Introduction
1. INTRODUCTION

1.1 ANTIBIOTICS

1.1.1 Definition

Antibiotics are chemical substances produced by various species of microorganisms that suppress the growth of other microorganisms and may eventually destroy them. This term also extends to synthetic antibacterial agents such as sulfonamides and quinolones that are not synthesised by microbes.

1.1.2 Classification and mechanism of action

The most common classification has been based on chemical structure and on their proposed mechanism of action (Sande et al., 1991) as follows:

a) drugs that inhibit synthesis of or activate enzymes that disrupt bacterial cell wall to cause loss of viability and often cell lysis, these include the penicillins and cephalosporins and also agents such as cycloserine, vancomycin, bacitracin and the imidazole antifungal agents.

b) drugs that act directly on the cell membrane of the microorganism, affecting permeability and leading to leakage of intracellular components. These include the detergents, polymyxin and colistimethate and the polyene antifungal agents.

c) drugs that affect the function of bacterial ribosome to cause a reversible inhibition of protein synthesis, these bacteriostatic drugs include chloramphenicol, erythromycin and clindamycin.
d) drugs that bind to the 30S ribosomal subunit of bacterial ribosomes and alter protein synthesis, which eventually leads to their cell death, these include aminoglycosides

e) drugs that affect nucleic acid metabolism such as rifamycins which inhibit DNA dependent RNA polymerase and the quinolones which inhibit DNA supercoiling and DNA synthesis

f) the antimetabolites including trimethoprim and the sulfonamides and lastly

g) nucleic acid analogs such as zidovudine, ganciclovir which halt viral replication

1.2 THE AMINOGLYCOSIDES: A BRIEF ACCOUNT

The aminoglycoside antibiotics include Gentamicin, Tobramycin, Amikacin, Netilicin. These drugs contain aminosugars linked to an aminocyclitol ring by O-glycosidic bonds. They are polycations and their polarity is in part responsible for the pharmacokinetic properties shared by all members of the group. The aminoglycosides are bactercidal in nature. Serious toxicity is a major limitation to the usefulness of the aminoglycosides and the same spectrum of toxicity is shared by all members of the group. Most notable are ototoxicity and nephrotoxicity (Sande and Mandell, 1991)

1.3 GENTAMICIN: AN INSIGHT

This is an important agent for the treatment of many serious gram negative bacillary infections. This is a broad spectrum antibiotic derived from species of the actinomycete Micromonospora purpurea
GENTAMICIN C

In gentamicin

\[ C_1, R_1 = R_2 = \text{CH}_3 \]
\[ C_2, R_1 = \text{CH}_3, R_2 = \text{H} \]
\[ C_{1a}, R_1 = R_2 = \text{H} \]

(Figure modified from Moeller, R. 1977)
1.3.1 Chemistry

The aminoglycosides consist of two or more aminosugar joined in glycosidic linkage to a hexose nucleus which is usually in the central position. This hexose is 2-deoxy streptamine which is characteristic of all aminoglycosides except streptomycin.

The aminoglycoside families are distinguished by the aminosugars attached to the aminocyclitol. The gentamicin family includes gentamicin C₁, C₁₈, and C₂, sisomycin, and netilmicin and contain a 3-aminosugar (Garrosamine). Variations in methylation of the other aminosugar results in different types of gentamicin. These modifications, however, have little effect on biological activity.

1.3.2 Therapeutic uses of gentamicin

Urinary tract infections. Over 90% of the lower urinary tract infections are cured by a single intramuscular dose of gentamicin (5 mg/kg Ronald et al., 1976, Varese et al., 1980).

Meningitis. Rahal and associates (1974) have recommended parenteral administration of gentamicin in combination with intrathecal injection of 4 to 12 mg every 18 hours for 5 to 10 days.

Gentamicin in combination with antipseudomonal penicillin is recommended for the treatment of granulocytopenia and infection with Pseud aeruginosa. Gentamicin is also well suited for the treatment of pseudomonas eye infections.
1.3.3 Untoward effects of gentamicin

The most important and serious side effects of the use of gentamicin are nephrotoxicity and irreversible ototoxicity. This is due to high concentration of gentamicin in the renal cortex and in the endolymph and perilymph of the inner ear (Davies et al., 1984). Gentamicin is said to be more nephrotoxic (Harold C Neu, 1991) when compared to other aminoglycosidic antibiotics like Streptomycin, Netilmicin, Kanamycin.

1.4 NEPHROTOXICITY

1.4.1 Biochemical events

Nephrotoxicity refers to the ability of an agent or drug to produce renal lesions or functional impairment. Antibiotic nephrotoxicity specifically refers to the ability of an antibiotic to produce renal lesions, usually tubulointerstitial or renal functional impairment. This is due to marked accumulation and avid retention of aminoglycoside antibiotic in the proximal tubular cell (Aronoff et al., 1983, Lietman and Smith, 1983).

The initial damage at this site is manifested by the excretion of enzymes of the renal tubular brush border (Patel et al., 1975). After several days there is a defect in renal concentrating ability, mild proteinuria and the glomerular filtration rate is reduced after several additional days (Schentag et al., 1979).

The biochemical events leading to tubular cell damage and glomerular dysfunction are poorly understood, but they may involve perturbations of the structure of cellular membranes, Aminoglycosides inhibit various phospholipases, sphingomyelinases, and ATPases and they affect the function of mitochondria and ribosomes (Silver-blatt, 1982,
Queener et al., 1983, Humes et al., 1984) Because of the ability of cationic aminoglycosides to interact with anionic phospholipids, these drugs may impair the generation of membrane-derived autacoids and intracellular second messengers such as prosta glandins, inositol phosphates and diacylglycerol. Derangements of prostaglandin metabolism might explain the relation between tubular damage and reduction in glomerular filtration rate (Ramsammy et al., 1985).

Morphological changes have been observed in glomerular endothelial cells (decreased number of endothelial fenestrations) in animals receiving aminoglycosides (Luft and Evans, 1980) and drug induced reduction in the glomerular capillary ultrafiltration coefficient (Baylis et al., 1977).

Eventually the aminoglycosides are internalized by pinocytosis, morphologically there is clear evidence of accumulation of drug in liposomes. Aminoglycosides are thereby trapped, concentrated (up to 50 times the plasma concentration) (Aronoff et al., 1983) and prepared for extrusion into the urine as multilamellar, phospholipid structures called 'myeloid bodies' (Silverblatt, 1982).

1.4.2 Mechanism of gentamicin induced nephrotoxicity

1.4.2.1 Cell membrane effects

The kidney is a highly differentiated organ with many different cell types of marked functional, morphological and biochemical heterogeneity. Many nephrotoxic agents exert their noxious effect in a well defined region of the kidney. This is due to the susceptibility of specific target areas in certain cells to a nephrotoxin, their capacity to
actively transport and concentrate a nephrotoxin, or their capacity to metabolize it, to a more toxic compound. The cells of the proximal convoluted tubule are the first that are exposed to the glomerular ultra filtrate in the tubular lumen. Because they actively transport many organic and inorganic compounds and are able to perform a wide range of drug metabolizing reactions, they are a primary target of many nephrotoxic agents (Boogard et al., 1989). This is due to the aminoglycoside concentrating capacity of these cells. (Kaloyanides and Pastoriza-Munzo, 1980).

Aminoglycosides are not metabolized in the body and are eliminated essentially by glomerular filtration and are partially reabsorbed by proximal tubular cells. Highly cationic aminoglycoside antibiotics interact initially with the proximal tubule cell at the brush border membrane (BBM) where they bind to anionic phospholipids including phosphatidyl inositol and phosphatidic acid. This binding involves the electrostatic interaction between the cationic antibiotic and the acidic phospholipids (Sastrasinh et al., 1982).

After binding to the BBM the antibiotic is internalised by adsorptive, energy-dependent pioncysotasis and concentrated in secondary lysosomes (Cojocel and Hook, 1983). Aminoglycoside elicited morphological lesions like structural changes and loss of brush border membrane, decrease in surface area of rat proximal convoluted tubule (Jones and Elliott, 1987) and formation of enlarged membrane filled secondary lysosomes (myeloid bodies). This reflects the route of entry into the proximal tubule cell (Humes et al., 1982; Kaloyanides and Pastoriza-Munoz, 1980).
Several hypotheses exist as to the nature of the primary aminoglycoside elicited proximal tubule cell lesion. One hypothesis suggests that the critical lesion may be the loss of lysosomal capacity to regulate normal turnover of cellular macromolecules and membranes and instability of lysosomal membranes (Humes et al., 1982).

Aminoglycoside induced changes in mitochondrial permeability and transport processes resulting in the loss of oxidative phosphorylation have been proposed as the critical lesion leading to cellular necrosis (Porter and Bennett, 1981).

Aminoglycoside exposure has been shown to alter proximal tubule cell plasma membrane permeability and transport processes (Lipsky and Lietman, 1980; Williams et al., 1981). The effect may be mediated by direct interaction of the antibiotics with BBM acidic phospholipids (Humes et al., 1982) which play an important role in maintaining plasma membrane integrity as well as a barrier and transport functions, (Lockwook., 1978) and cell hormone interactions (Michell, 1982; Farese, 1983). Aminoglycoside elicited alterations in the metabolism of BBM acidic phospholipids resulting in loss of membrane integrity and critical disruption of all intracellular processes.

Of potential importance is the ability of aminoglycosides and other amines to compete with and displace calcium from anionic phospholipids (Lullmann and Vollmer, 1982) and interact directly with the calcium regulatory protein calmodulin and inhibition of renal Na⁺, K⁺-ATPase (Williams et al., 1984). The inhibition is probably due to an interaction at the cytoplasmic face of the basolateral membrane. This diminished pump activity would also lead to cell injury.
The above mechanism of cell injury brought about by gentamicin strongly suggests that renal cortex is the major site where these proximal events of injury occur. Cortex differs from medulla not only anatomically, but also qualitative differences in metabolism exist due to differences in the tissue stores of substrates and due to compartmentalisation of enzymes.

1.5 CARBOHYDRATE METABOLISM IN THE RENAL MEDULLA

Here the utilisation of glucose occurs by glycolysis. Glycolysis is defined as the formation of lactate from glucose or glycogen. In this process glucose is converted to pyruvate which enters the tricarboxylic acid cycle for further oxidation and energy production. Kean et al. (1961) reported that anaerobic glycolysis as the major source of energy for renal medullary work and the increase in lactate concentration within the medulla has been suggested to be due to an increased rate of medullary glycolysis (Capraro et al., 1961). Since the glycogen stores are minimal, glycolysis occurs primarily from glucose (Schlender, 1973) and the increased activity of hexokinase suggests that the main site of lactate production from glucose is the inner medulla and papilla (Ross et al., 1986). The high glycolytic rate and relatively low medullary pO₂ has led to the suggestion that the medulla has an exclusively anaerobic type of metabolism.

1.6 CARBOHYDRATE METABOLISM IN THE RENAL CORTEX

Krebs et al. (1966) have probed many of the biochemical and physiological phenomena that may affect the gluconeogenic capacities of the renal cortex. Renal cortical
tissue has low glycogen and glucose concentration and has been chosen as the organ for *gluconeogenesis*.

Gluconeogenesis is the de novo synthesis of glucose from non-carbohydrate precursors. The three energy barrier reactions of glycolysis catalysed by

a) pyruvate kinase  
b) phosphofructokinase  
c) hexokinase

are side stepped by reactions catalysed by

a) pyruvate carboxylase and phosphoenolpyruvate carboxy kinase  
b) fructose 1,6 diphosphatase  
c) glucose 6-phosphatase

In the cortical tissue the $pO_2$ is higher than the $pO_2$ of most other tissues and the oxygen availability in the cortex is more than adequate for complete support of aerobic metabolism (Sugano *et al*., 1974).

### 1.7 EFFECT OF GENTAMICIN ON THE ENERGY MECHANISMS

The above differences in energy metabolism between the renal cortex and medulla led to the speculation that there might be changes in energy metabolism of the cell. To substantiate it there are evidences which suggest that gentamicin brings about cell injury, eventually affecting the energy mechanisms of a cell. Normally when a toxin enters a cell a likely target for disruption of cell function might be the energy producing mechanisms. Either glycolytic or oxidative pathways could be involved, which in turn lead to a failure
of energy supply and an eventual failure of the processes that utilize this energy. For example, transport processes.

Although gentamicin has important effects on the cell membrane, it also causes deterioration of mitochondrial function as well. Reduced mitochondria respiration, a deficiency that may cause a complete disruption of energy dependent systems in the metabolism has also been reported (Vera Roman et al., 1975; Kluwe and Hook, 1978).

High doses of Kanamycin, another aminoglycoside antibiotic has been shown to inhibit the glycolytic pathway in the kidney (Tachibana et al., 1976).

Pertaining to the effect of gentamicin on the energetic mechanism of the cell, gentamicin is reported (Hono et al., 1986) to inhibit dose dependently Na⁺ dependent D-glucose transport in a non-competitive manner in both outer cortical and outer medullary preparations. The inhibition may be due to the alteration in the fluidity of the lipid bilayer or the two distinct D-glucose transport sites may have different susceptibilities to gentamicin toxicity or the phosphotidyl inositol contents in the outer cortical or outer medullary BBM may differ.

Masayuki et al (1987) suggested that gentamicin might interact with the glucose carrier in the brush border membrane to inhibit the glucose uptake and it might also exert its effect indirectly through stimulation of sodium uptake.
1.8 SCOPE OF THE PRESENT INVESTIGATION

Aminoglycoside antibiotics are widely used in the treatment of gram negative infections (Bennett, 1983). Nephrotoxicity is a common complication of aminoglycoside antibiotic therapy (Kahlmeter and Dahlander, 1984) and the clinical use of gentamicin is complicated primarily by a high incidence of nephrotoxicity (Moore et al., 1984). Although risk factors for developing aminoglycoside nephrotoxicity have been identified, strategies for obviating this complication have met with limited success.

The pathogenesis of aminoglycoside nephrotoxicity can be viewed as a two-step process. The first step involves the transport and accumulation of antibiotic in high concentration by renal proximal tubular cells (Kaloyerides, 1984) and the second step involves the adverse interactions between these polycationic drugs with one or more critical intracellular processes (Humes et al., 1982).

One potential strategy for mitigating the risk of aminoglycoside nephrotoxicity is to inhibit the uptake of drug by renal proximal tubular cells. A second potential strategy is to inhibit the adverse interactions between drug and intracellular processes. A few compounds have been identified which reduce the risk of gentamicin-induced nephrotoxicity.

1.8.1 Compounds that afford protection against gentamicin nephrotoxicity

In the late 1970's the protective effect of D-glucarates was reported by Furuno et al. (1976). D-Glucaro-δ-Lactam, an anion was shown to exhibit protective effect against aminoglycoside induced nephrotoxicity in rats (Tetsutaro-Niizato et al., 1986).
Calcium has also been reported to diminish aminoglycoside binding by renal mitochondria (Kornguth et al., 1980) Calcium loading is said to reduce gentamicin binding and uptake by the renal tubular brush border Calcium loading is associated with a slower accumulation of gentamicin in the renal cortex attenuating experimental nephrotoxicity (Benett et al., 1982, Quarum et al., 1984)

Polyaminoacids including polyaspartic acid (PAA) have been reported (Williams et al., 1986) to provide protection against the development of aminoglycoside nephrotoxicity PAA protects by competing with gentamicin for a common binding site on the renal brush border and/or basolateral membranes But, surprisingly the total amount of gentamicin accumulated by the kidney cortex is actually increased after co-administration with polyaspartic acid (Beauchamp et al., 1986, Gilbert et al., 1989)

However the actual mechanisms of action is far from clear and presently no literature is available as to their roles pertaining to intermediary metabolism

One of the major sites of action of gentamicin is inhibition of D-glucose transport across the renal brush border membrane where the energy mechanisms are affected (Horio et al., 1986) Apart from this, gentamicin inhibits gluconeogenesis in the kidney cortex (Michalik et al., 1991)

Our present drug of choice, lipoic acid was shown to play a vital role in carbohydrate metabolism by Belly et al in as early as 1955 offat Jayanthi et al (1991) have proved that lipoic acid stimulated the activities of glycolytic enzymes both in liver and kidney and inhibited gluconeogenic enzymes in these tissues Extensive works have
Schematic representation of proximal tubular cell, indicating functional relationships between intracellular ATP concentration and α-MG uptake. ATP is the driving force for the Na⁺/K⁺-ATPase which creates a sodium gradient across the cell membrane allowing Na⁺/glucose cotransport to proceed as a secondary active transport process

(Boogard et al., 1989)
been carried out to study the effect of lipoic acid in diabetic rats (Nataraj et al, 1984, Wagh et al, 1987)

Lipoic acid was shown to increase glucose utilisation and it did not interfere with the action of insulin and its action was additive to that of insulin (Haugaard et al, 1970, Gandhi et al, 1985)

Biber et al (1979) have reported that one or several free -SH groups, located either on the cytoplasmic side of the membrane or within the membrane itself are essential for concentrative D-glucose transport. Oxidation, alkylation or chelation of these -SH groups leads to a specific increase in the Na⁺ permeability of the membrane and in turn to a rapid dissipation of the Na⁺ gradient, driving concentrative D-glucose transport (Murer et al, 1974)

Haugarrd et al (1970) have also reported that lipoic acid might provide a source of sulphydryl groups that allows the tissue to metabolize glucose and possibly other substances at a faster rate

The present project is aimed to study the effect of gentamicin on certain glycolytic, gluconeogenic and TCA cycle enzymes in the kidney and liver tissues of rats along with the role of DL α-lipoic acid
Lipoic acid

\[
\begin{align*}
\text{H}_2 & \quad \text{C} \quad \text{H} \\
\text{H}_2\text{C} & \quad \text{S} \quad \text{S} \quad \text{C} \quad \text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{C}^\circ \\
& \quad \text{O} \\
\end{align*}
\]

\(\alpha\)-Lipoic acid

\[
\begin{align*}
\text{H}_2 & \quad \text{C} \quad \text{H} \\
\text{H}_2\text{C} & \quad \text{S} \quad \text{S} \quad \text{C} \quad \text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{C}^\circ \quad \text{C}=\text{O} \\
& \quad \text{O} \\
\end{align*}
\]

Lipoamide

1.9 CHEMISTRY AND FUNCTION

Trivial names "pyruvate oxidation factor" (POF), "acetate replacing factor" and B.R. Factor were used to designate the biologically active substances prior to its isolation and identification. Lipoic acid is insoluble in water but highly soluble in chloroform and benzene. It is a sulfur containing organic acid and chemically it is \(6,8\), dithio octanoic acid containing 2 sulphur atoms and 8 carbon atoms. It plays an active role in oxidative phosphorylation (Haddock, 1977). The well defined role of lipoic acid is that of a prosthetic group, in multienzyme which catalyze the oxidative decarboxylation of pyruvate and \(\alpha\)KG to produce acetyl coenzyme A, succinyl coenzyme A and NADH respectively.
1.9.1 Distribution and metabolism

Lipoic acid exists predominantly in the protein bound form as the α-aminolysyl amide (Reed, 1966) and is widely distributed among microorganisms like yeast and bacteria, in animals and in green plants.

Lipoic acid was observed to be present predominantly in brain, liver, kidney, intestine and skeletal muscles of rats (Karpov et al., 1977). The metabolism of lipoic acid might be affected during thiamine deficiency (Patterson et al., 1956). Octanoic acid and to a smaller extent oleic acid are precursors for lipoic acid biosynthesis in the rat. The reaction occurs predominantly in the microsomal fraction of the rat liver (Spoto et al., 1982). Lipoate like octanate is transported into mitochondria like any other fatty acid through a carnitine - independent system.

Shih et al (1972) observed that B-oxidation of lipoate to bisnor lipoate or tetranorlipoate was the major degradative pathway for L (1-6)¹⁴ C lipoate in the bacterium Pseudomonas putida. It suggests that peroxidative enzymes of fatty acid metabolism are involved in lipoic acid oxidation.

1.9.2 Isolation

Studies indicate that lipoic acid is not found in the free form but it is lightly bound to protein in covalent linkage through its carboxyl group. Thus it is not extractable by hot water or by lipid solvents and is released only by hydrolysis with acid or alkali or crude proteolytic enzymes.
1.9.3 Biological functions

Lipoic acid is a vital co-factor in the multienzyme complexes that catalyze the oxidative decarboxylation of αKG and branched chain α-Ketoacids. The oxidative decarboxylation catalysed by the complex pyruvate dehydrogenase enzyme system comprises of 3-specific enzymes

(a) Pyruvate decarboxylase (prosthetic group TPP)
(b) Lipoate-reductase-transacetylase (prosthetic group Lipoamide)
(c) Dihydrolipoate dehydrogenase (prosthetic group - FAD)

Wagh et al (1987) reported that lipoic acid or dithioloctanoic acid can substitute for Co A in a wide array of enzymes involved in fatty acid oxidation and biosynthesis of fatty acids, triglycerides and phospholipids completely or partially.

DTO replaces CoA in the following reactions.

Fattyacid + DTO + ATP → Fatty acyl DTO
Acetate + DTO + ATP → Ac DTO
Ac Ac + DTO + ATP → AC AC DTO
Succ + DTO + ATP → Succ DTO
Malonate + DTO + ATP → Mal DTO
Pyr + DTO + NAD⁺+H⁺→ Ac DTO + NADH
L-Gly-3-P + Fatty Acyl DTLO → TG + PL
Belly and Williams (1955) reported significant increases in growth rate and efficiency of food utilization in rats and chicks on purified type diets supplemented with minute amounts of synthetic lipoic acid. The role of lipoic acid in intermediary metabolism may not be confined to its co-factor role in α-ketoacid dehydrogenases but may replace CoA for activating fatty acids prior to acylation with α-glycerol-3 phosphate (Wagh et al., 1987).

1.9.4 Lipoic acid in diseases

Lipoic acid being a co-factor in the multienzyme complex has been observed to correct various conditions like hyperglycemia, ketonemia, reduced glycogen and fat synthesis in diabetic rats. (Nataraj et al., 1984)

Reduced lipoic acid protects against microsomal peroxidation when offered in combination with oxidized glutathione. The effect of infusion of co-factors like CoA, NAD⁺, Lipoic acid in acute hepatitis has been observed in double blind experiment (Colombia et al., 1969) where increase in liver cell fatty degeneration was small with co-factor administration. Thiocetic acid has been found beneficial in treatment of cardiac diseases. (Benda, 1965). Eskelson et al., (1969) reported that lipoic acid has a synergistic inhibitory effect on cholesterol synthesis under in vitro conditions. Injection of lipoic acid in the tumor bearing (Walker carcinoma) rats restored the activity of pyruvate dehydrogenase to normal with in 1 hour, while repeated injections prolonged life expectancy by 25 percent (Kaprov et al., 1977).
Thiostic acid has been successfully used to treat patients suffering from the hepatotoxic effects of mushroom poisoning (Ehrenthal and Prellwitz, 1986). Pharmacological doses are also effective in acute and chronic poisoning of mercuric chloride, arsenite, arsenobenzoates, carbon tetrachloride (Segre, 1956). Gotz et al (1986) have suggested the usefulness of lipoic acid in the preventive medication for recurrent calcium oxalate stone formation. Lipoic acid has thus been found useful in the treatment of various disorders.

The objective of this work was to see whether the administration of lipoic acid is able to reduce the nephrotoxic effect produced by gentamicin along with their effects on carbohydrate metabolism.