Introduction
(A) GENERAL

The kidney is a vital organ, which plays an essential role in health, diseases and overall development and growth. The main function of the kidney is to maintain total body fluid volume, its composition and thus plays a major role in the maintenance and regulation of acid-base balance of the body fluids including the blood. This is accomplished collectively by the presence of several millions of functional units of the kidney known as “nephron”. A nephron consists of a glomerulus with an extended tubular structure. The renal functions are mainly characterized by the reabsorption abilities of the tubules which run through several anatomically distinct parts of the kidney.

The structure of the mammalian kidney apparently looks very homogenous, however, it can be viewed as a composite of several organs/segments, geometrically, functionally and metabolically [1]. Thus each nephron consists of a group of organs/segments arranged in series coursing through four concentric tissue planes namely, the cortex, outer and inner zones of the outer medulla and the inner medulla [1]. Each concentric tissue plane from cortex to inner medulla also possesses individual “organ” characteristics with respect to their ionic contents and characteristics of metabolism (metabolic rates) among the above tissue zones [1]. The renal tubular structure documented to be the site of whole kidney functions can be characterized by transport processes which occur in the mammalian kidney. It has been demonstrated that these transport processes, mostly of ions and solutes, are somehow driven by metabolic energy yielding reactions [2]. The transport of sodium ions (Na⁺) is considered to be the major work function in mammalian kidney, as in
the absence of any Na⁺ transport, the transport of other solutes such as amino acids and hexoses approaches zero [3,4,5]. Fromter et al. [6] suggested that one third of the net transepithelial sodium flux is transported actively. Atleast 80% of this active transport depends on adenosine triphosphate (ATP) [7] indicating a linkage between transport processes and energy producing metabolic reactions [8,9,10,11]. The active transport performed by the kidney has been shown to be associated with oxygen consumption rate which is usually involved in producing energy for such transport systems. Earlier studies demonstrated a linear relationship between sodium transport and oxygen consumption [12,13], however, a direct relationship with the energy production or utilization could not be confirmed.

Fatty acids, glucose and their metabolites e.g. intermediates of citric acid cycle, amino acids, lactate etc. are known to contribute to the energy supply of the kidney in various mammals including man [8]. The rate of metabolism of the above substrates by one or the other pathways seems to be dependent on the availability of oxygen in any particular zone of the kidney [8,14-18]. There appears to be a reverse cortico-medullary gradient for tissue oxygen tension (PO₂) i.e. PO₂ in inner medulla is far lower then cortical tissue [19-22]. Available evidences so far indicate that aerobic metabolism is more prevalent in renal cortex while anaerobic metabolism in renal outer and inner medulla [14].

Nephron which consists of various subsegments shows distinct structural and functional differences. Thus nephron heterogeneity also adds to the variation in the function of kidney as a whole. Both inter- and intra-nephronal heterogeneity exist in
the mammalian kidney that depends on the origin and location of the nephrons in cortical region of kidney [23,24]. The nephrons which originate from the glomeruli located in the superficial cortex are known as “superficial nephrons” while the nephrons originating from the deep cortical region are known as “deep” or “juxtamedullary nephrons”. These populations of nephrons have been found to be distinct structurally and functionally [23].

Inter- and intra-renal heterogeneity of proximal tubules (PT)

Structural and/or functional differences between proximal convoluted tubules (PCT) of superficial cortex and juxtamedullary cortex represent inter-nephron heterogeneity, whereas, differences between early segment and late segment of proximal tubule of a single nephron represent intra-nephron or axial heterogeneity. The nephron classification system fundamentally is based on the cortical location of their glomeruli and/or on the length of their loops of Henle. According to the recent view, nephrons are classified into three groups: Superficial cortical nephrons which have glomeruli located approximately 0.5 to 1mm below the capsular surface, Midcortical nephrons with glomeruli situated in the midcortex deep to the superficial nephrons but above the juxtamedullary nephrons, and Juxtamedullary nephrons with glomeruli located immediately above the corticomedullary junction. Generally, the most superficial nephrons have “short loops” (or even cortical loops) and deep nephrons have long loops. Besides the differences in glomerular diameter, proximal tubular length, filtration rate, epithelial permeability and transport characteristics, transepithelial voltage differences and distribution of various enzyme activities are other factors that contribute to distinguish different nephron populations [25,26]. In
inter-nephron heterogeneity, proximal convoluted tubules of superficial nephrons always touch the surface of the kidney, while convolutions from midcortical nephrons do so infrequently [27] and then tend to run perpendicular to the cortical surface, whereas proximal convolutions from juxtamedullary nephron run perpendicular to and intertwine with medullary rays. In most mammals juxtamedullary nephrons have longer proximal tubules (pars recta included) than superficial nephrons [28-31]. Juxtamedullary-proximal convolutions are approximately 25% longer and have a significantly greater diameter than superficial convolutions [27].

In intra-nephron or axial heterogeneity, the proximal tubules have been divided into three distinct morphological subsegments namely, S₁, S₂ and S₃. The early PCT both in superficial and juxtamedullary nephrons is defined as S₁-segment and can be identified by its attachment with glomeruli on one side. The cells are tall (10-12 μm) having a long (~3 μm) brush border and extensive interdigitations between lateral cell and margin of adjacent cells [27,32]. S₂ is defined as the late superficial proximal convoluted tubule, early superficial proximal straight tubule and late juxtamedullary proximal convoluted tubule. Thus it includes rest of the convolutions and entire pars recta (straight portion) in cortical portion of the kidney. Cells of S₂ segments are shorter, with shorter brush border and less extensive lateral and basal infoldings than S₁ cells [27,32]. In contrast, S₃ is located principally in the outer stripe of outer medulla and terminal superficial proximal straight tubule and entire juxtamedullary proximal straight tubule. S₃ is identified by its medullary location and by its connection with thin limbs on distal part. Superficial pars recta is long and contains S₂ and S₃ cell types whereas juxtamedullary-pars recta is short and made of
predominantly S₃ subsegments [27]. All S₃-subsegments (pars recta), as they descend from cortex into the outer stripe of the outer medulla, change from the S₂ to S₃ cell type. Thus the outer stripe of the outer medulla contains proximal tubular cells but only the S₃ type [27]. S₃ cells are cuboidal and have the longest brush border, fewest mitochondria, least basolateral invaginations and least developed endocytic apparatus of all three proximal tubular cell types [33]. Peroxisomes are most numerous in S₃ cells [33].

Besides morphologic differences, the functional inter- and intra-nephronal differences have also been observed in proximal tubule. Functional differences mostly coincide with the morphological subdivisions. In early PCT (S₁-segment) oxidative metabolism, Na⁺-K⁺ATPase activity and active transport are relatively high, allowing very efficient Na-coupled net reabsorption of glucose, amino acids, phosphate and net secretion of hydrogen ions [23]. In S₂ cells, glucose, Pi and H⁺ transport capacities are still noticeable and progressively decrease along proximal convolution [23]. Finally, in S₃, Na⁺-K⁺ATPase activity, Na⁺ transport is found to be relatively low however the transport of organic acids and bases is main concern of the S₃ segments. Proximal heterogeneity also exists in regard to hormonal action and some other adaptive changes [34]. In the proximal tubule, the apical cell border is well developed and closely packed microvilli form the brush border. The luminal membrane contains a number of specific carrier systems for the reabsorption or transport of ions and solutes [35]. It is well established that in proximal tubule, brush border membrane is the major site by which most of the solutes are reabsorbed [36-
39]. It is also the site for the regulation of the reabsorptive properties by the adaptive changes and by various stimuli [36].

**Acute Renal Failure (ARF)**

The term “Acute renal failure” denotes a dramatic clinical situation in which both the kidneys stop their function within a short period of time or immediately depending on the severity of ARF. Since, the major function of the kidney is to maintain the composition of extracellular body fluids by filtration and reabsorption processes, the loss of renal function is reflected by oligonuria and a steady rise in the concentration of urea and creatinine in plasma [40,41]. The major causes of ARF are either ischemia or toxic insult to the kidneys [42-51].

Acute renal failure is a process rather than a state. It begins with cellular damage initiated by primary toxic insult or ischemia and it continues until renal function and structure have essentially recovered due to reperfusion of blood or by the administration of various drugs and hormones [41,52-74]. Although, the time course of injury and recovery overlap and are variable due to degree of damage, nevertheless, ARF can be grossly divided into three phases; pathogenic phase, manifestation phase and recovery phase. In the first phase, a progressive disintegration and necrosis especially of tubular epithelial cells has been observed, leading to the functional loss of the kidney which is manifested by the reduction of inulin clearance [40]. In the second phase, long lasting effects are observed that severely affect the clearance of both creatinine and inulin and which can continue for several days after recovery begins, depending on the degree of renal damage. Other
additional adverse effects are not observed and the recovery processes are not being initiated in this phase. In the recovery phase there is an increase in concentrating ability of the kidney with eventual normalization of kidney function.

A variety of experimental models have been used to study ARF. In experimental animals, toxic ARF can be induced by various agents such as heavy metals [42,43], chemicals [75-82] and drugs [44,83]. Generally ARF caused by drugs and chemicals is much more severe and irreversible and the recovery sometimes is not possible [40]. The pathophysiologic mechanism of ARF has been investigated extensively in the last few decades [52]. Although a number of structural and functional changes involved in ARF were observed, but due to the varying experimental conditions in which they were observed, no single possible pathogenic mechanism to explain ARF could be concluded [52]. Four major possible causes of ARF have been generalized which include renal vasoconstriction, glomerular permeability, tubular obstruction and tubular leakage [52]. Several preventive measures have also been utilized [41,52-74] but a definite answer for the pathogenesis and its control however remains the topic of future studies.

The physiological role of the kidney in the production of urine involves the selective reabsorption and secretion of solutes and fluids [36,37,84,85]. Tubular reabsorption of sodium, the major work function of the kidney, is dependent on active ion transport, an energy dependent process which in the kidney is primarily dependent on ATP supplied by oxidative metabolism [7-11]. In fact, most of the metabolic work of the renal cell is directed towards the production of ATP for the
support of active reabsorption of ions and solutes [12,13]. A close relationship has been observed between ion transport and cell metabolism in extension with the O2 consumption in the kidney [12,13]. The oxygen tension or consumption in the kidney has been demonstrated to be different in the nephron subsegments distributed in metabolically different kidney tissues such as the cortex and medulla [1,8,11,14,17,18]. ARF due to toxic insult or ischemia, results in the depressed metabolic activity because of limited O2 supply through the blood, curtailing energy production and leading to the loss of active reabsorption and secretion processes in the kidney [49-51,76-78,86].

Pathophysiology of ARF

Toxic agents are known to cause acute or chronic renal failure leading to the partial or total loss of normal excretory functions of the kidney. Acute renal failure is accompanied by a simultaneous but steady rise in plasma creatinine and urea concentrations [40,41]. Classical concepts regarding the cause of the loss of renal functions in ARF include tubular leakage across the damaged epithelium, tubular obstruction by cast formation or interstitial compression, a decrease in renal blood flow (RBF) and glomerular membrane permeability [52]. However, due to lack of support for a satisfactory explanation for the loss of renal function following toxic insult, some of the above factors were considered as not the only basis for initiating the damage caused by toxic insult or ischemia. Raised intratubular pressure indicative of tubular obstruction was not consistently present [87]. A decrease in RBF was frequently observed, but was often lacking [88]. A reduction in glomerular permeability was able to explain only a depression in filtration rate but not the
decrease in tubular function [89-91]. Thus the extensive research of the past 40 years using morphology, clearance and micropuncture techniques were unable to provide a universal view regarding the mechanism for the pathogenesis of ARF. The other reason for not reaching a consensus could be due to the fact that most of the evidences are based on structural alterations rather than on functional ones. Some recent experimental evidence shows that ARF induced by ischemia or administration of toxic agents is characterized by a progression of well-defined events [40,92-94]. Initially, the epithelial cells lining the proximal tubule (the principal site of damage along the nephron) exhibit varying degree of sublethal cell injury that can ultimately culminate in cellular necrosis.

(B) AMINOGLYCOSIDE NEPHROTOXICITY

Renal dysfunction or disease may result from the clinical use of antibacterial agents. The clinical and morphological expression of antibiotic nephrotoxicity represents a spectrum of alterations ranging from acute renal failure and tubulointerstitial nephrotoxicity to selected renal tubular disorders.

Molecular and pharmacologic aspects of Aminoglycoside Nephrotoxicity

Aminoglycosides are highly polar cations (average pKa 8.0 or greater) composed of various sugar molecules in glycosidic linkage with amino-group containing side chains. Aminoglycosides act by inhibiting the synthesis of bacterial proteins via interference with the activity of ribosomes. Aminoglycosides have low lipid solubility and a low capacity for penetrating membranes; all aminoglycosides are poorly absorbed from gut, when given intramuscularly or intravenously, aminoglycosides are
rapidly distributed throughout the extracellular fluid. Because of their polar nature (Fig. 1), these are largely excluded from most cells.

![Structure of gentamicin](image)

**Fig. 1: Structure of gentamicin**

The potential for aminoglycoside nephrotoxicity seems to depend on the number of ionizable amino groups ($\text{NH}_2^+$) contained on their molecule and on the derived cationicity. The cationic charge of the aminoglycoside is correlated with the degree of aminoglycoside and membrane interaction and interference with mitochondrial function [95]. Although a good correlation exists between the cationicity and specific (renal, oto or neurotoxicities) or whole animal toxicity [96,97] but aminoglycosides with similar cationic charges may exhibit different clinical or experimental toxicities [98]. Soberon *et al.* [99] showed that aminoglycosides containing equal number of aminogroups (five $\text{NH}_2^+$ groups) exhibit different nephroxicities. Therefore factors other than the molecular cationic charge are of importance. These may include charge orientation or position and an inherent propensity of the aminoglycoside molecular structure for causing toxic injury to intracellular organelles [99,100].
Renal transport of aminoglycosides

Aminoglycosides do not undergo metabolism in the body and are rapidly excreted by the kidney in experimental animals and/or in humans. The excretion being faster in rats than humans. The kidney is the major route by which aminoglycosides are eliminated from the body [101,102]. Binding of aminoglycosides to plasma protein plays only a minor role in restricting ultrafiltration, however, the degree of protein binding may give conflicting results regarding net tubular transport of aminoglycoside [103,104]. Following a single injection, 60 to 80% of the drug is recovered in the urine unchanged over the subsequent 24 h period [105]. Once in the tubular lumen, aminoglycosides undergo cellular uptake by the proximal convoluted tubule and pars recta. Uptake of GM has been demonstrated by means of microinjection and micropuncture techniques [104,106-108], autoradiograph experiments [109-111], isolated renal cortical slices [104,112,113], tubule suspensions [111,114] and in profused kidneys [115] in either rats or rabbits. Overwhelming evidence supports the net reabsorption of GM along the proximal tubule. Due to small but substantial aminoglycoside uptake, the concentration of aminoglycosides in renal cortex was several folds higher than plasma in humans [116-118] and in experimental animals [118-121]. However, the accumulation in renal medulla and papilla is considerably less. In one study, urinary fractional excretion of GM exceeded delivery of the drug to the superficial distal tubule, suggesting secretion beyond this segment of the nephron [106]. Sheth et al. [107] suggests this heterogeneity for GM transport by demonstrating secretion of GM in the deep nephrons. In the superficial nephrons, reabsorption is the predominant direction of net transport, whereas, net secretion predominates in the juxtamedullary nephron [107]. Although the overall transport
favours net luminal reabsorption, there is possibility that tubular secretion may be important under certain circumstances. Pastoriza-Munoz et al. [108] showed that in Sprague-Dawley rats, netilmicin undergoes both net secretion along the early proximal convoluted tubule and net reabsorption along the pars recta. However GM has higher absorptive flux and lower secretory flux along proximal tubule. These differences in transport explain the higher accumulation of GM in renal cortex. The steps leading to cellular uptake and accumulation of GM in the proximal tubule have been elucidated by means of autoradiographic studies [109-111]. They are schematically depicted in Fig. 2 and can be summarized as follows: The first step is binding of the cationic aminoglycoside to anionic sites on the brush border membranes of the proximal tubular cells [104,110,122,123]. The binding of aminoglycoside to the BBM is mediated in part by electrostatic interaction with acidic phospholipid, predominantly to phosphotidylinositol (PI) [123-125]. However, recently it has been suggested that uptake may also involve the protein receptor gp\textsuperscript{330} [126], also called megalin [127]. Megalin is a member of the low density lipoprotein (LDL) receptor super family that includes the LDL receptor, the very low-density lipoprotein receptor etc. The membrane bound aminoglycosides are then engulfed by absorptive endocytosis (pinocytosis) into small vesicles that fuse with lysosomes. These transfer the aminoglycosides to secondary lysosomes, where storage occurs. The uptake and intracellular accumulation of aminoglycosides in lysosomes has been demonstrated by Tulkens et al. [128-130] in cultured rat fibroblasts and renal tubular cells. Uptake via the basolateral membrane of the proximal tubular cells, although, generally considered to be minor route of aminoglycoside cellular penetration, may contribute to the overall renal cortical accumulation of these drugs and to their
**Fig. 2**: Possible pathways of aminoglycoside transport and mechanisms of aminoglycoside cellular injury. After binding on brushborder, aminoglycosides (AG) are taken up in pinocytotic vesicles (PV), which separate from the luminal membrane. These vesicles fuse with primary lysosomes (PL), and AG are subsequently transferred to secondary lysosomes (SL). The AG may interfere with lysosomal digestion of phospholipids and proteins, inducing formation of myeloid bodies (MB). Later AG labilize lysosomes that may release their lytic content into cytosol or may discharge lysosomal residues outside of the cell via exocytosis. In addition to endocytosis, the existence of other uptake mechanisms at the luminal and contraluminal membrane (*dashed arrows*) may contribute to direct alterations in structure and function of cellular organelles, such as mitochondria (M). In the upper right corner, both a schema of the binding of cationic AG with anionic receptors (phosphatidylinositol, PI) of the brush border membrane and subsequent internalization of the AG-membrane receptor complex is shown. On the *lower right corner*, the assumed effect of gentamicin in blocking phospholipase C and in altering the turnover of the membrane-bound receptor is also shown.
toxicity [106-108,113,118]. Once in the renal tubular cell, it appears that aminoglycosides are in a tightly bound and non-exchangeable pool, which is the cause for their long half-life in the renal cortex [117,120,131]. Since these compounds do not undergo metabolism in the tubular cells, their elimination is accompanied by exocytosis into the tubular lumen.

Morphologic patterns of Aminoglycoside Nephrotoxicity

Aminoglycosides produce acute tubular cell necrosis, largely of the proximal convoluted tubules and pars recta (S_1 and S_2 segments) [117,132-135]. Although the morphologic changes have been extensively studied in animals, findings in human kidney show the same general pattern. An increase in the number and size of secondary lysosomes: cytosegrosomes or phagosomes constitute the earliest morphologic evidence of toxicity [132]. In the rat, these changes are seen 48 h after GM administration and may extend in late toxicity to the distal tubule [132]. However, they have been shown in humans just 4 h after the GM administration with normal renal function [136]. These cytosegrosomes are primary lysosomes that have fused with endocytic or autophagic vacuoles. Many of these secondary lysosomes contain myeloid bodies [136]. Myeloid bodies probably represent autophagic vacuoles that result from sequestration of membrane and organelle fragments damaged during cellular handling of potential toxins including aminoglycosides undergoing lysosomal processing and digestion [137]. However, the lysosomal alterations, including the formation of myeloid bodies, do not necessarily imply that the person or animal under study has been exposed to these compounds.

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Furthermore, they are not specific, since many other toxins are capable of inducing their formation [137].

Following the initial lysosomal morphologic alterations of the proximal tubular cells, a decrease in the number and height of the microvilli of brush border membrane, cytoplasmic vacuolation, and dilation of the ER appear [134]. Depending on the intensity of the lesions, various cellular debris begin to appear in the tubular lumen. Simultaneously mitochondrial swelling also becomes prominent and as cellular injury evolves, initially patchy and later extensive tubular cell necrosis with tubular obstruction becomes apparent.

Since vascular endothelial cells contain negatively charged binding sites and are capable of endocytosis, it is conceivable that glomerular cells may incorporate aminoglycosides [105]. Scanning electron microscope shows that structural alterations of the glomerular endothelial cells may play a causative role in the development of renal dysfunction in ischemia and nephrotoxic acute renal failure (ARF) in humans and animals [90,138,139]. Other studies have revealed both decrease in the size and area of endothelial fenestrae and swelling of endothelial cells following experimental aminoglycoside nephrotoxicity [140-142]. However, more recent quantitative investigations cast some doubt on these earlier findings [143]. Thus presumptive role of the glomerular endothelium in the early pathogenesis of GM-induced acute renal failure remains unsolved.
Cellular Mechanisms of Aminoglycoside Toxicity

The final pathways by which aminoglycosides cause tubular cell necrosis remains to be established, but major cellular targets for their action are the plasma membranes, lysosomes and mitochondria. GM exerts a direct inhibitory effect on renal cortical mitochondrial oxidative phosphorylation [95,144]. Mitochondrial dysfunction also occurs during GM nephrotoxicity in vivo before functional or morphologic evidence of severe renal damage appears [145]. GM is also a competitive inhibitor of mitochondrial Ca\(^{2+}\) uptake [146]. This indicates the potential for aminoglycosides to alter membrane function and thereby, to contribute to toxic cell injury through its interaction with divalent cations. Renal cortex ion composition in dogs treated with GM showed an early cellular depletion of potassium, magnesium and phosphorous [147] which probably explain the potassium and magnesium wasting seen in GM-treated animals [148]. Decrease in sodium and calcium content and decrease in activity of Na\(^{+}\)-K\(^{+}\)ATPase may occur in later phase of GM toxicity [147].

Changes in both the integrity and function of cellular structures have been described as associated with aminoglycoside treatment and they undoubtedly are key factors in the pathophysiologic events leading to cellular toxicity. It is possible that the tubular cells die because of the simultaneous occurrence of multiple changes. In addition to luminal binding and transport of GM, an increase in total phospholipid content and selected phosphoinositides, particularly phosphatidylinositol (PI) have been reported in rat renal cortex after GM treatment [123,124,149]. Similar results were obtained in cultured rat fibroblasts and in rat and human renal tubular cells.
Subcellular fractionation studies of rat renal cortex have shown that the increase in phosphatidylinositol (PI) induced by GM involved multiple cell membranes, including mitochondria, brush border, endoplasmic reticulum and lysosomes in proximal tubular cells [150]. The mechanism of the increase in PI levels is not entirely elucidated, but it is attributed to aminoglycoside-induced inhibition of phospholipases A and C [151,152]. Hostetler and Hall [152] demonstrated aminoglycoside inhibition of lysosomal phospholipases A and C isolated from the rat kidney cortex. Comparative inhibition of these phospholipases by other aminoglycosides approximated their known toxicities [151,153].

Aminoglycosides are typical "lysosomotropic agents" [154]; that is agents taken up in lysosome either by pinocytosis or by any other mechanism. These agents may stay within the lysosomes and may act either by altering the lysosomal content or the lysosomal ability to degrade engulfed substances, thus inducing lysosomal storage disorders. They may also alter the properties of the lysosomal membrane, thus inducing either an increase or decrease in its permeability towards exogenous substances or even to lysosomal enzymes, which may cause cell injury by escaping the organelle. GM inhibits lysosomal enzymes sphingomyelinase and phospholipases A and C, which are responsible for the early steps in the catabolism of phospholipids and thus lead to accumulation of all major phosphoinositides [128-130,149,151-153] leading to the formation of myeloid bodies. Thus the GM toxicity has evolved as a lysosomal phospholipidosis or lysosomal overloading phenomena, which is an early manifestation of aminoglycoside-induced cellular damage [124,128-130]. As per Feldman et al. [124] (Fig. 2), it appears that within the lysosomes, aminoglycosides
might interfere with catabolism of the membrane receptor by directly inhibiting phospholipase C, by modifying substrate-enzyme affinity or by increasing the intralysosomal pH above the range of the enzyme. The relation of the aminoglycoside-induced renal phospholipidosis to cellular dysfunction and necrosis remains unsolved. It is possible that rupture of the lipid-overloaded lysosomes, with release of potent lysosomal hydrolases into the cytosol, or depletion of critical substrates due to suppressed lysosomal catabolism may be responsible for ultimate cellular damage and death [124]. Other mechanisms of cellular injury have also been suggested since membrane phospholipids are involved in regulating membrane permeability to calcium [155,156].

As hypothesized earlier that phosphoinositides function as luminal membrane receptors for aminoglycosides in an animal model that was characterized by resistance to GM nephrotoxicity [157]. Teixeira et al. [158] demonstrated that the Sprague-Dawley rats with untreated streptozotocin-induced diabetes mellitus are functionally and morphologically protected from GM-induced acute renal failure [159]. This resistance was associated with a decreased accumulation of GM by the renal cortex [158] and it was present as early as five days after the induction of experimental diabetes [160]. Other investigators have also recently confirmed that the untreated diabetic rats injected with GM had renal cortical PI levels significantly lower than those of equally injected nondiabetic animals [157]. Moreover, the renal cortex PI content in diabetic animals was lowered even before GM administration [157] which was due to abnormalities of inositol metabolism [161]. Non-diabetic glycosuric animals and the insulin-treated diabetic rats [162] exhibited normal PI
renal cortical levels and so were unprotected from the GM-induced nephrotoxic effects. Thus the lowered content of phosphoinositides, particularly PI, limits the apical membrane binding and subsequent intracellular transport of GM. This results in lower renal cortical accumulation and prevention of aminoglycosides-induced nephrotoxicity. The intriguing observation that recovery from GM-induced nephrotoxicity occurs with continued drug administration remains unexplained [163,164], but it may be related to the resistance displayed by newly regenerated tubular cells to GM toxic effects.

(C) PATHOPHYSIOLOGY OF URANYL NITRATE-INDUCED ARF

Uranyl Nitrate (UN)-induced acute renal failure (ARF) best approximates the basic criterion for an acceptable experimental nephrotoxic model [165]. These criteria are: ease of induction of acute renal failure; reproducible alterations in renal function which were predictable at any given time point in the course of acute renal failure; and parallels in the pathophysiologic manifestations of the model of experimental acute renal failure with those observed in clinical acute renal failure [165-169].

In common with other ARF models, the most frequent pathological lesion observed in the kidney as a result of UN administration is acute necrosis affecting the S3 segment of the proximal tubule. Functional changes reported following single acute nephrotoxic doses of UN include a decrease in the glomerular filtration rate (GFR) [170] and renal blood flow (RBF) [167] and increase in urine osmolality [169], electrolyte excretion [171] together with diuresis [172]. UN also causes an increased level of plasma renin activity [173] elevated blood urea nitrogen (BUN)
and increased plasma creatinine concentration [174]. A combination of pathophysiological mechanisms, including haemodynamic alterations, alteration in glomerular capillary permeability, tubular obstruction and tubular backpressure are believed to contribute to the pathogenesis of ARF [167,175]. However despite extensive study by conventional bioanalytical procedures, the mechanism of UN-induced renal dysfunction and biochemical sequelae of UN exposure remains poorly understood.

The administration of UN to rats causes morphologic changes detectable as early as 1h following injection that become progressively more severe during the next 5 days. Initially, the entire epithelium of cortical proximal tubules shows subtle changes. Subsequently, severe lesions and necrosis occurs in a predictable pattern along the tubule in the following order: P₃C (Cortical P₃)>P₂C> P₃M (Medullary P₃) [176]. The renal corpuscle and portions of the nephron distal to the proximal tubule develops abnormalities by the end of day 5 after treatment.

The systemic distribution of uranium after its administration may account for several of its characteristic effects on renal structure and function. After injection uranium complexes with bicarbonate in the blood [177] this complex filters freely at the glomerulus, and as much as 50 percent of the dosage given is excreted in urine in the first 24 hours [178]. Uranium deposited in bone has a half-life of 50 to 60 days [179] and is held in reversible equilibrium with that in blood [177]. Once filtered, uranyl ligands become progressively more concentrated and interact with the luminal plasma membrane of lateral segments of the proximal tubule, resulting in the
inhibition of active transport of sodium, chloride, amino acids and hexoses [177]. The
general morphologic changes associated with sublethal cell injury induced by uranium
compounds are not well known inspite of the fact that uranium-induced ARF is the
major experimental model. However, it has been shown that the earliest site to
become necrotic is the portion of the proximal tubule at the corticomedullary
junction, and injury progresses proximally and distally from this site [180]. Necrosis
of the pars recta [169,180-182] and change in the glomerular epithelial cells
[139,165,183] within days after injection of UN has also been well documented.

It has been suggested that the tubular injury escalates progressively for at least 5
days following injection of UN. Thus, the pattern of injury caused by uranium more
closely resembles that of cisplatin rather than mercury as has long been assumed. In
the cisplatin model of ARF, proximal tubular necrosis is widespread five days after
administration of the agent [184]. Mercury, in contrast, causes peak necrosis 24 h
later, with epithelial regeneration well under way within 5 days of treatment. Only
minimal evidence of repair is seen within 5 days after UN administration. The
progressive degeneration of the nephron might be attributed to the fact that bone acts
as a reservoir from which uranium is continually released [183], resulting in the slow,
prolonged exposure of the kidney to the nephrotoxin.

Within a few hours of injection of UN, the P3C segment begins to show its unique
sensitivity to the metal [180]. Diane [176] documented that necrosis extends
proximally up to pars recta, and that P3M region was the last to undergo necrosis.
The particular susceptibility of the P3C segment is poorly understood. Inner cortical
ischemia as a factor is doubtful since the cortical distribution of blood remains constant following administration of UN [167]. There is evidence that uranium selectively attacks the adluminal surface of the epithelium of this segment [185]. Moreover it has been emphasized that the metabolic activities within the P3C segment is heterogeneous in its distribution. It is possible that either uranium is secreted at the P3C segment or that some aspect of the metabolism of the cells at this site, reflected at the basal or luminal plasma membrane as perhaps an increase in electronegativity, preferentially attracts uranium. Cast formation in thin limbs of loops of Henle at the day 5-time interval is also reported by Diane [176]. The extent of the casts alone would preclude normal flow of filtrate and can be expected to cause considerable obstruction. Tubular obstruction must, therefore, be considered as a possible factor contributing to functional deficits during the later maintenance phase of UN-induced ARF. This cast formation due to uranium probably causes proteinuria in both the initiation [186] as well as maintenance [180, 186-188] phase of UN-induced ARF.

In summary, UN causes widespread changes throughout the kidney. These changes begin to manifest themselves shortly after exposure of the animal to uranium and become progressively more severe for the subsequent 5 to 6 days. Uranium has a predilection for the pars recta of the proximal tubule but most of the nephron eventually becomes involved in pathologic changes.

(D) PROTECTION AND PREVENTION OF ARF

It appears from the above discussion that the pathophysiology and/or
biochemistry of ARF is not yet fully clear. A potential repair, prevention and/or protection would also be a more rational approach for better understanding the pathogenesis as well as for the treatment of ARF. Reperfusion of blood and certain chemicals, drug or hormones have been used in recent past demonstrating a partial recovery from toxic shock or ischemia [41,53-74]. Some attempts have been made in the past to reverse the drug or ischemia-induced alterations by using glucose, essential and non-essential amino acids and their α-keto derivatives which could increase the survival rate after the ARF episode [189]. Solez et al. [190] reported a beneficial effect of propranolol and chloridine. Also, mannitol, furosemide, dopamine, prostaglandins and bradykinin have been shown to prevent the functional defect in some models of ARF. Recently, calcium channel blockers like verapamil have been included in the above group [191-193].

Various other agents like epidermal growth factor, EGF [57-59,72,194], endothelin [41,60], atrial natriuretic factor [61,62], defibrotide [64,65], glycine and glutathione [81], insulin like growth factor1 IGF1 [53,71], platelet-activating factor [195], platelet-activating factor antagonist BN52021 [196], fasting [197], pentoxifylline analogue HWA448 [198], fleroxacin [199] and fish oil [200] have been shown to control renal failure induced due to toxic insult and/or ischemia. Various hydroxyl radicals scavengers like superoxide dismutase [67], dimethylthiourea (DMTU) and allopurinol [69,70,73], carvidol [201] or iron chelators [202], when administered just prior to the ischemic event or toxic insult, have also been reported to ameliorate renal function.
Above attempts which were made to ameliorate the function of ARF kidney, generally resulted in the improvement of GFR and RBF, decreased serum creatinine and BUN with increased inulin as well as creatinine clearance and in the enhancement of cellular regeneration [34-74,203]. The above efforts, however, failed to provide any generalized mechanism for the damage or repair of the ARF kidney in particular.

**Protection and/or prevention against GM and UN nephrotoxicity**

Due to their broad spectrum of activity against aerobic gram-negative and gram-positive organisms, their chemical stability, and their rapid bactericidal action, aminoglycoside antibiotics remain of considerable value for the treatment of variety of life-threatening infections. However, nephrotoxicity frequently occurs as an adverse effect of aminoglycoside dosing especially GM [204], which is most commonly used. The reduction of the toxicity of these drugs remains a concern among clinicians and efforts have been made towards a better assessment of potential risk factors. An alternative strategy is the pre- or coadministration of inhibitors, but the clinical use of these is still questionable. Poly-L-aspartic acid has recently been shown to protect against aminoglycoside nephrotoxicity without reducing the cortical accumulation of aminoglycosides [205-210]. A series of studies by Kisore et al. [205,211,212] demonstrate that poly-L-asp and not poly-L-glu is an effective protectant against all measurable acute and sub-acute signs of aminoglycoside-induced alterations in rats but these studies also point out to the possibility of mild lysosomal thesaurismosis to develop under these conditions. The latter would necessitate further toxicological evaluation. With respect to poly-L-glu, these studies also demonstrate that not all polyanionic peptides can be used for the purpose of
nephroprotection in vivo and thereby clearly show the limits of the in vitro investigations (where poly-L-glu behaved similar to poly-L-asp in binding the drug and displacing it from anionic phospholipids or renal membrane vesicles), for the design and screening of this type of nephroprotectants [205].

Recent studies have shown that daptomycin, a lipopeptide antibiotic active against methicillin resistant staphylococci and other clinically important aerobic, facultative, and anaerobic gram-positive bacteria, protected proximal tubular cells against GM-induced renal toxicity in the presence of a similar or even an increased accumulation of the aminoglycosides in the renal cortex [213-215]. The mechanism of this inhibition of toxicity is still unknown [216]. Adnan et al. [217] showed that methimazole, a sulfur-containing drug commonly used to treat hyperthyroidism protects against kidney damage induced in rats, mice, and dogs by cisplatin, an antitumor drug. Furthermore, methimazole has been shown to protect rats against nephrotoxicity elicited by cephaloridine, S-(1-2 dichlorovinyl)-L-cysteine and 2-bromohydroquinone, the metabolites of trichloroethylene and bromobenzene, respectively, and GM [217-219].

Investigations into thyroid-induced nephrotic effect lately have acquired growing interest owing to the demonstration of a positive influence of thyroid hormone upon various renal partial functions, occurring not only in normal or hypothyroid individual but also in the individual suffering from kidney disease [220]. The theory upon which these studies are based is that there is a thyroxine-mediated effect on tubular cells, resulting in an increase in Na⁺ reabsorption in the proximal tubule and loop of Henle,
this will then signal the juxtaglomerular apparatus to release preglomerular or 
vasoconstriction and thereby increase RBF and GFR [220]. The protective effect of 
thyroxine (T₃) on GM- and uranyl nitrate-induced ARF, two dissimilar nephrotoxic 
models of ARF, indicates that protection is likely to be nonspecific [43]. Further, the 
finding suggests that the following plasma membrane changes may be important in 
thyroid hormone protection: (1) a stimulation of Na⁺-K⁺ATPase activity, (2) an 
alteration in the brush border membrane structure and (3) accelerated repair and/or 
regeneration of injured membranes [221-222].

The precise cellular mechanisms responsible for the beneficial effect of thyroxine 
cannot be determined from the earlier studies. However, this hormone has been 
shown to stimulate gluconeogenesis [223] and Na⁺-K⁺ATPase activity in the renal 
cortex [224]. In fact, T₃ can influence protein synthesis in the renal cortex so as to 
account for an increase in both the number and activity of Na⁺-K⁺ATPase units and 
to promote glucose and amino acid uptake [225] by epithelial cells. Each of these 
effects would be expected to aid the repair and regeneration of injured tubular 
epithelial cells. It is tempting to speculate that thyroxine and the adenine nucleotides 
may share a common molecular mechanism, such as repletion of cellular nucleotides, 
or repair of cell membranes by restoration of Na⁺-K⁺ATPase pumps. Both of these 
effects would augment cell volume regulation and enhance recovery from acute renal 
injury. However, Whittem et al. suggest that thyroid hormone does not protect 
against GM-induced proximal tubule epithelial injury at a cellular level despite a 
reduction of cellular GM uptake [226].
Since nephrotoxins or ischemia consequently cause depletion of tissue level of ATP, ADP and AMP [54], it has been proposed that restoration of ATP synthesis by the damaged kidney can have a beneficial effect. Seigel et al. [55,56] showed that administration of adenine nucleotides (ATP, ADP and AMP) combined with magnesium chloride, ameliorates the renal function after the initiation of acute renal failure. One of the reason for the decrease in ATP, ADP or AMP in renal tissue can be depleted Pi level due to reduced Pi reabsorption across the apical membrane, the primary site of action for most of the nephrotoxins. The Na-Pi co-transport across luminal brush border membrane is the rate-limiting step in proximal tubular Pi reabsorption [38]. Thus the studies of Na-Pi co-transport and its modulation by various stimuli is considered to be useful for the protection against ARF. The long-term dietary phosphate deprivation [37,38] in response to feeding by low phosphate diet (LPD) and the administration of thyroid hormones [227] are the two experimental maneuvers known to elicit the most pronounced long term adaptive increase [37] in the capacity of renal BBM for Na⁺ gradient-energized Na⁺-Pi cotransport. As the adaptive modulation of Pi transport is virtually always manifested as an increase or decrease in \( V_{\text{max}} \) of Na⁺ gradient-dependent transport of Pi across BBM without change in apparent \( K_m \) for Pi (\( K_m Pi \)), it has been proposed that in a more restricted biochemical sense the long term adaptive changes are due to an increase or decrease in number of Na-Pi symporters, also called carriers. Yet, it should be considered that the increase in \( V_{\text{max}} \) may also be due to an enhanced rate of translocation of Pi, together with Na⁺ by Na-Pi symporters already existing in BBM, with or without an increase in their number. Yusufi et al. [228] showed that in response to treatment with T₃ the greater number of Na-Pi symporters with normal
affinity (K_mPi) are inserted into BBM. The T_3 evoked increase in Na^+ gradient-dependent ³²Pi uptake by BBMV, which was blocked by Act and CHK; thus indicating that Na-Pi symporters newly inserted into BBM are probably de novo synthesized in response to T_3. In contrast, in adaptation to LPD, the increase in Na^+ gradient-dependent Pi transport kinetically similar to the increase in response to T_3, is due to a faster rate of Pi plus Na^+ translocation [228] across BBM by Na-Pi symporters that exist in BBM in the same number and have same affinity for Pi as in the control state.
SCOPE OF THE THESIS

Aminoglycosides are potent, water-soluble antibiotics with peak concentration dependent bactericidal activity against many pathogenic gram-negative bacilli. Being highly water soluble, they do not cross biological membranes readily. For systemic therapy, they must therefore be given by intravenous, intraperitonial or intramuscular injection. For the same reason, once in the body, they are largely confined to extracellular spaces, have correspondingly small volumes of distribution (Vd) and are mainly eliminated unchanged in urine. Their penetration into cerebrospinal fluid, bronchial secretions and vitreous humour is very meager. Thus ordinarily, their efficacy does not extend to these tissues or to the intracellular pathogens.

GM is the usual all purpose agent of choice which exhibits enduring antibacterial activity (especially against gram-negative bacilli) even many hours after tissue concentrations become negligible. However, like other aminoglycosides, GM is also endowed with a concentration-dependent liability to produce (i) ototoxicity (that commonly becomes irreversible) [229-231] and (ii) reversible nephrotoxicity [232]. Both of these toxic manifestations show a positive correlation with conventionally determined high trough concentration. GM concentration dependent efficacy and toxicity confer a narrow therapeutic index (range).

To account for the nephrotoxicity following GM course and recovery on long-term treatment, it should be appreciated that in reality, GM pharmakinetics and pharmacodynamics are very complex. Due to this complexity, the reverence and mystique conferred on so-called ‘toxic’ trough concentrations or concentration-
dependent toxicity seems justified in some aspects while illogical in others [204,233]. The ambiguity still persists in the relationship between GM toxicity and/or efficacy and its peak or trough values. This ambiguity casts shadow on the efficacy and safety of single dose vs. multiple dose in GM treatment.

GM or other nephrotoxins cause structural tubular changes accompanying the development of acute renal failure (ARF) in experimental animals. Loss of microvilli, increased apical vacuolization, mitochondrial swelling and eventual cell necrosis occur at specific sites within the proximal tubule during experimental ARF both in the ischemic model [234] and in nephrotoxic models induced by mercuric chloride, cisplatin and some antibiotics [235-237]. While the morphology associated with each of these models of ARF has been described in detail, the changes associated with sublethal cell injury induced by uranium compounds are unknown inspite of the fact that uranium-induced ARF is a major experimental model [170]. The present investigation was designed to more carefully characterize renal structural changes produced by UN and GM throughout both the initiation and maintenance phase of ARF. Single or multiple doses of GM or UN treatment result in profound alterations in tubular cell functions, metabolism and structural integrity. Studies involving toxic and/or ischemic ARF have shown that renal proximal tubular cells are severely affected [46,234] and undergo dynamic transformations [234]. The damage that occurred to proximal tubular cells, the main functional site where most of the fluid, ions and molecules are reabsorbed, is poorly understood [238,239]. Both the early damage due to GM administration or recovery during long course of treatment are difficult to follow. However, their knowledge is very much essential for better
understanding of the pathophysiology of ARF. It has been suggested that the proximal tubular brush border membrane (BBM) is badly affected by toxic or ischemic insult and is mainly responsible for the loss of excretory functions of kidney. The effect of nephrotoxins has been manifested in rats mainly by the disappearance of brush border microvilli [240,241] or by occasional irreversible interiorization of microvilli and subsequent loss into the lumen leading to cellular necrosis and ARF [47]. The proximal tubular cell membrane has been found to be histologically regenerated after long-term treatment of GM or UN. Physiologic alterations of BBM due to toxic insult implicating surface membrane dysfunction include, loss of polarity, changes in the fluidity, loss of selective permeability, reductions in the activity of BBM associated enzymes and decrease in proximal tubular fluid, sodium, glucose and cation reabsorption. Some studies demonstrated that the loss of activities of BBM-enzymes: AlkPase, GGtase and LAP show direct relationship with enzymuria. Studies by Herminghuysen et al. [242] and Desmonliere & Camber [243] showed an increase in urinary GGtase activity in ischemia or intoxicated rats indicating the alterations in the BBM and the sloughing of the enzyme components to accumulate in the urine. Increased fractional sodium excretion together with reduced renal oxygen consumption was also observed [242].

Heterogeneity of the proximal tubule in terms of both structure and function is well known [243,244]. It has been shown that proximal tubule which consists of S1 (pars convoluta), S2 and S3 (pars recta) subsegments, differ in the distribution of marker enzyme [245,246], reabsorptive properties of various solutes and differential response to various drug, hormones and dietary stresses [247-254]. Cronin et al. [43]
has reported that these nephron subsegments were also differentially affected by GM and/or UN. While the surface nephron (S1-subsegments) showed susceptibility to GM, the deeper nephrons (S3-subsegments) were severely damaged by UN. It has been further reported that nephron functions, after early intoxication, were markedly suppressed in surface nephron by GM, the suppression was to a smaller extent in middle nephrons by both GM and UN and severely depressed in the deep ones by UN administration. GM and other aminoglycoside antibiotics-induced toxic insult showed specific effects in proximal convoluted tubular (PCT) segments [237,255] while proximal straight tubule (PST-pars recta) segment was shown to be more sensitive to oxygen deprivation, ischemia or toxic insult due to UN. Molitoris et al. [49,50] have implicated variations in lipid contents or fluidity of the membrane as one of the major reasons for toxic and ischemic ARF [256,257].

Studies of transport functions showed reduction in the Na-dependent glucose [49,50] and organic cations [51] in nephrotoxic or ischemic ARF. The transport of Pi, which involves in the maintenance of energy for many renal functions [85,86] has not been studied so far in nephrotoxic ARF. The transport of Pi in renal proximal tubule is known to be regulated at the brush border membrane site [38]. The feeding of low Pi diet (LPD) and the treatment of thyroid hormone resulted in the increased transport of Pi by differential mechanisms [228]. Thyroxine has been demonstrated to be beneficial in drug-induced as well as ischemic ARF [43,63].

It can be suggested that the study of known nephrotoxins like UN in parallel with clinically relevant nephrotoxins such as GM in the laboratory can provide clues to the
pathogenesis of toxic nephropathy as well as to renal injuries produced by other etiologic agents. In view of this, the present work was carefully designed and undertaken to study the damage caused by nephrotoxins to the structure and functions of the renal cortical proximal tubules. For better understanding of the mechanism of the pathogenesis of GM- and UN-induced ARF and its possible reversal, the following studies were performed:

(1) The natural history was studied during the course of GM and UN administration.

The serum and urine parameters viz. creatinine, phospholipid, cholesterol, ionic concentrations (Na⁺, Mg²⁺, K⁺) along with proteinuria, enzymuria and glycosuria were carefully monitored during the GM and UN administration.

(2) The activities of some marker enzymes considered to be of structural and functional importance of the proximal tubular BBM eg. AlkPase, Maltase, LAP and GGtase were determined in BBMVs isolated from WC or SC and JMC tissues of GM- or UN-treated and control rats. The activities of enzymes belonging to other organelles were also determined after GM or UN administration.

(3) The activities of various enzymes of metabolic importance especially related to energy metabolism (in particular, of glucose metabolism) were determined in different tissue zones of the kidney eg. whole cortex, superficial cortex, juxtamedullary cortex and medulla isolated from GM- or UN-treated rats.

(4) Transport of Pi was determined and further characterized in the BBMVs isolated from GM- or UN-treated rats under different conditions.

(5) The protective/preventive effect of thyroid hormone (T₃) and LPD were explored on BBM Pi transport affected by GM or UN.
The present studies showed specific alterations due to GM and UN treatment on the above parameters. These studies would be helpful in further understanding the pathogenesis of GM- and UN-induced toxic ARF and its possible prevention or protection.