0×10^4
	1.5	5.5×10^4	7.0×10^4	6.3×10^4
	2	2.2×10^4	2.8×10^4	2.5×10^4
	2.5	3.2×10^3	3.0×10^3	3.1×10^3
	3	4.0×10^2	4.3×10^2	4.2×10^2
	4	9.0×10^1	8.0×10^1	8.5×10^1

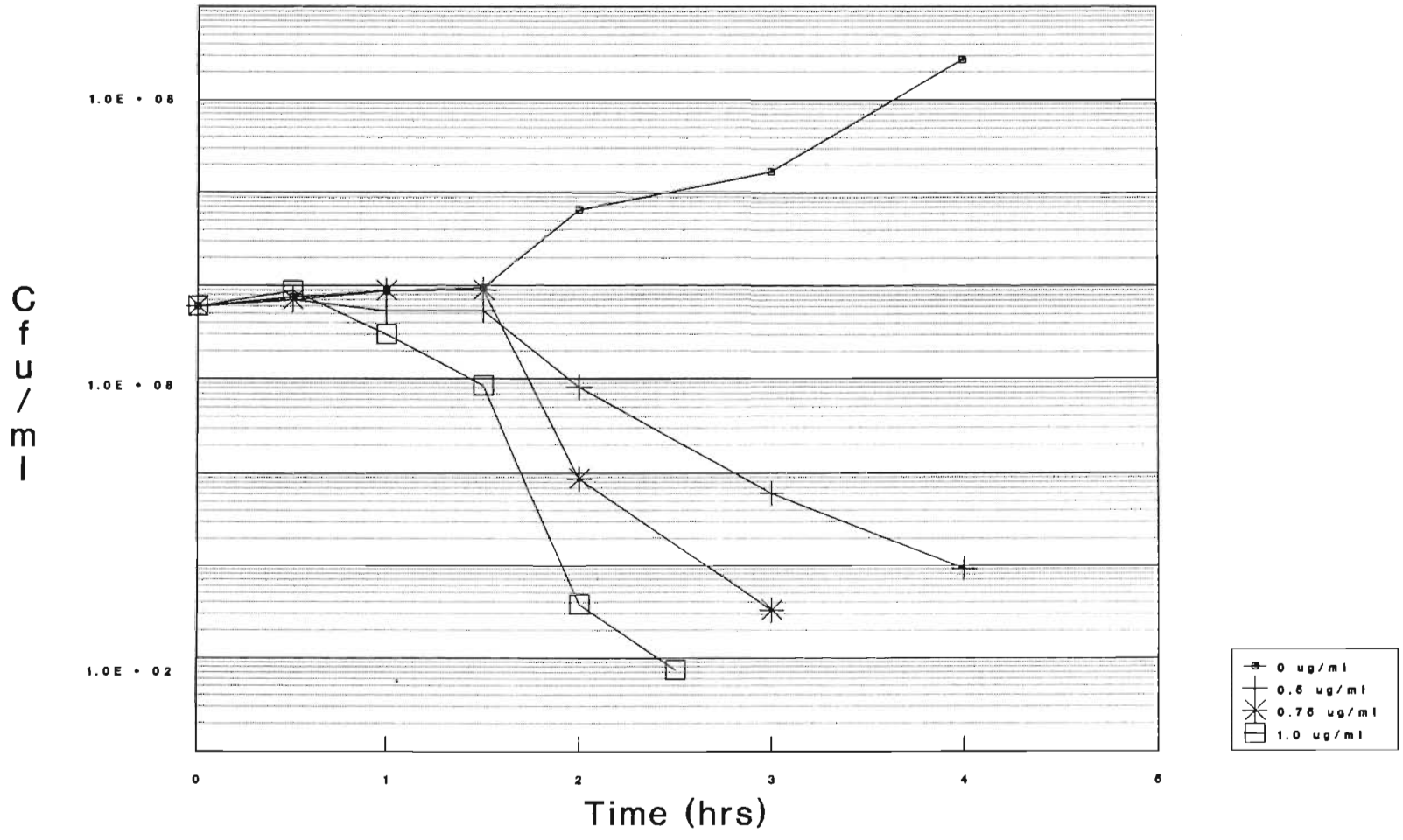


ID.14 Killing curve

ID 15

ORGANISM E.COLI

Drug concen = tration (μg/mℓ)	Time (hr)	Sample 1	Sample 2	Average
0	0	6.0×10^5	2.0×10^5	4.0×10^5
	0.5	8.6×10^5	3.0×10^5	5.8×10^5
	1	8.0×10^5	7.3×10^5	7.7×10^5
	1.5	7.7×10^5	1.0×10^6	8.9×10^5
	2	6.2×10^6	3.0×10^6	4.6×10^6
	3	1.2×10^7	1.8×10^7	1.5×10^7
	4	2.9×10^8	8.3×10^7	1.9×10^8
0.5	0.5	6.0×10^5	4.0×10^5	5.0×10^5
	1	5.0×10^5	5.2×10^5	5.1×10^5
	1.5	3.0×10^5	4.0×10^5	3.5×10^5
	2	7.0×10^4	6.0×10^4	6.5×10^4
	3	5.0×10^3	3.0×10^3	4.0×10^3
	4	9.0×10^2	8.0×10^2	8.5×10^2
0.75	0.5	6.0×10^5	4.0×10^5	5.0×10^5
	1	8.0×10^5	7.0×10^5	7.5×10^5
	1.5	7.5×10^5	8.5×10^5	8.0×10^5
	2	8.5×10^3	6.0×10^3	7.3×10^3
	3	8.5×10^2	5.0×10^2	6.8×10^2
1.0	0.5	7.0×10^5	8.0×10^5	7.5×10^5
	1	2.5×10^5	1.5×10^5	2.0×10^5
	1.5	7.3×10^4	7.0×10^4	7.2×10^4
	2	2.8×10^2	2.0×10^2	2.4×10^2
	2.5	5.0×10^2	6.0×10^2	5.5×10^2



ID.15 Killing curve

APPENDIX D

ID 1

ORGANISM K. PNEUMONIAE

During antibiotic exposure

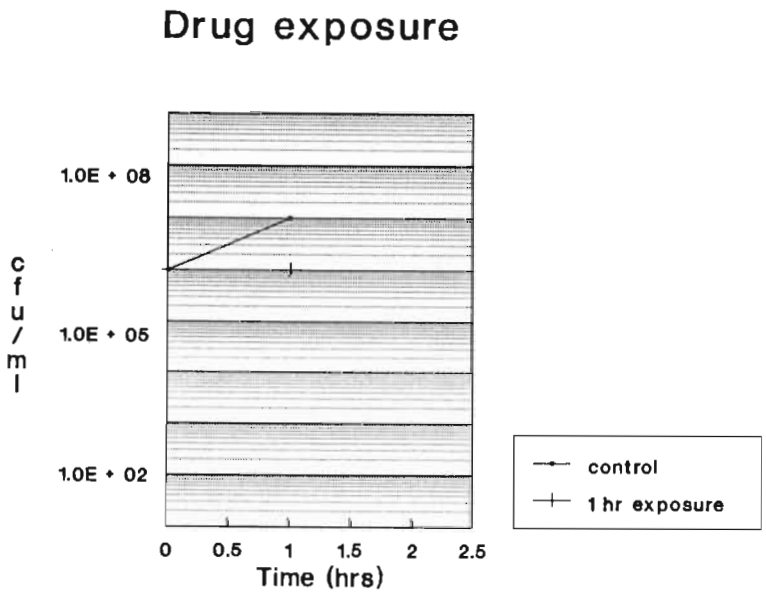
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	1.3×10^7	9.5×10^6	1.1×10^7	-	-	-
1	7.9×10^8	8.7×10^8	8.3×10^8	4.0×10^6	8.5×10^6	6.3×10^6
2	6.2×10^9	7.1×10^9	6.7×10^9	2.0×10^6	1.5×10^6	1.8×10^6

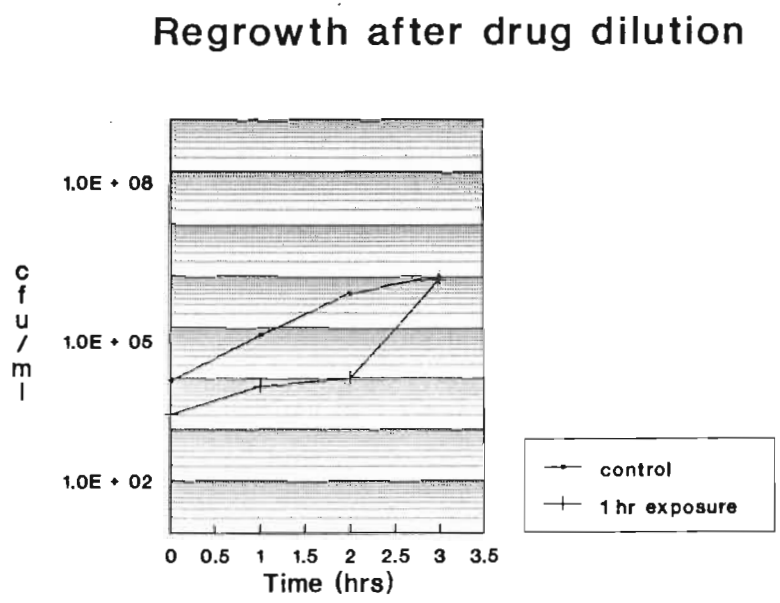
1 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	2.0×10^5	4.6×10^5	3.3×10^5	3.4×10^5	3.0×10^5	3.2×10^5
1	1.8×10^6	2.0×10^6	1.9×10^6	6.0×10^5	6.3×10^5	6.2×10^5
2	1.0×10^7	1.2×10^7	1.1×10^7	4.8×10^6	4.7×10^6	4.8×10^6
3	7.6×10^7	7.9×10^7	7.8×10^7	5.8×10^7	6.0×10^7	5.9×10^7

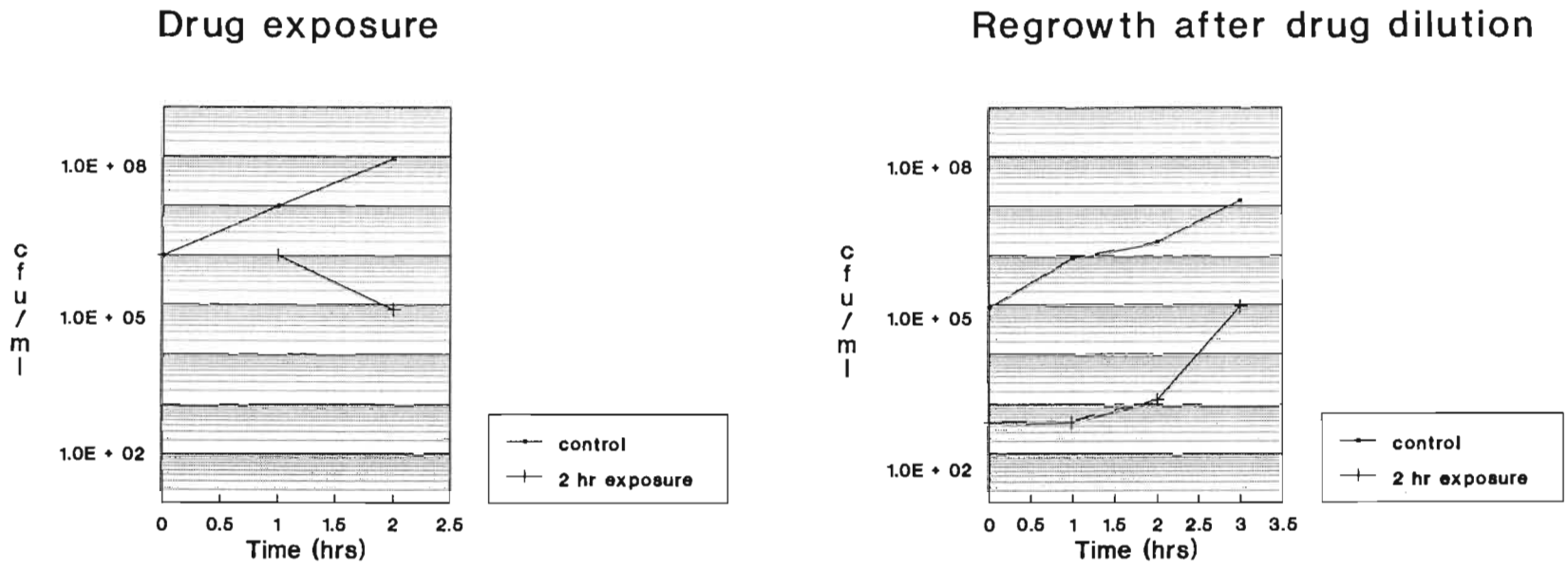
2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	2.1×10^3	2.0×10^3	2.1×10^3	1.4×10^3	1.6×10^3	1.5×10^3
1	1.1×10^4	1.3×10^4	1.2×10^4	3.5×10^3	4.0×10^3	3.8×10^3
2	6.2×10^4	7.9×10^4	7.1×10^4	9.5×10^3	8.9×10^3	9.2×10^3
3	7.6×10^5	7.9×10^5	7.8×10^5	5.0×10^4	5.4×10^4	5.2×10^4





ID.1 Post antibiotic effect after 1 hour drug exposure



ID.1 Post antibiotic effect after 2 hour drug exposure

ID 2

ORGANISM K. PNEUMONIAE

During antibiotic exposure

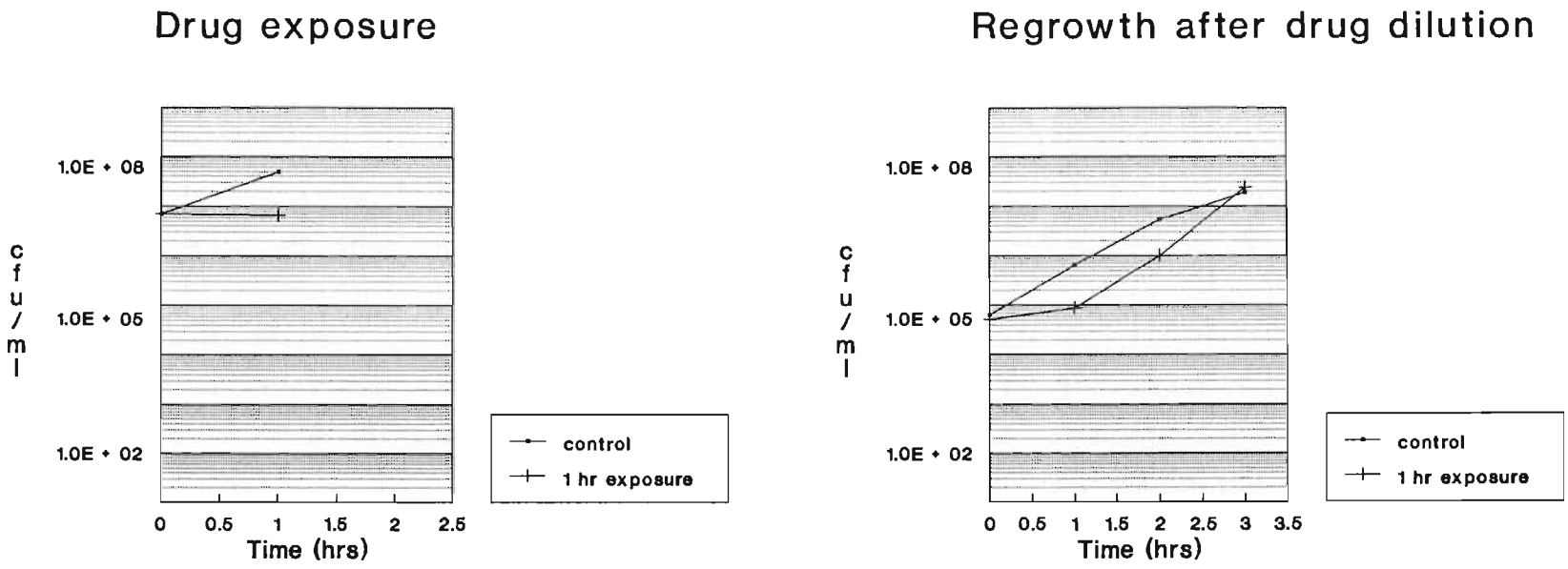
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.2×10^6	5.0×10^6	5.1×10^6	-	-	-
1	3.0×10^7	2.5×10^7	2.8×10^7	4.6×10^6	4.8×10^6	4.7×10^6
2	1.0×10^8	9.0×10^7	9.5×10^7	4.0×10^6	4.2×10^6	4.1×10^6

1 Hour exposure

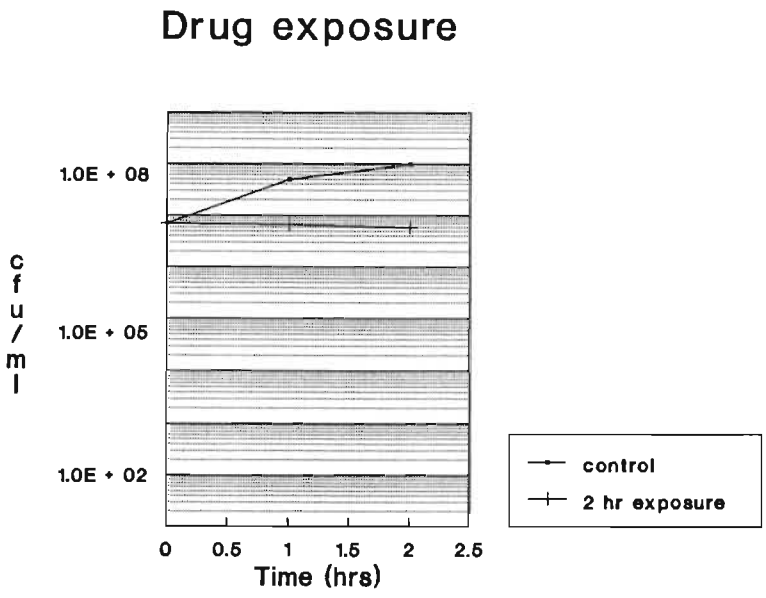
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	4.0×10^4	4.5×10^4	4.3×10^4	3.4×10^4	3.5×10^4	3.5×10^4
1	5.0×10^4	4.5×10^4	4.8×10^4	8.0×10^4	7.0×10^4	7.5×10^4
2	4.0×10^6	3.0×10^6	3.5×10^6	1.3×10^6	8.0×10^5	1.1×10^6
3	2.0×10^7	1.2×10^7	1.6×10^7	1.5×10^7	2.0×10^7	1.8×10^7

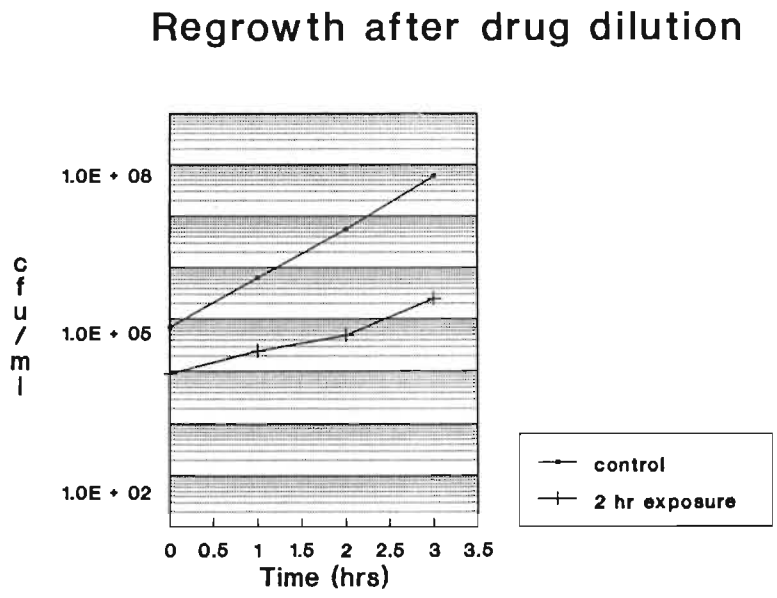
2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	6.0×10^4	4.0×10^4	5.0×10^4	8.0×10^3	8.4×10^3	8.2×10^3
1	6.0×10^5	3.0×10^5	4.5×10^5	1.5×10^4	2.0×10^4	1.8×10^4
2	6.2×10^6	2.0×10^6	4.1×10^6	3.0×10^4	3.4×10^4	3.2×10^4
3	5.0×10^7	3.0×10^7	4.0×10^7	2.6×10^5	1.2×10^5	1.9×10^5



ID.2 Post antibiotic effect after 1 hour drug exposure





ID.2 Post antibiotic effect after 2 hour drug exposure

ID 3

ORGANISM K. PNEUMONIAE

During antibiotic exposure

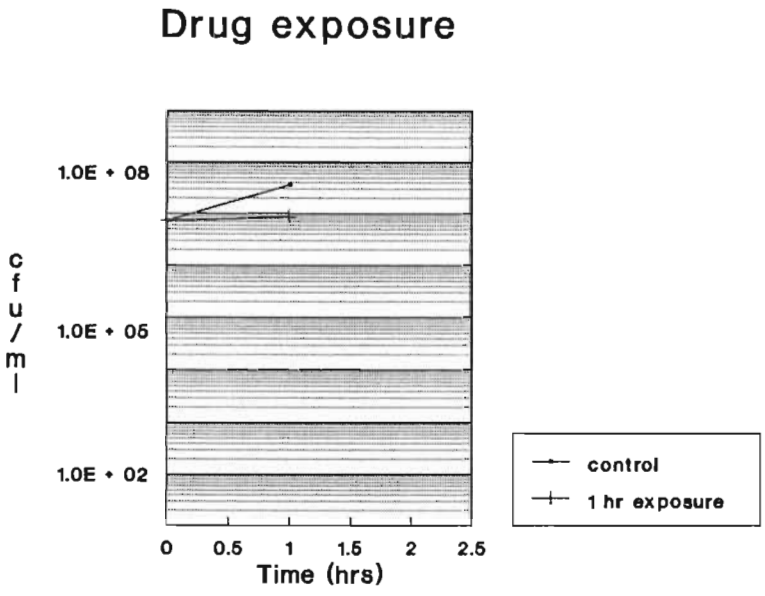
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.1×10^6	5.5×10^6	5.3×10^6	-	-	-
1	5.2×10^7	4.6×10^7	4.9×10^7	7.5×10^6	6.8×10^6	7.1×10^6
2	6.0×10^7	7.3×10^7	6.7×10^7	1.0×10^6	3.0×10^6	2.0×10^6

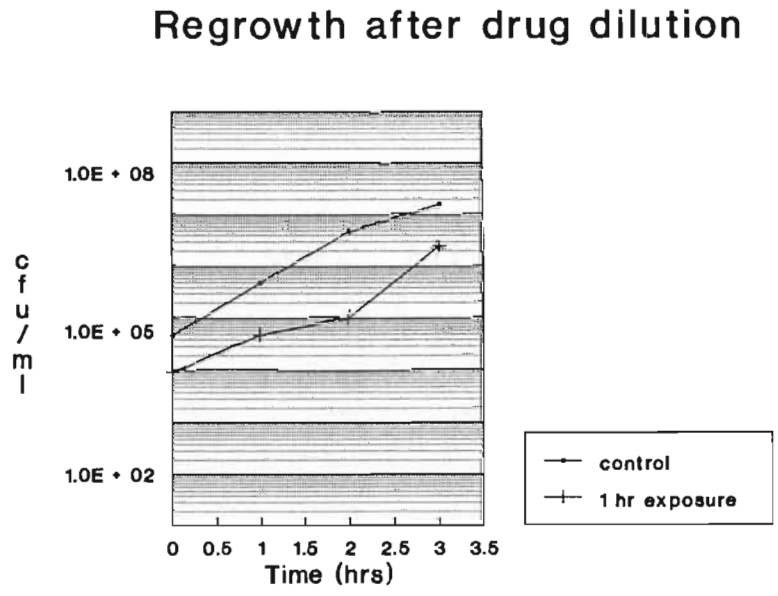
1 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.0×10^4	1.5×10^4	3.3×10^4	9.5×10^3	7.0×10^3	8.3×10^3
1	6.0×10^5	1.5×10^5	3.8×10^5	2.0×10^4	1.8×10^4	1.9×10^4
2	5.0×10^6	1.6×10^6	3.3×10^6	1.3×10^5	1.0×10^5	1.2×10^5
3	1.4×10^7	1.5×10^7	1.5×10^7	1.4×10^6	2.0×10^6	1.7×10^6

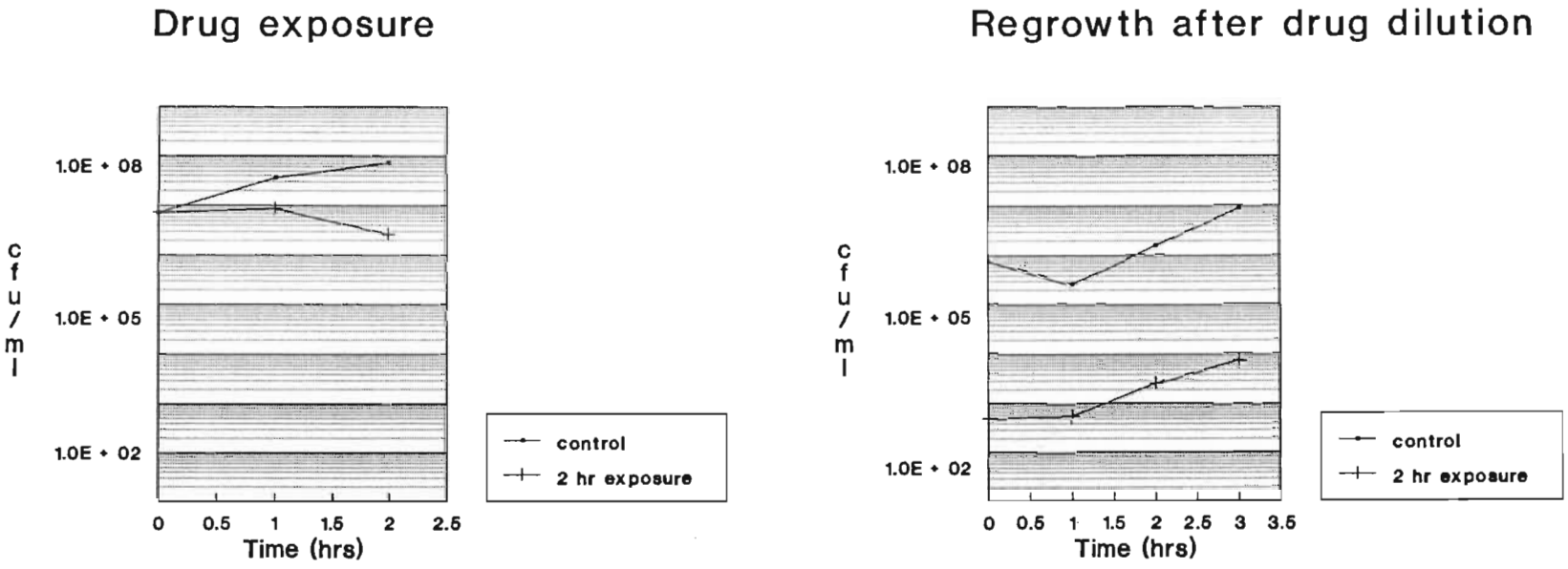
2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	2.0×10^5	4.0×10^5	3.0×10^5	4.5×10^2	2.0×10^2	3.3×10^2
1	1.0×10^6	3.0×10^6	2.0×10^6	4.5×10^2	2.7×10^2	3.8×10^2
2	1.0×10^7	3.0×10^7	2.0×10^7	1.3×10^3	1.0×10^3	1.2×10^3
3	6.3×10^7	8.8×10^7	7.6×10^7	6.0×10^3	5.0×10^3	5.5×10^3





ID.3 Post antibiotic effect after 1 hour drug exposure



ID.3 Post antibiotic effect after 2 hour drug exposure

ID 4

ORGANISM K. PNEUMONIAE

During antibiotic exposure

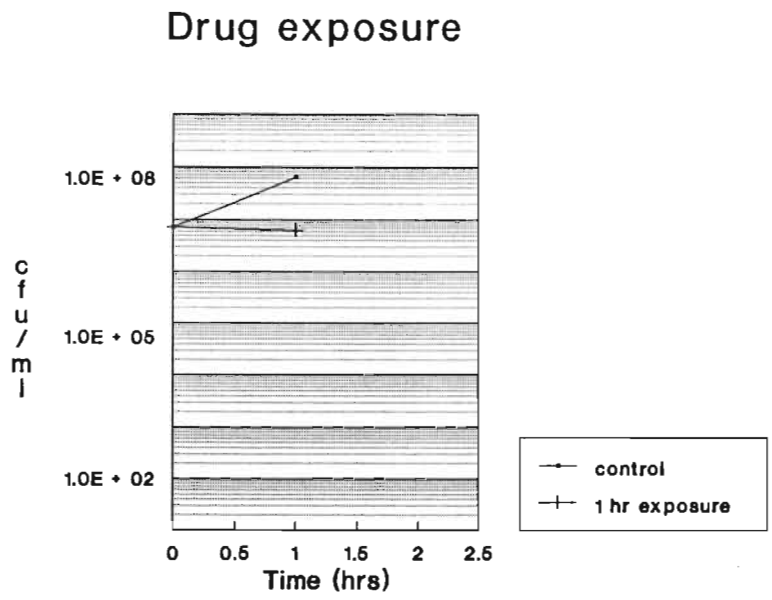
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.0×10^{6}	5.7×10^{6}	5.4×10^{6}	-	-	-
1	4.0×10^{7}	5.0×10^{7}	4.5×10^{7}	4.6×10^{5}	3.8×10^{6}	2.1×10^{6}
2	2.5×10^{8}	6.0×10^{8}	4.3×10^{8}	3.0×10^{2}	2.0×10^{2}	2.5×10^{2}

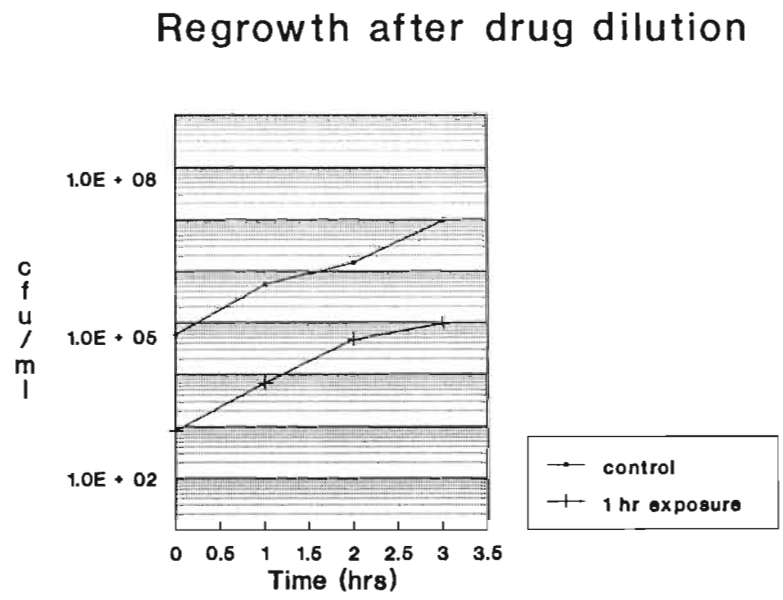
1 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	3.6×10^{4}	4.0×10^{4}	3.8×10^{4}	5.8×10^{2}	8.0×10^{2}	6.9×10^{2}
1	4.0×10^{5}	3.0×10^{5}	3.5×10^{5}	6.7×10^{2}	3.8×10^{3}	2.2×10^{3}
2	1.2×10^{6}	1.5×10^{6}	1.4×10^{6}	2.0×10^{3}	4.3×10^{4}	2.3×10^{4}
3	9.3×10^{6}	9.0×10^{6}	9.2×10^{6}	9.3×10^{3}	9.5×10^{4}	5.2×10^{4}

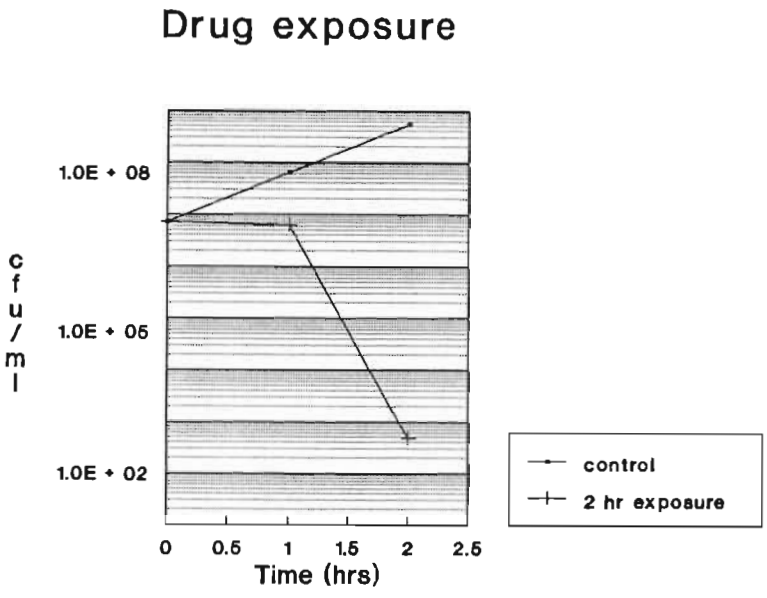
2 Hour exposure

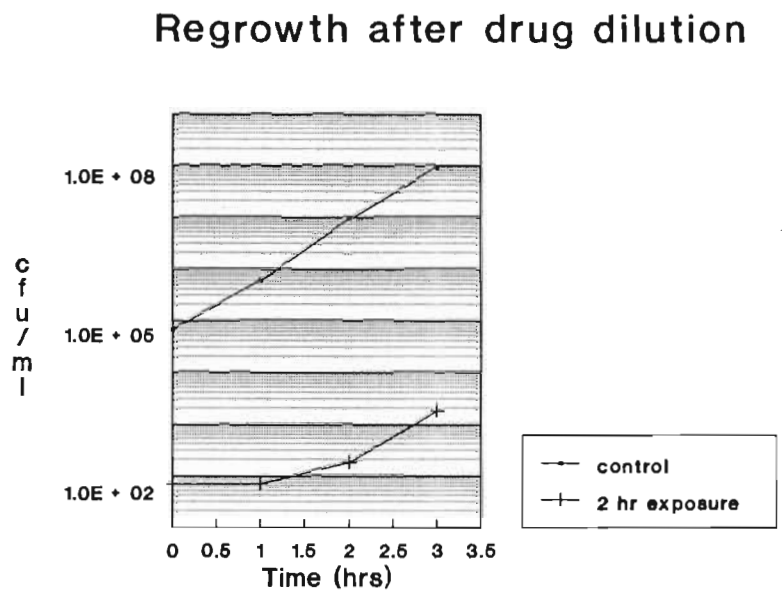
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	4.0×10^{4}	6.0×10^{4}	5.0×10^{4}	5.0×10^{1}	5.2×10^{1}	5.1×10^{1}
1	8.5×10^{5}	7.0×10^{5}	7.8×10^{5}	5.0×10^{1}	4.6×10^{1}	4.8×10^{1}
2	8.0×10^{6}	8.3×10^{6}	8.2×10^{6}	1.5×10^{2}	1.7×10^{2}	1.6×10^{2}
3	7.5×10^{7}	8.5×10^{7}	8.0×10^{7}	1.7×10^{3}	1.3×10^{3}	1.5×10^{3}





ID.4 Post antibiotic effect after 1 hour drug exposure





ID.4 Post antibiotic effect after 2 hour drug exposure

ID 5

ORGANISM K. PNEUMONIAE

During antibiotic exposure

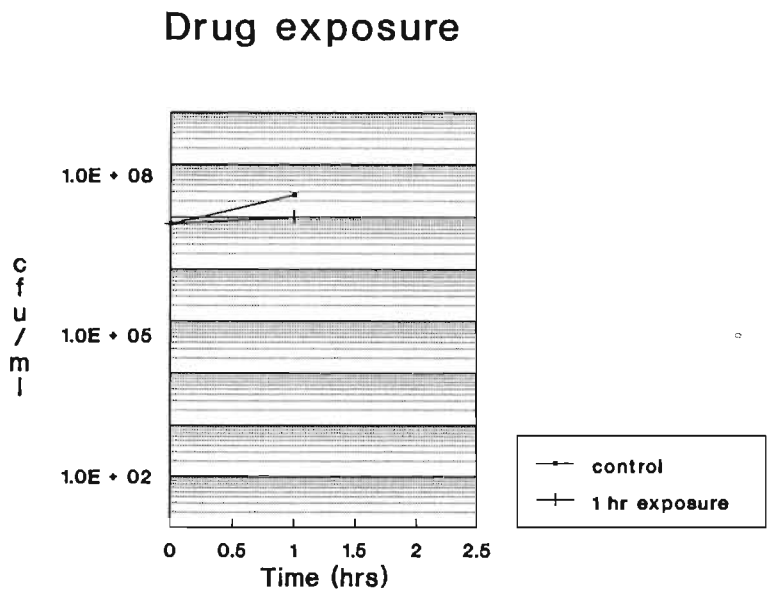
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	6.2×10^6	5.0×10^6	5.6×10^6	-	-	-
1	3.0×10^7	1.1×10^7	2.1×10^7	1.3×10^7	1.0×10^7	1.2×10^7
2	1.7×10^8	6.0×10^8	3.9×10^8	1.0×10^6	1.3×10^6	1.2×10^6

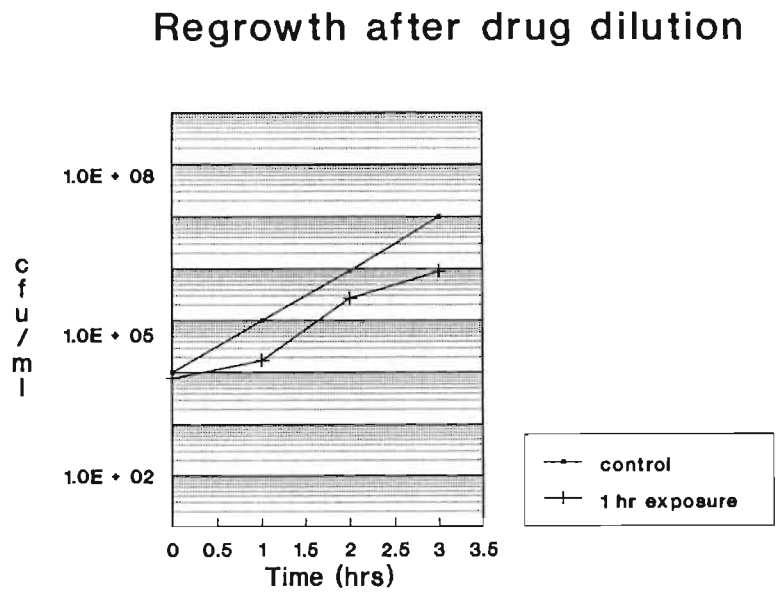
1 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	1.0×10^4	1.3×10^4	1.2×10^4	5.0×10^3	6.8×10^3	5.9×10^3
1	1.2×10^5	1.1×10^5	1.2×10^5	1.5×10^4	1.7×10^4	1.6×10^4
2	9.5×10^5	7.5×10^5	8.5×10^5	1.2×10^5	2.8×10^5	2.0×10^5
3	1.1×10^7	9.0×10^6	1.0×10^7	7.2×10^5	8.0×10^5	7.6×10^5

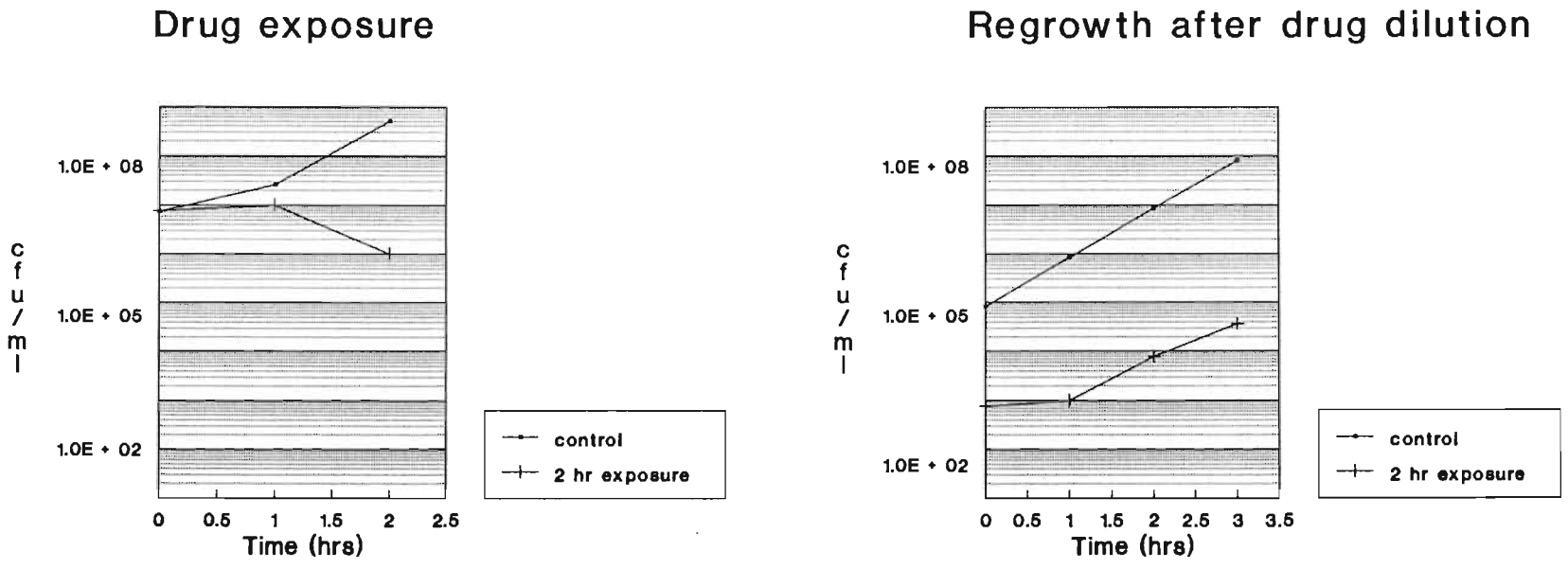
2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	6.0×10^4	7.0×10^4	6.5×10^4	4.4×10^2	6.0×10^2	5.2×10^2
1	6.6×10^5	7.0×10^5	6.8×10^5	1.1×10^3	1.3×10^3	1.2×10^3
2	7.5×10^6	7.0×10^6	7.3×10^6	7.0×10^3	5.0×10^3	6.0×10^3
3	7.0×10^7	5.0×10^7	6.0×10^7	4.0×10^4	1.3×10^4	2.7×10^4





ID.5 Post antibiotic effect after 1 hour drug exposure



ID.5 Post antibiotic effect after 2 hour drug exposure

ID 6

ORGANISM K. PNEUMONIAE

During antibiotic exposure

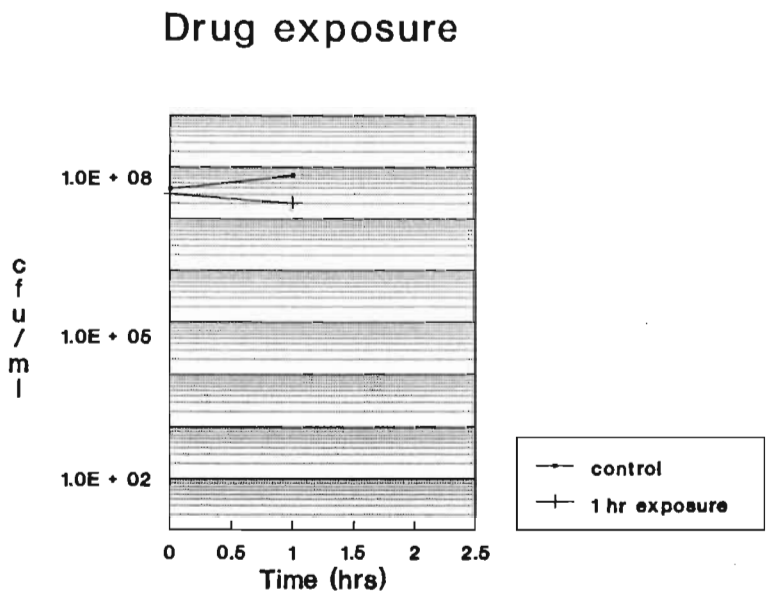
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	1.4×10^7	3.5×10^7	2.5×10^7	-	-	-
1	5.5×10^7	4.5×10^7	5.0×10^7	1.3×10^7	2.0×10^7	1.7×10^7
2	7.0×10^8	8.5×10^7	3.9×10^8	1.2×10^6	9.0×10^5	1.1×10^6

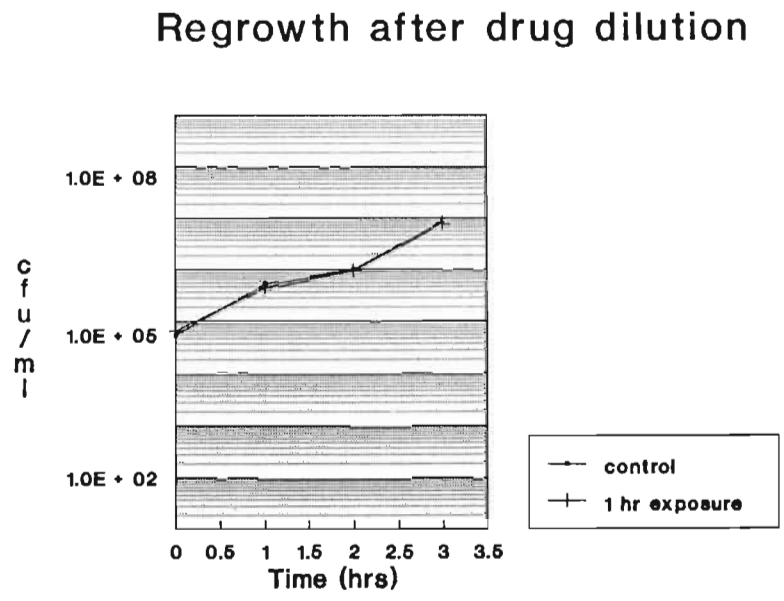
1 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	3.5×10^4	3.3×10^4	3.4×10^4	6.0×10^4	3.3×10^4	4.7×10^4
1	4.0×10^5	2.8×10^5	3.4×10^5	3.5×10^5	2.0×10^5	2.8×10^5
2	1.5×10^6	7.0×10^5	1.1×10^6	9.7×10^5	8.0×10^5	8.9×10^5
3	9.5×10^6	7.0×10^6	8.2×10^6	4.8×10^6	8.0×10^6	6.4×10^6

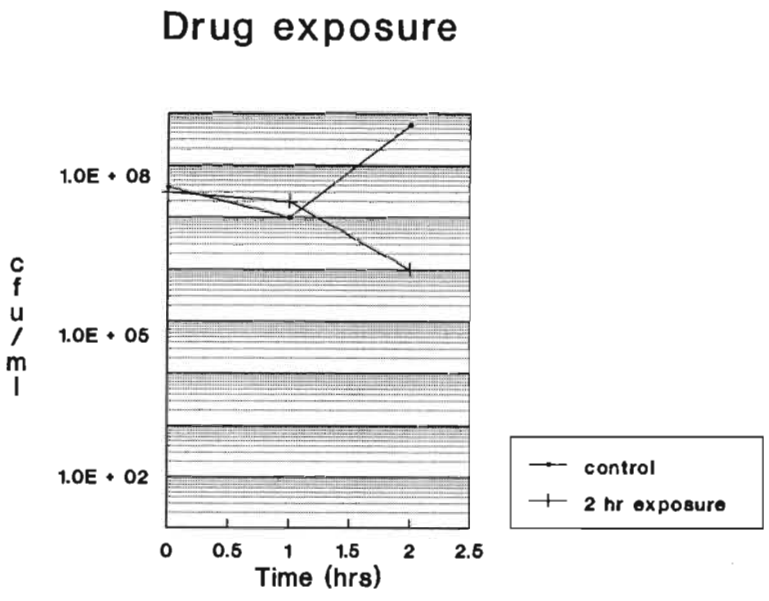
2 Hour exposure

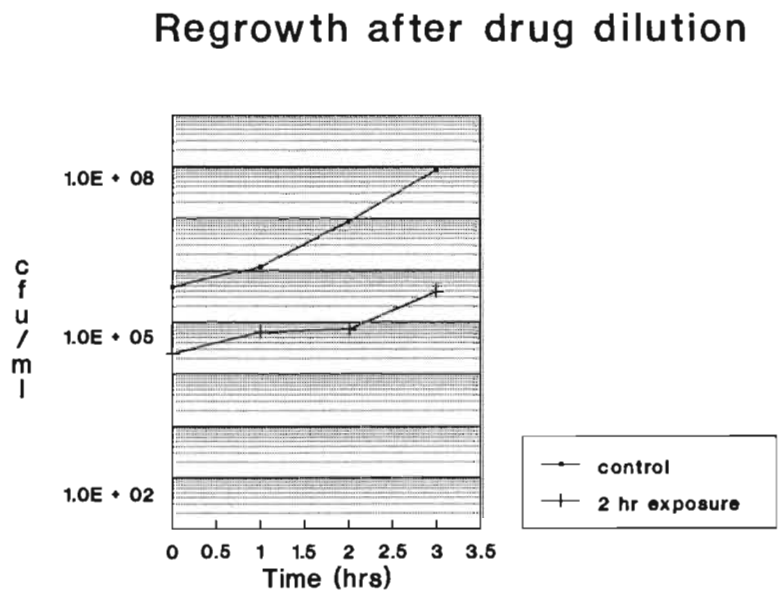
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	4.0×10^5	2.2×10^5	3.1×10^5	2.5×10^4	1.1×10^4	1.8×10^4
1	2.0×10^6	6.0×10^5	1.3×10^6	3.5×10^4	5.0×10^4	4.3×10^4
2	1.2×10^7	3.8×10^6	7.9×10^6	5.0×10^4	6.0×10^4	5.5×10^4
3	9.0×10^7	5.0×10^7	7.0×10^7	3.5×10^5	1.5×10^5	2.5×10^5





ID.6 Post antibiotic effect after 1 hour drug exposure





ID.6 Post antibiotic effect after 2 hour drug exposure

ID 7

ORGANISM K. PNEUMONIAE

During antibiotic exposure

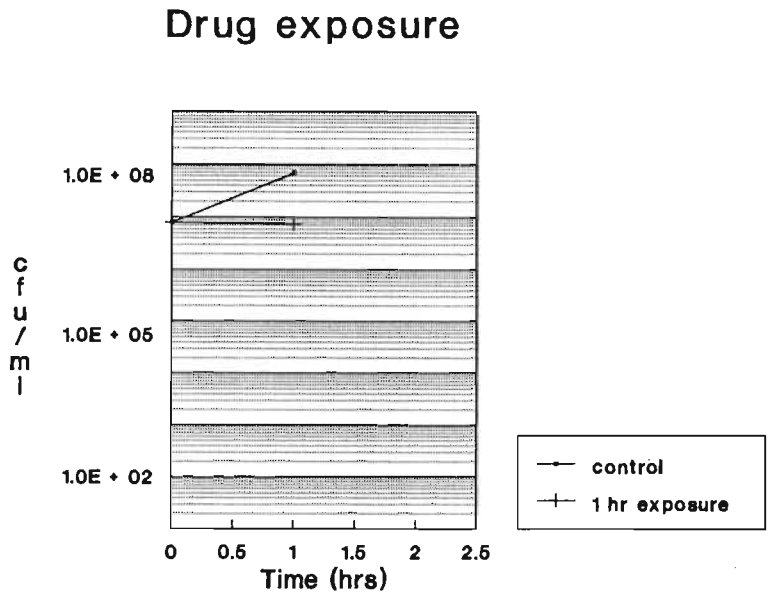
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	4.3×10^6	8.7×10^6	6.5×10^6	-	-	-
1	2.5×10^7	7.1×10^7	4.8×10^7	3.5×10^6	8.7×10^6	6.1×10^6
2	7.6×10^8	1.3×10^8	4.5×10^8	1.0×10^6	5.0×10^6	3.0×10^6

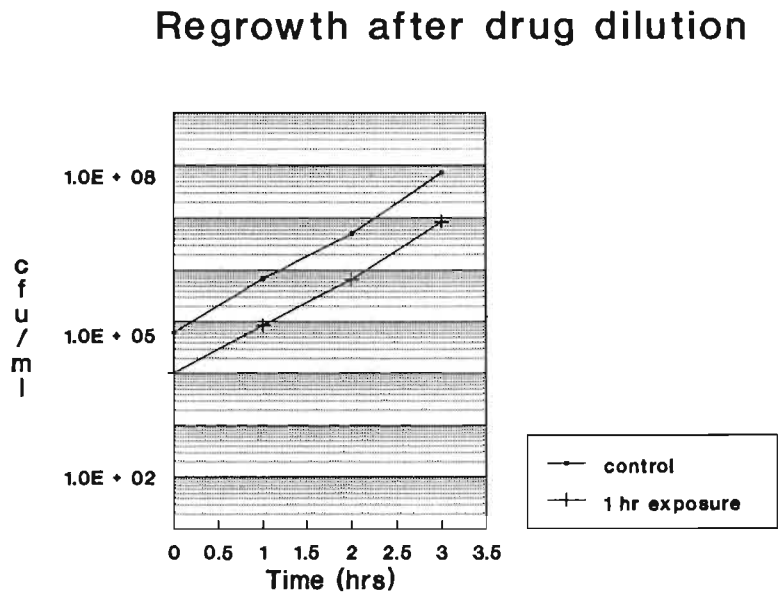
1 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	3.4×10^4	5.0×10^4	4.2×10^4	1.5×10^4	1.0×10^4	1.3×10^4
1	3.5×10^5	6.5×10^5	5.0×10^5	7.9×10^4	7.1×10^4	7.5×10^4
2	2.5×10^6	3.7×10^6	3.1×10^6	3.0×10^5	7.1×10^5	5.1×10^5
3	3.0×10^7	8.9×10^6	1.9×10^7	6.0×10^6	7.9×10^6	7.0×10^6

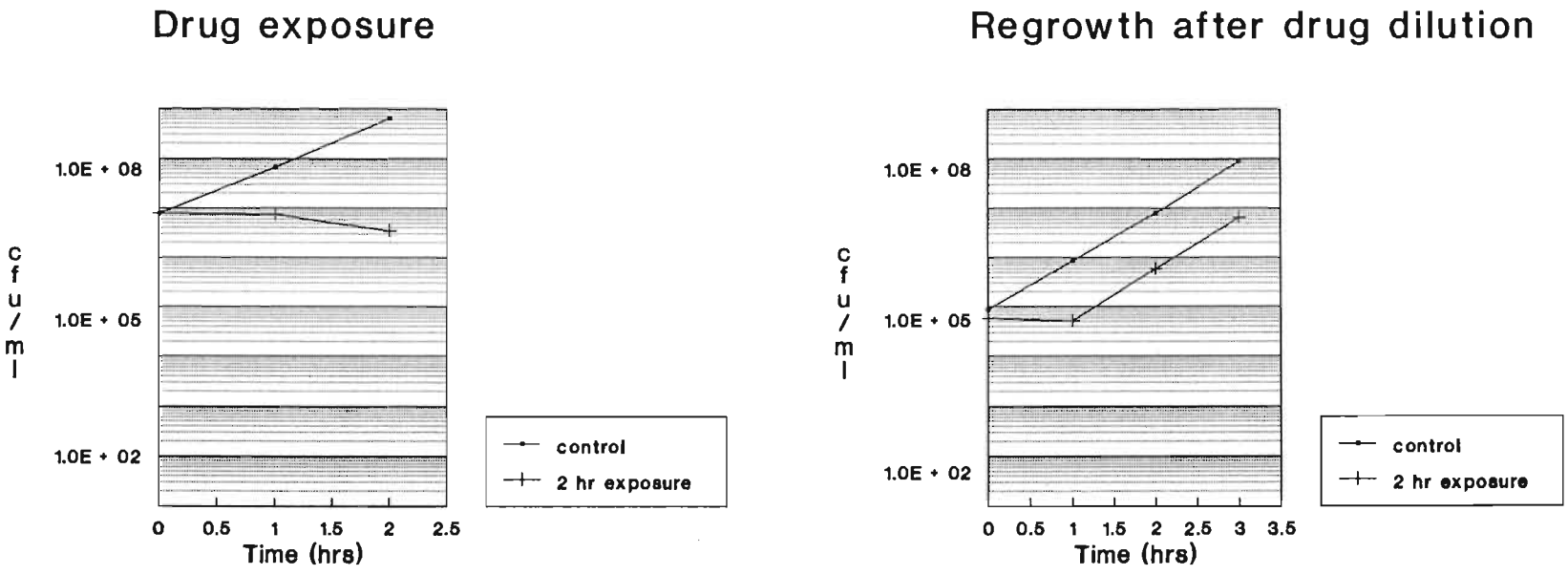
2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	7.1×10^4	7.9×10^4	7.5×10^4	2.0×10^4	7.1×10^3	4.6×10^4
1	6.2×10^5	8.5×10^5	7.4×10^5	3.5×10^4	3.2×10^4	3.4×10^4
2	5.0×10^6	7.4×10^6	6.2×10^6	5.0×10^5	3.0×10^5	4.0×10^5
3	6.2×10^7	9.3×10^7	7.8×10^7	4.0×10^6	5.0×10^6	4.5×10^6





ID.7 Post antibiotic effect after 1 hour drug exposure



ID.7 Post antibiotic effect after 2 hour drug exposure

ID 8

ORGANISM K. PNEUMONIAE

During antibiotic exposure

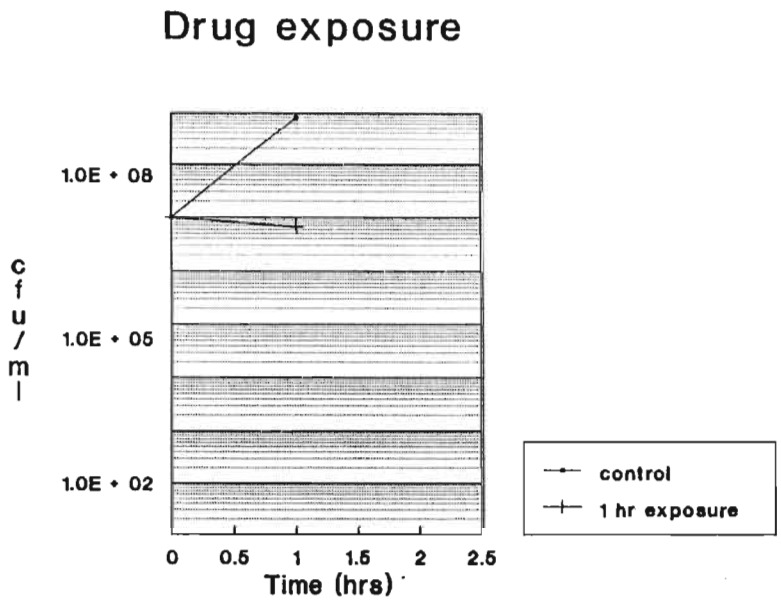
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	1.2×10^7	1.0×10^7	1.1×10^7	-	-	-
1	1.2×10^8	1.0×10^8	1.1×10^8	7.0×10^6	3.0×10^6	5.0×10^6
2	1.0×10^9	1.3×10^9	1.2×10^9	8.8×10^5	8.0×10^5	8.4×10^5

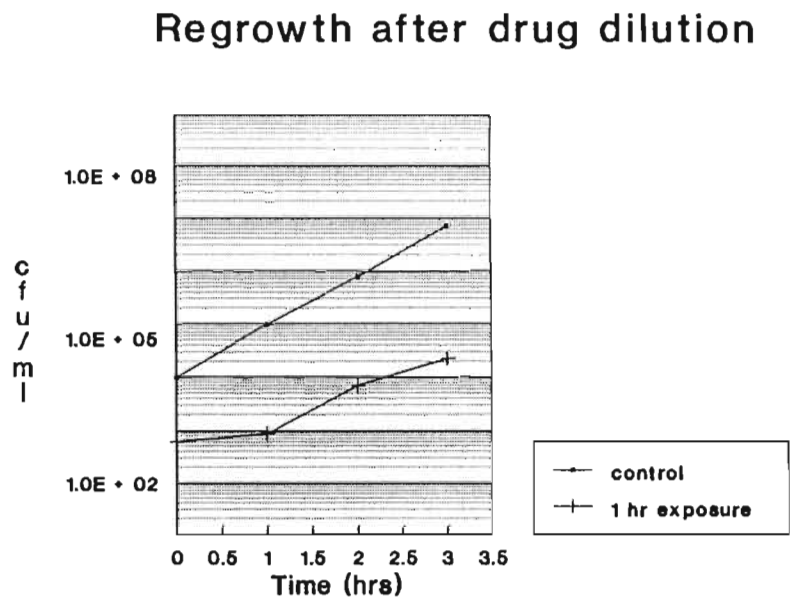
1 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	1.0×10^4	1.2×10^4	1.1×10^4	4.8×10^2	3.6×10^2	4.2×10^2
1	1.4×10^5	1.0×10^5	1.2×10^5	8.0×10^2	7.5×10^2	7.8×10^2
2	6.5×10^5	6.0×10^5	6.3×10^5	7.0×10^3	3.6×10^3	5.3×10^3
3	6.0×10^6	4.3×10^6	5.2×10^6	3.2×10^4	9.0×10^3	2.1×10^4

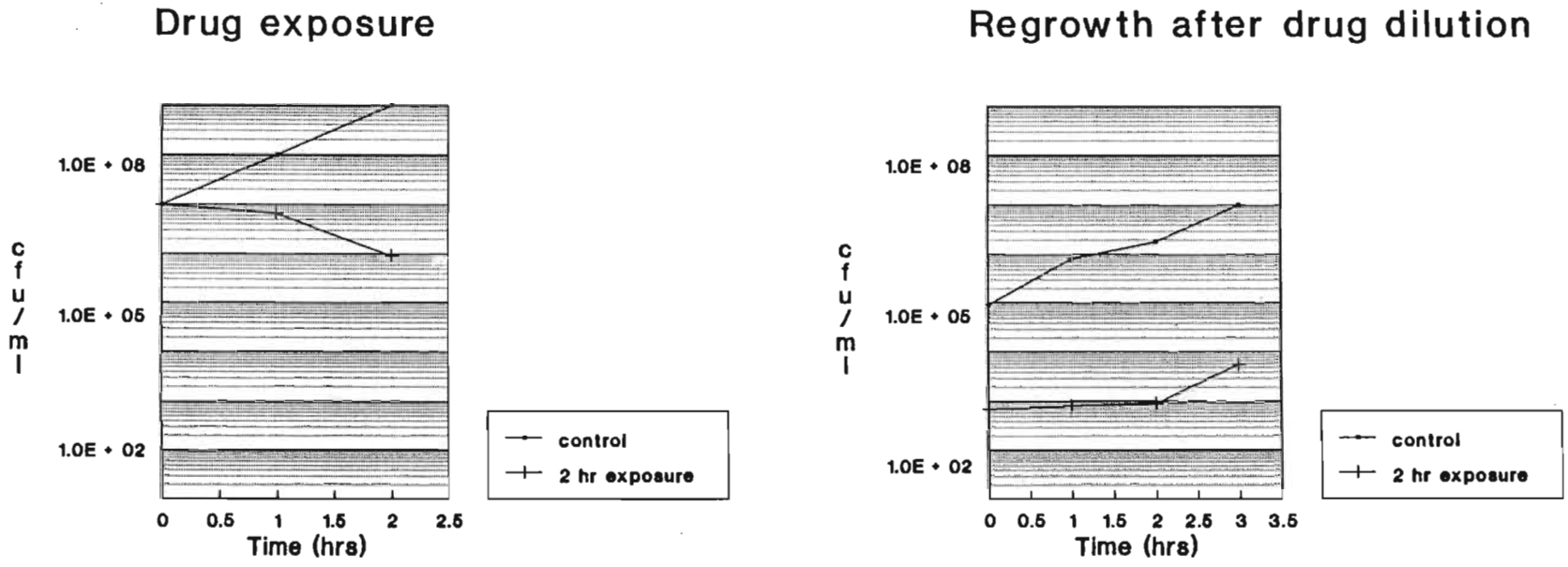
2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	7.5×10^4	8.3×10^4	7.9×10^4	5.6×10^2	4.0×10^2	4.8×10^2
1	7.5×10^5	5.0×10^5	6.3×10^5	8.0×10^2	4.6×10^2	6.3×10^2
2	1.5×10^6	1.7×10^6	1.6×10^6	9.0×10^2	6.0×10^2	7.5×10^2
3	1.2×10^7	9.0×10^6	1.1×10^7	5.6×10^3	2.6×10^3	4.1×10^3





ID.8 Post antibiotic effect after 1 hour drug exposure



ID.8 Post antibiotic effect after 2 hour drug exposure

ID 9

ORGANISM E. COLI

During antibiotic exposure

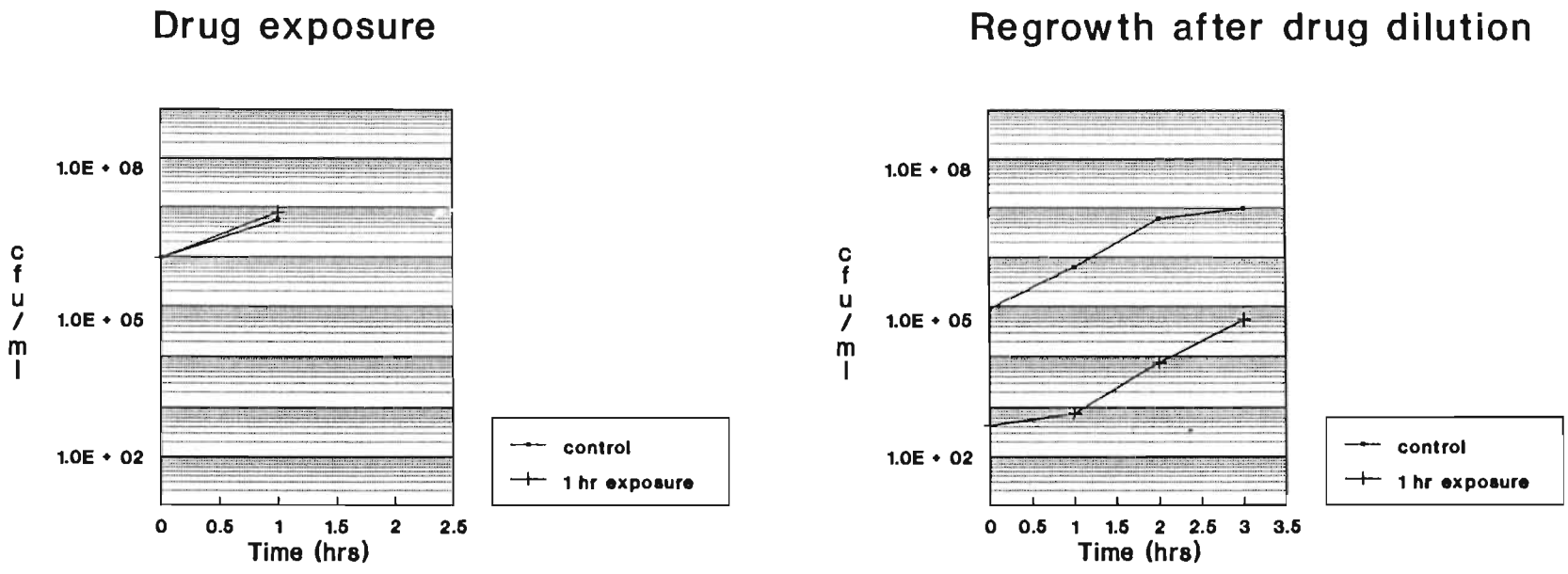
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	8.0×10^5	1.0×10^6	9.0×10^5	-	-	-
1	5.2×10^6	2.8×10^6	4.0×10^6	4.7×10^6	4.0×10^6	4.4×10^6
2	7.7×10^7	7.0×10^7	7.4×10^7	1.1×10^5	3.3×10^5	2.2×10^5

1 Hour exposure

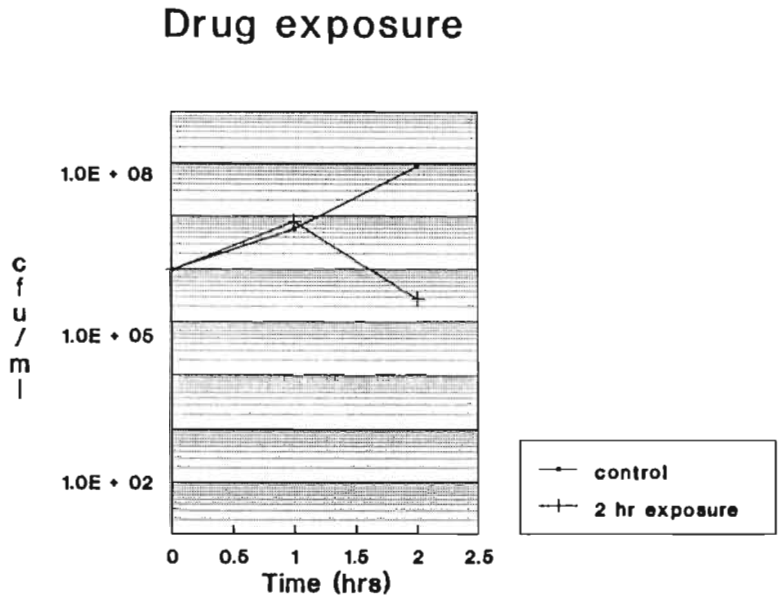
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	7.5×10^4	8.2×10^4	7.9×10^4	2.5×10^2	2.7×10^2	2.6×10^2
1	3.7×10^5	4.8×10^5	4.3×10^5	5.5×10^2	5.1×10^2	5.3×10^2
2	3.8×10^6	4.5×10^6	4.2×10^6	4.2×10^3	3.8×10^3	4.0×10^3
3	1.2×10^7	1.0×10^7	1.1×10^7	2.5×10^4	5.0×10^4	3.8×10^4

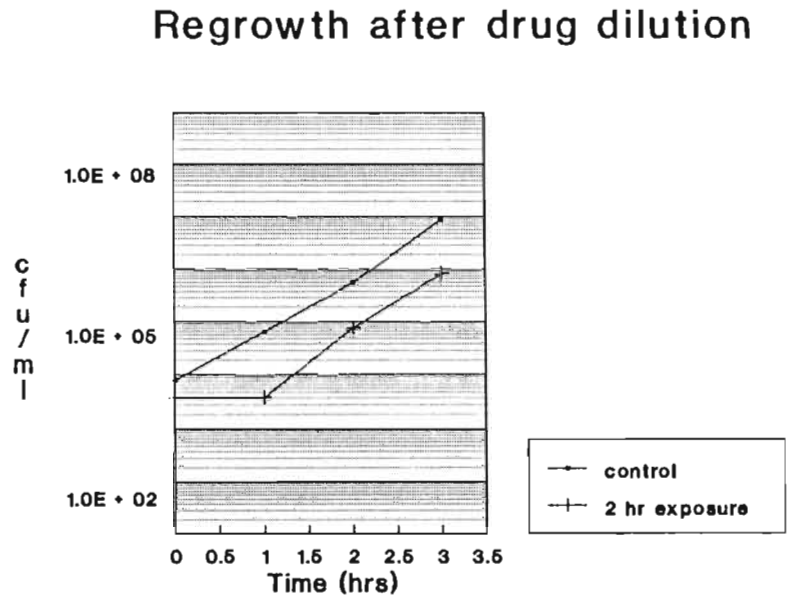
2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.5×10^3	6.0×10^3	5.8×10^3	2.7×10^3	2.1×10^3	2.4×10^3
1	4.2×10^4	5.0×10^4	4.6×10^4	7.3×10^3	6.8×10^3	7.1×10^3
2	3.0×10^5	4.4×10^5	3.7×10^5	2.8×10^4	3.3×10^4	3.1×10^4
3	7.5×10^6	7.0×10^6	7.3×10^6	6.5×10^5	7.5×10^5	7.0×10^5



ID.9 Post antibiotic effect after 1 hour drug exposure





ID.9 Post antibiotic effect after 2 hour drug exposure

ID 10

ORGANISM E. COLI

During antibiotic exposure

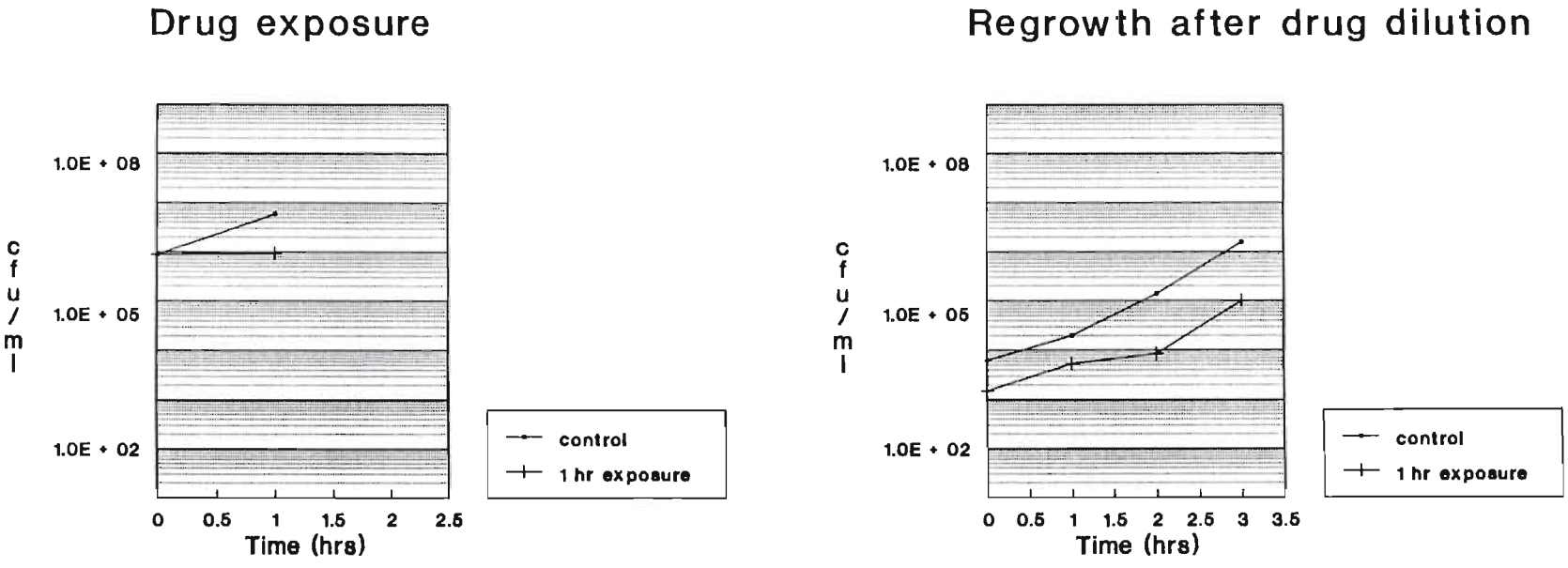
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	8.3×10^5	6.8×10^5	7.6×10^5	-	-	-
1	3.5×10^6	4.3×10^6	3.9×10^6	1.1×10^6	6.8×10^5	8.9×10^5
2	1.1×10^7	1.3×10^7	1.2×10^7	7.0×10^3	6.0×10^3	6.5×10^3

1 Hour exposure

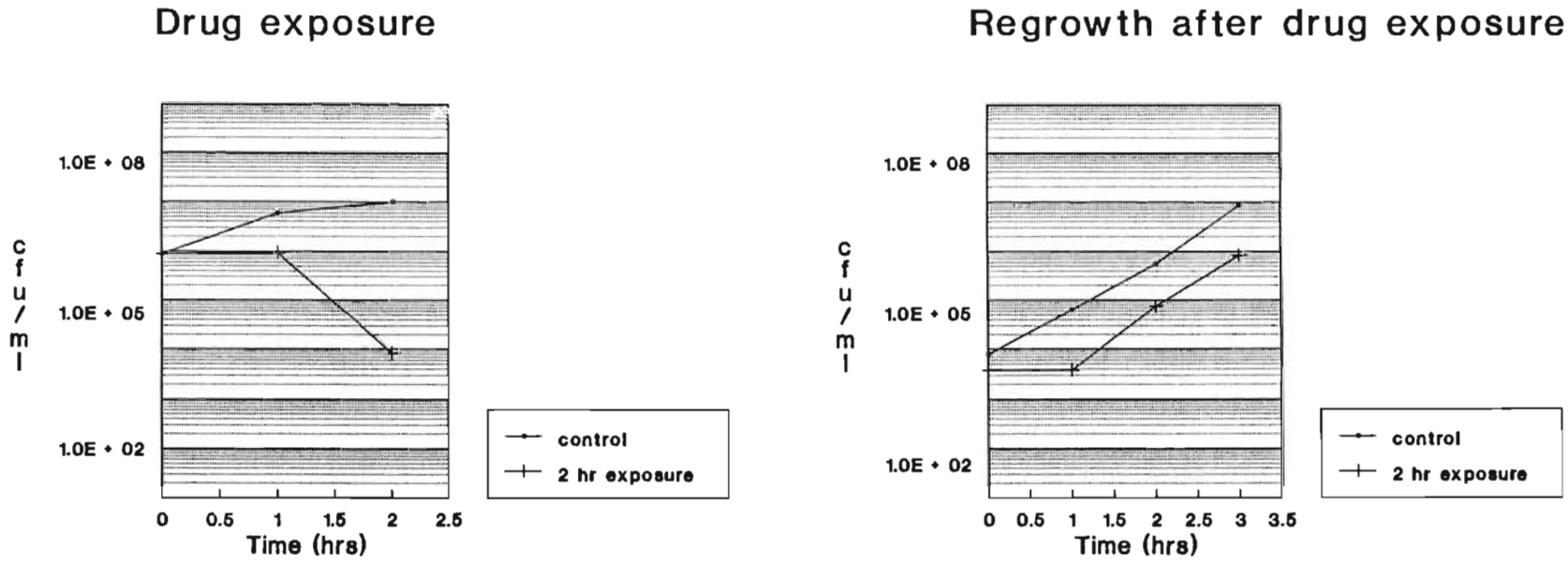
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	3.5×10^3	5.0×10^3	4.3×10^3	2.3×10^3	8.8×10^2	1.6×10^3
1	1.7×10^4	2.5×10^4	2.1×10^4	4.5×10^3	2.5×10^3	3.5×10^3
2	1.6×10^5	1.2×10^5	1.4×10^5	7.8×10^3	6.0×10^3	6.9×10^3
3	1.4×10^6	1.5×10^6	1.5×10^6	1.0×10^5	1.3×10^5	1.2×10^5

2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	4.2×10^4	4.0×10^4	4.1×10^4	1.0×10^2	1.2×10^2	1.1×10^2
1	1.2×10^5	2.3×10^5	1.8×10^5	1.5×10^2	1.6×10^2	1.6×10^2
2	9.7×10^5	1.3×10^6	1.1×10^6	6.3×10^2	4.5×10^2	5.4×10^2
3	1.4×10^7	7.0×10^6	1.1×10^7	1.0×10^3	1.5×10^3	1.3×10^3



ID.10 Post antibiotic effect after 1 hour drug exposure



ID.10 Post antibiotic effect after 2 hour drug exposure

ID 11

ORGANISM E. COLI

During antibiotic exposure

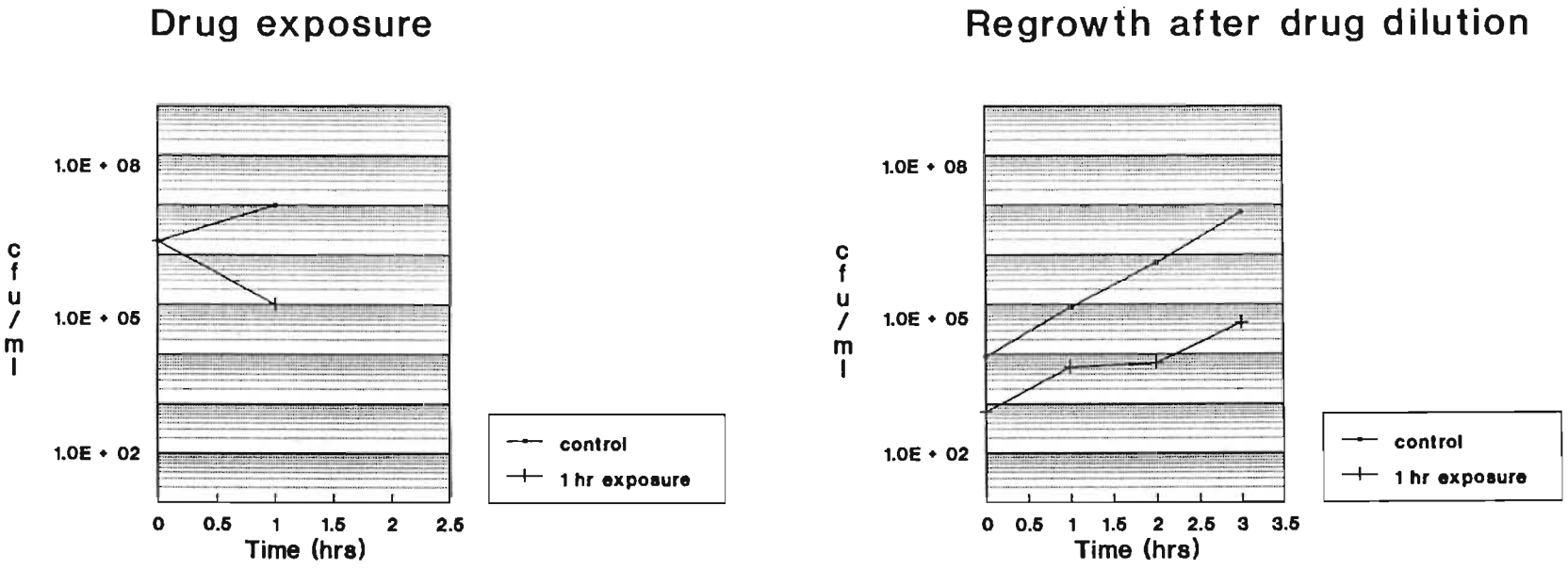
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	2.2×10^6	1.1×10^6	1.7×10^6	-	-	-
1	7.5×10^6	1.3×10^7	1.0×10^7	2.7×10^5	6.0×10^4	1.7×10^5
2	4.8×10^7	7.8×10^7	6.3×10^7	7.0×10^4	4.3×10^4	5.7×10^4

1 Hour exposure

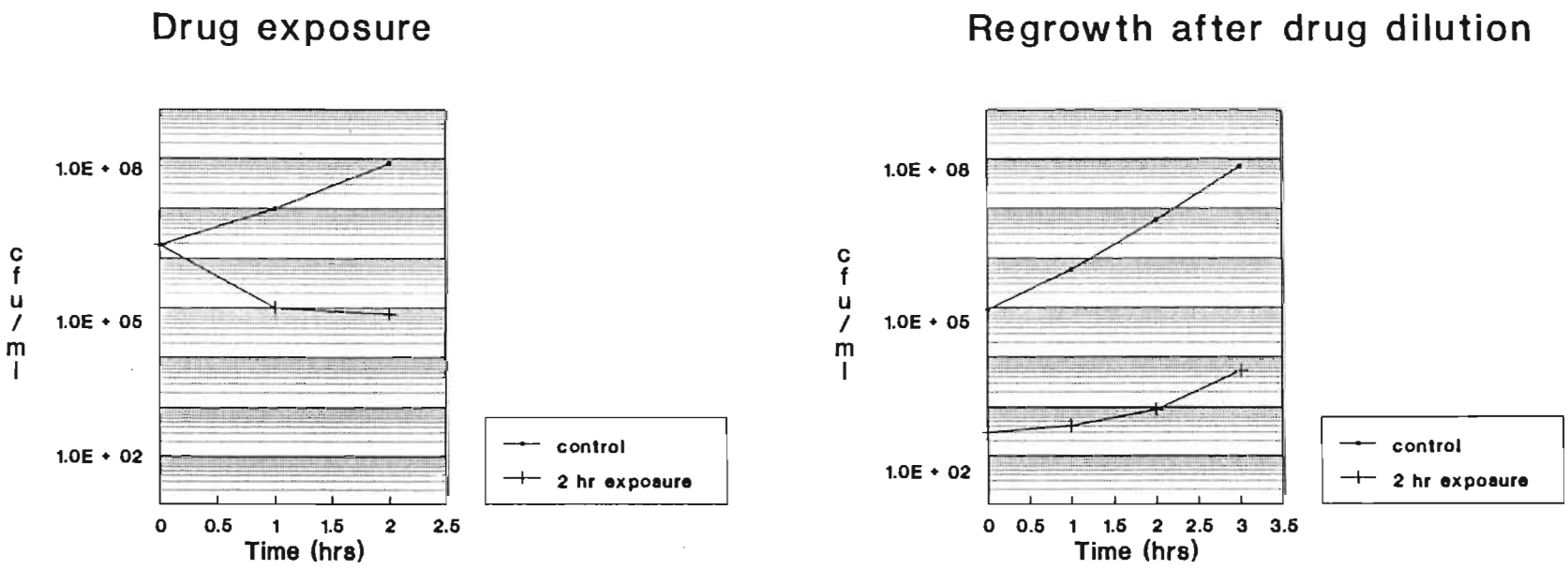
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	1.0×10^4	6.3×10^3	8.2×10^3	1.5×10^3	1.5×10^2	8.3×10^2
1	1.0×10^5	5.3×10^4	7.7×10^4	2.5×10^3	5.0×10^2	1.5×10^3
2	6.0×10^5	3.8×10^5	4.9×10^5	9.0×10^3	2.5×10^3	5.8×10^3
3	4.7×10^6	5.3×10^6	5.0×10^6	1.0×10^5	7.0×10^3	5.4×10^4

2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	7.8×10^4	9.0×10^4	8.4×10^4	2.4×10^2	1.6×10^2	2.0×10^2
1	3.3×10^5	4.5×10^5	3.9×10^5	2.0×10^2	3.3×10^2	2.7×10^2
2	4.5×10^6	3.5×10^6	4.0×10^6	6.8×10^2	8.0×10^2	7.4×10^2
3	6.7×10^7	4.0×10^7	5.4×10^7	4.2×10^3	2.8×10^3	3.5×10^3



ID.11 Post antibiotic effect after 1 hour drug exposure



ID.11 Post antibiotic effect after 2 hour drug exposure

ID 12

ORGANISM E. COLI

During antibiotic exposure

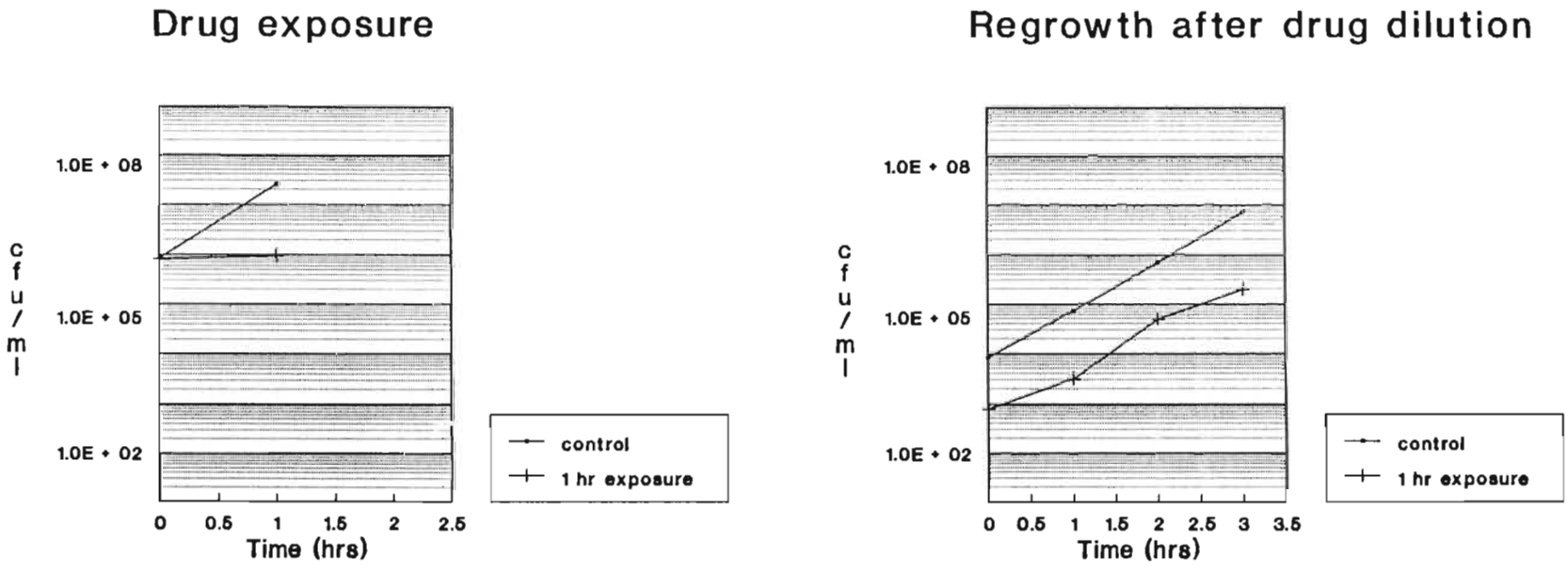
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.5×10^5	1.0×10^6	7.8×10^5	-	-	-
1	4.2×10^6	7.5×10^7	4.0×10^7	9.3×10^5	9.5×10^5	9.4×10^5
2	1.4×10^7	8.0×10^8	4.1×10^8	3.0×10^2	2.0×10^2	2.5×10^2

1 Hour exposure

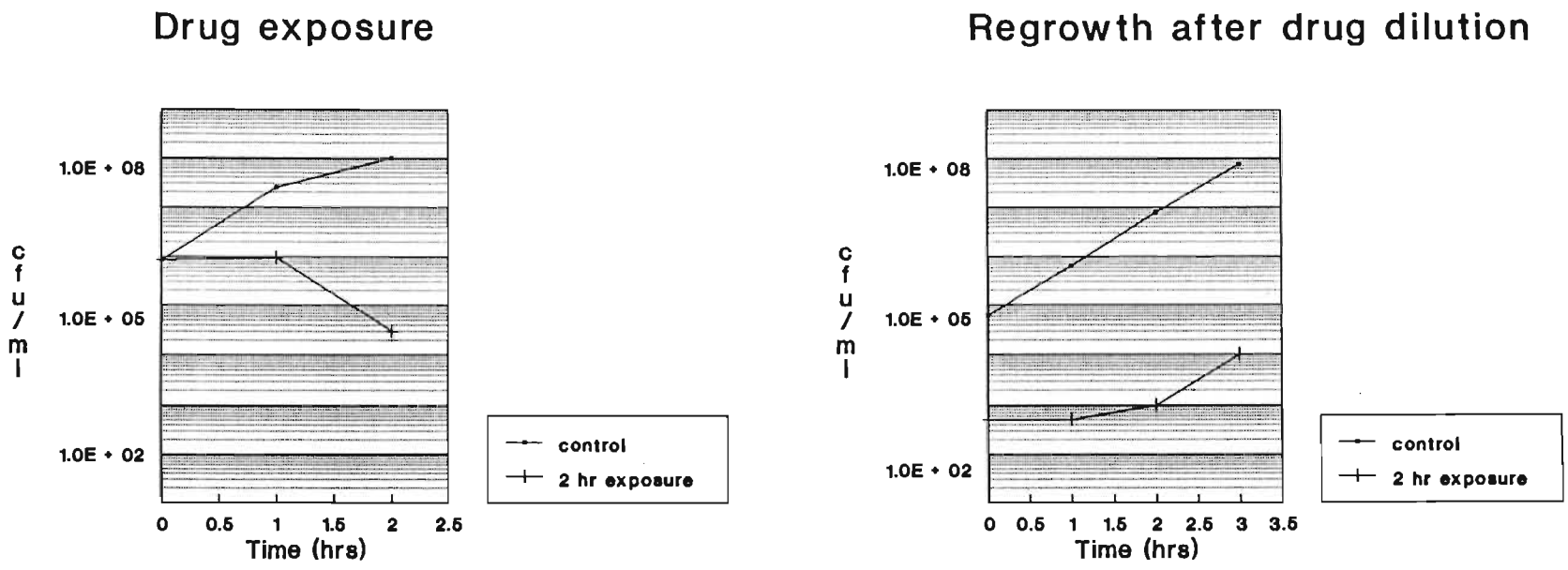
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.0×10^3	1.0×10^4	7.5×10^3	9.0×10^2	8.0×10^2	8.5×10^2
1	3.0×10^4	1.0×10^5	6.5×10^4	1.7×10^3	2.5×10^3	2.1×10^3
2	4.0×10^5	6.0×10^5	5.0×10^5	2.0×10^4	1.0×10^4	1.5×10^4
3	8.5×10^6	3.3×10^6	5.9×10^6	1.0×10^5	2.0×10^5	1.5×10^5

2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.0×10^4	3.5×10^4	4.3×10^4	2.0×10^3	5.0×10^1	1.0×10^3
1	5.0×10^5	4.0×10^5	4.5×10^5	2.2×10^3	5.1×10^1	1.1×10^3
2	5.0×10^6	7.0×10^6	6.0×10^6	1.1×10^4	1.2×10^2	5.6×10^3
3	4.3×10^7	7.0×10^7	5.7×10^7	1.1×10^5	1.2×10^3	5.6×10^4



ID.12 Post antibiotic effect after 1 hour drug exposure



ID.12 Post antibiotic effect after 2 hour drug exposure

ID 13

ORGANISM E. COLI

During antibiotic exposure

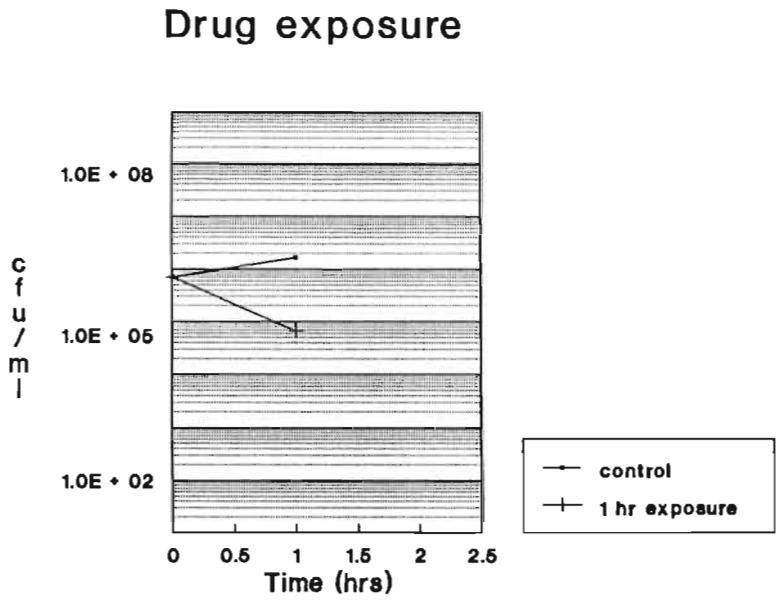
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.2×10^5	4.8×10^5	5.0×10^5	-	-	-
1	1.5×10^6	1.4×10^6	1.5×10^6	5.7×10^4	4.0×10^4	4.9×10^4
2	4.0×10^7	5.5×10^7	4.8×10^7	4.0×10^3	8.0×10^2	2.4×10^3

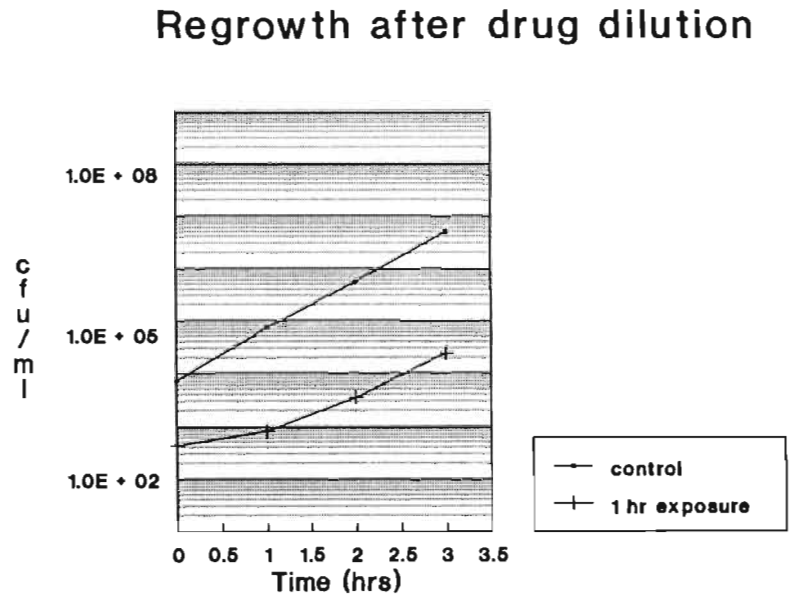
1 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	4.0×10^3	7.0×10^3	5.5×10^3	2.5×10^2	3.0×10^2	2.8×10^2
1	4.0×10^4	7.0×10^4	5.5×10^4	6.0×10^2	8.0×10^2	7.0×10^2
2	1.2×10^6	9.3×10^6	5.3×10^6	2.0×10^3	2.5×10^3	2.3×10^3
3	1.2×10^7	9.3×10^7	5.3×10^7	1.1×10^4	3.0×10^4	2.0×10^4

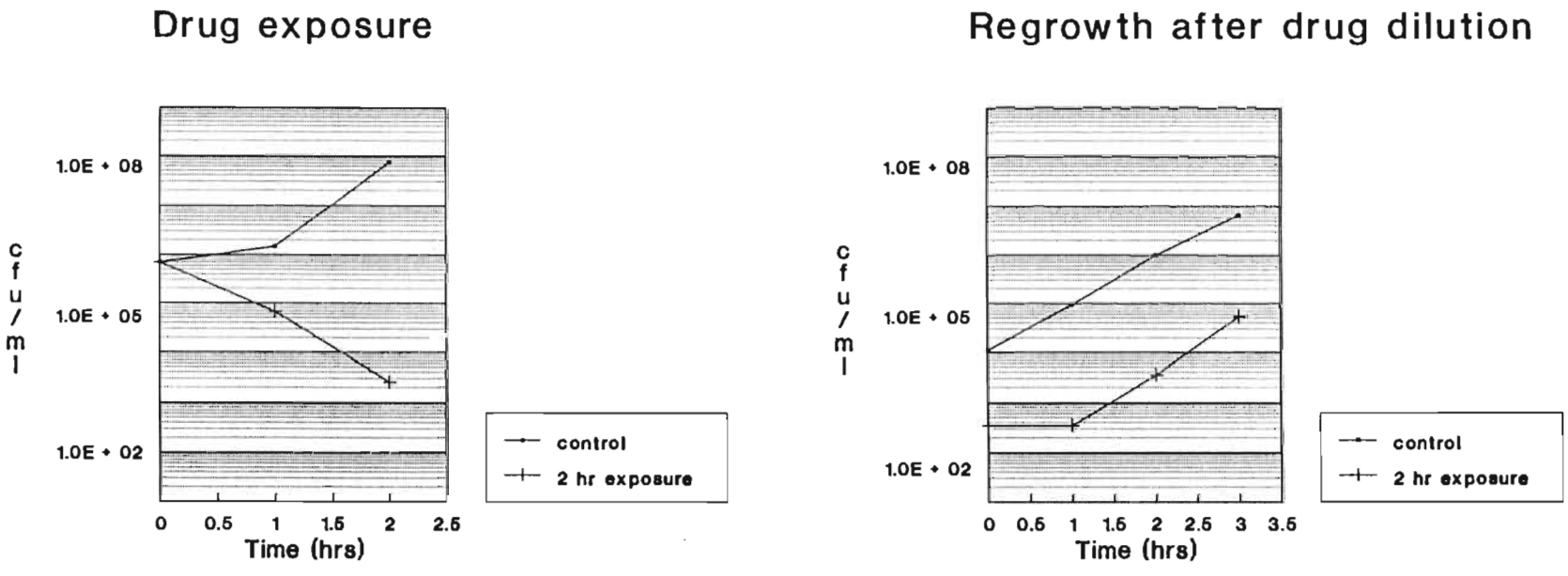
2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	1.7×10^4	1.0×10^4	1.4×10^4	2.0×10^2	2.5×10^2	2.3×10^2
1	1.1×10^5	8.0×10^4	9.5×10^4	2.0×10^2	2.5×10^2	2.3×10^2
2	1.2×10^6	8.8×10^5	1.0×10^6	2.0×10^3	2.4×10^3	2.2×10^3
3	4.2×10^6	5.3×10^6	4.8×10^6	3.0×10^4	4.0×10^4	3.5×10^4





ID.13 Post antibiotic effect after 1 hour drug exposure



ID.13 Post antibiotic effect after 2 hour drug exposure

ID 14

ORGANISM E. COLI

During antibiotic exposure

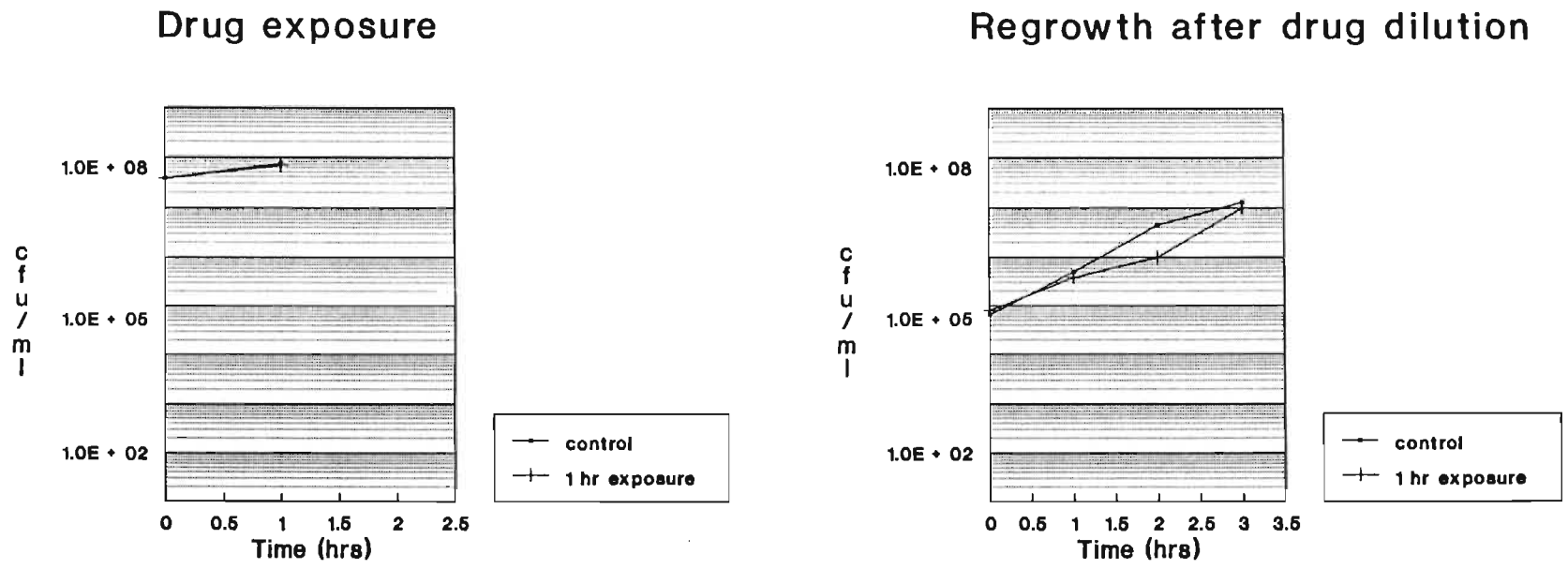
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	3.3×10^7	1.8×10^7	2.6×10^7	-	-	-
1	5.0×10^7	6.3×10^7	5.7×10^5	4.8×10^7	4.6×10^7	4.7×10^7
2	3.5×10^8	2.3×10^8	2.9×10^8	5.0×10^6	2.5×10^6	3.8×10^6

1 Hour exposure

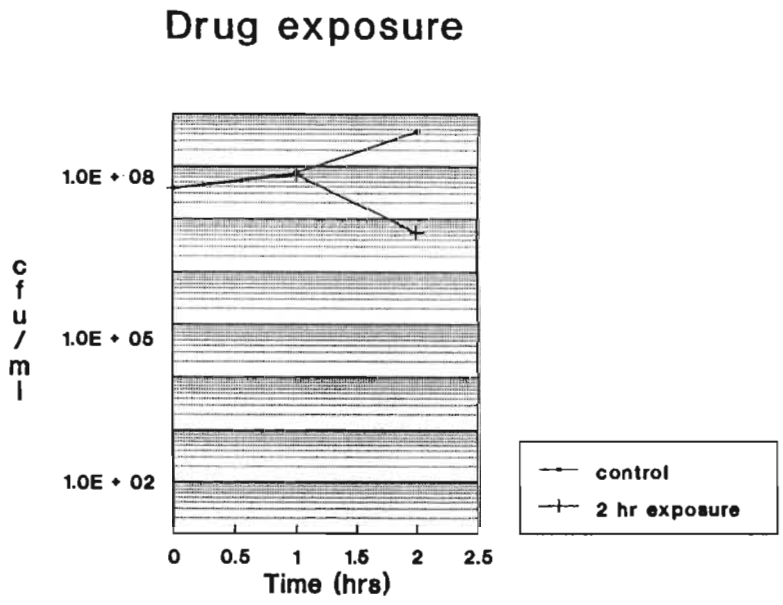
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	5.3×10^4	4.0×10^4	4.7×10^4	7.5×10^4	5.0×10^4	6.3×10^4
1	5.0×10^5	2.0×10^5	3.5×10^5	2.5×10^5	2.3×10^5	2.4×10^5
2	1.8×10^6	4.5×10^6	3.2×10^6	1.2×10^6	1.4×10^6	1.3×10^6
3	1.1×10^7	1.8×10^7	1.5×10^7	1.1×10^7	1.4×10^7	1.3×10^7

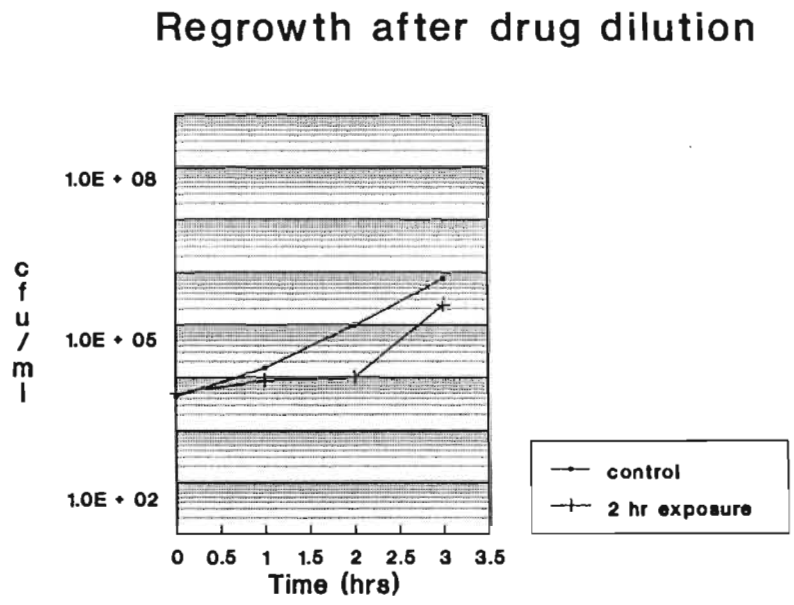
2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	2.9×10^3	3.1×10^3	3.0×10^3	3.1×10^3	2.7×10^3	2.9×10^3
1	1.6×10^4	1.3×10^4	1.5×10^4	7.5×10^3	7.1×10^3	7.3×10^3
2	9.5×10^4	9.3×10^4	9.4×10^4	1.0×10^4	1.5×10^4	1.3×10^4
3	6.0×10^5	5.5×10^5	5.8×10^5	2.0×10^5	2.6×10^5	2.3×10^5



ID.14 Post antibiotic effect after 1 hour drug exposure





ID.14 Post antibiotic effect after 2 hour drug exposure

ID 15

ORGANISM E. COLI

During antibiotic exposure

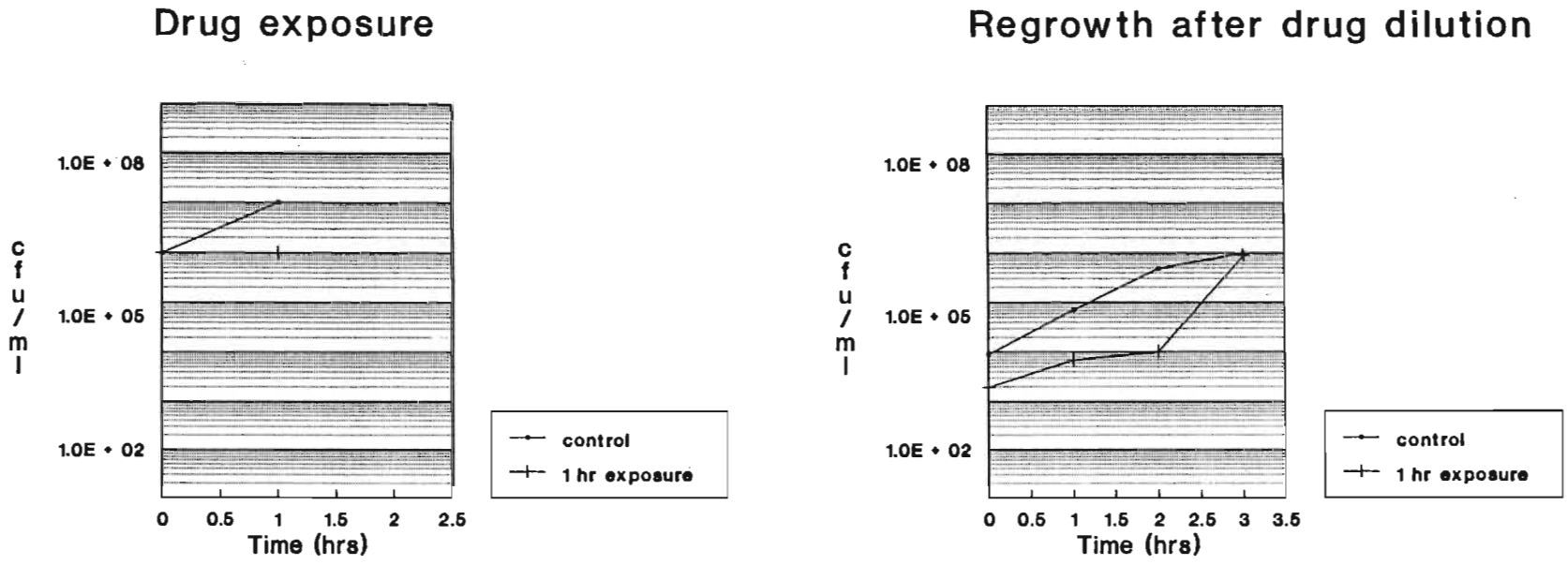
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	1.2×10^6	1.0×10^6	1.1×10^6	-	-	-
1	1.3×10^7	8.3×10^6	1.1×10^7	9.0×10^5	1.5×10^6	1.2×10^6
2	6.7×10^7	8.5×10^7	7.6×10^7	7.5×10^4	5.0×10^4	6.3×10^4

1 Hour exposure

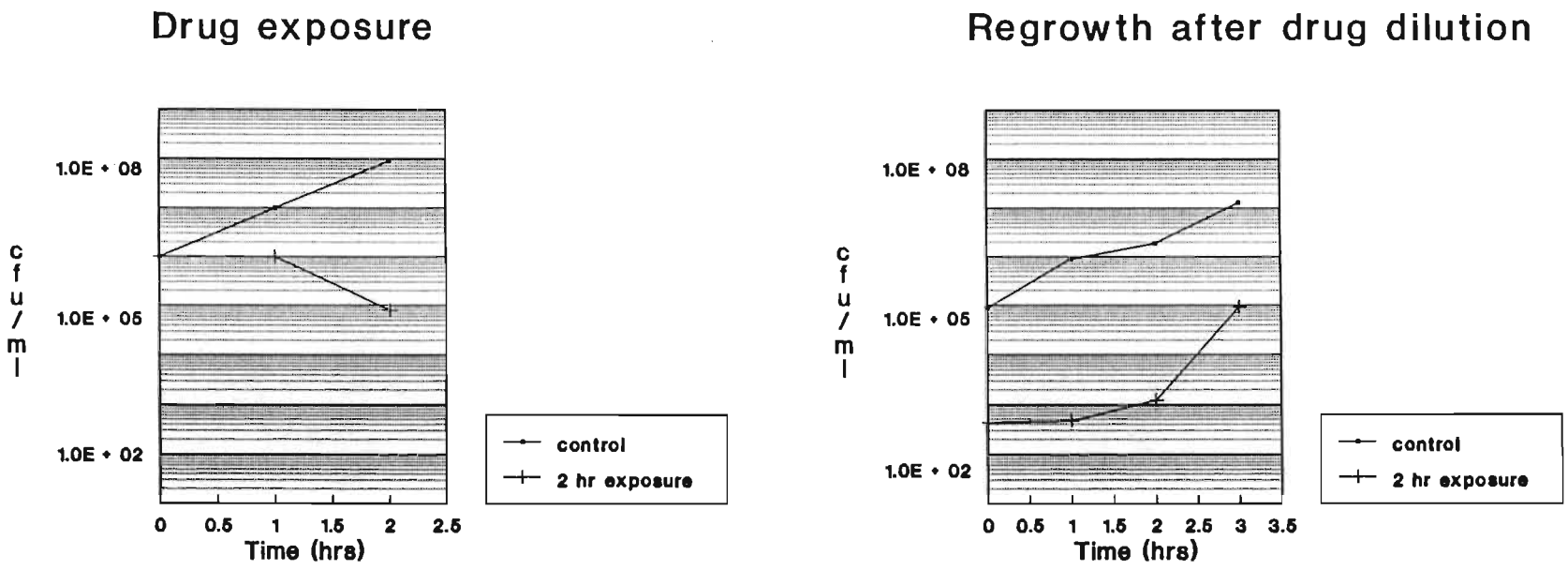
Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	8.2×10^3	8.0×10^3	8.1×10^3	1.3×10^3	1.8×10^3	1.6×10^3
1	8.5×10^4	7.7×10^4	8.1×10^4	4.5×10^3	5.5×10^3	5.0×10^3
2	9.5×10^5	8.3×10^5	8.9×10^5	1.4×10^4	1.1×10^4	1.3×10^4
3	1.2×10^6	1.6×10^6	1.4×10^6	1.3×10^5	1.2×10^5	1.3×10^5

2 Hour exposure

Time	Control 1	Control 2	Average	Sample 1	Sample 2	Average
0	7.0×10^4	8.2×10^4	7.6×10^4	2.2×10^2	2.0×10^2	2.1×10^2
1	8.5×10^5	7.0×10^5	7.8×10^5	1.3×10^2	2.8×10^2	2.1×10^2
2	5.5×10^6	6.0×10^6	5.8×10^5	1.3×10^2	1.5×10^2	1.4×10^2
3	6.5×10^7	7.5×10^7	7.0×10^7	3.8×10^3	9.0×10^3	8.5×10^3



ID.15 Post antibiotic effect after 1 hour drug exposure



ID.15 Post antibiotic effect after 2 hour drug exposure