

SPECIFIC NEPHROTOXICANTS

Heavy Metals

Many metals, including cadmium, chromium, lead, mercury, platinum, and uranium, are nephrotoxic.

It is important to recognize that the **nature** and **severity** of metal nephrotoxicity **varies** with respect to **its form**.?

For example, **salts of inorganic mercury** produce a greater degree of renal injury and a lesser degree of neurotoxicity than do **organic mercury** compounds.

Metals may cause toxicity through their ability to bind to **sulfhydryl** groups.

For example, the affinity of mercury for sulfhydryl groups is very high and is about 10 orders of magnitude higher than the affinity of mercury for carbonyl or amino groups.

Thus, metals may cause renal cellular injury through their ability to **bind to sulfhydryl groups of critical proteins within the cells** and thereby inhibit their normal function.

Mercury

Humans and animals are exposed to **elemental mercury vapor, inorganic mercurous and mercuric salts, and organic mercuric** compounds through the environment.

Administered elemental mercury is rapidly oxidized in erythrocytes or tissues to inorganic mercury, and thus the tissue distribution of elemental and inorganic mercury is similar.

Due to its high affinity for sulfhydryl groups, virtually all of the Hg^{2+} found in blood is bound to cells—**albumin**, other **sulfhydryl**-containing proteins, **glutathione**, and **cysteine**.

The kidneys are the primary target organs for accumulation of Hg^{2+} , and the S 3 segment of the proximal tubule is the initial site of toxicity.

As the dose or duration of treatment increases, the S 1 and S 2 segments may be affected.

Renal uptake of Hg^{2+} is very rapid with as much as 50% of a nontoxic dose of Hg^{2+} found in the kidneys within a few hours of exposure.

Considering the fact that virtually all of the Hg^{2+} found in blood is bound to an endogenous ligand, it is likely that the luminal and/or basolateral transport of Hg^{2+} into the proximal tubular epithelial cell is through cotransport of Hg^{2+} with an endogenous ligand such as glutathione, cysteine, or albumin, or through some plasma membrane Hg^{2+} -ligand complex.

Current evidence indicates that at least two mechanisms are involved in the proximal tubular uptake of Hg^{2+} (Fig. 1).

One mechanism appears to involve the apical activity of γ -glutamyl transpeptidase, cysteinylglycinase, and the transport of Cys–S–Hg–S–Cys through one of more amino acid transporters.

Basolateral membrane transport is likely to be mediated by the organic anion transport system.

The acute nephrotoxicity induced by HgCl_2 is characterized by **proximal tubular necrosis and AKI within 24 to 48 hours after administration.**

Early markers of HgCl_2 –induced renal dysfunction include an increase in the urinary excretion of brush-border enzymes such as **alkaline phosphatase** and γ -GT, suggesting that the brush border may be an initial target of HgCl_2 .

As injury progresses, tubular reabsorption of solutes and water decreases and there is an increase in the urinary excretion of glucose, amino acids, albumin, and other proteins.

Associated with the increase in injured proximal tubules is a decrease and progressive decline in the **GFR.**

The reduction in GFR results from the glomerular injury, tubular injury, and/or vasoconstriction.

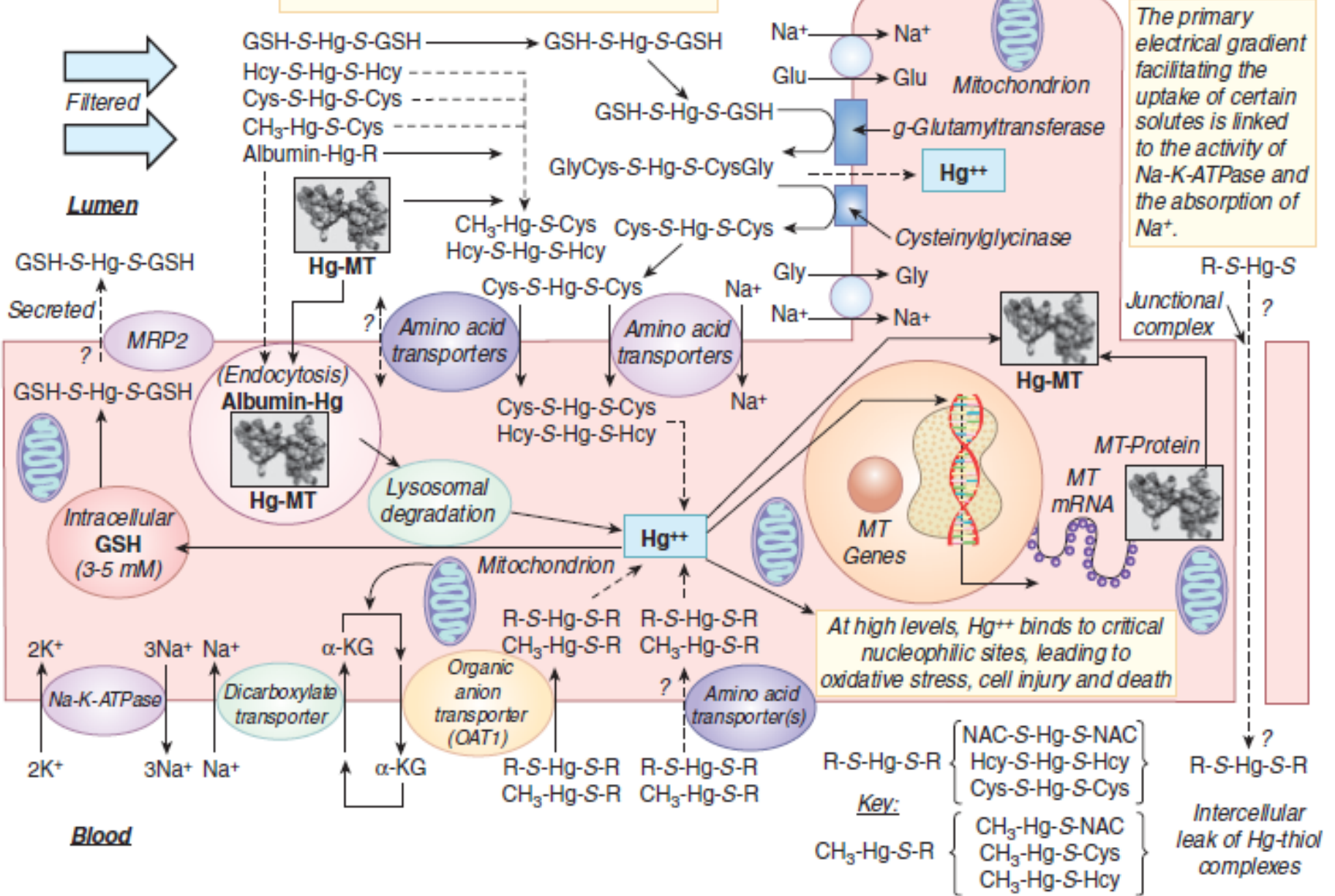
If the decline in renal function is not too severe, the remaining proximal tubular cells undergo a proliferative response and renal function returns over time.

Chelation therapy with 2,3-dimercaptopropane-1-sulfonate or 2,3-mesodimercaptosuccinic acid is used for the treatment for mercury-induced nephrotoxicity.

Changes in mitochondrial morphology and function are very early events following HgCl_2 administration, supporting the hypothesis that **mitochondrial dysfunction** is an early and important contributor to inorganic mercury-induced cell death along the proximal tubule.

Other studies have suggested that **oxidative stress and dysregulation of Ca^{2+} homeostasis** plays an important role in HgCl_2 -induced renal injury.

Handling of Hg by proximal tubular cells



The primary electrical gradient facilitating the uptake of certain solutes is linked to the activity of Na-K-ATPase and the absorption of Na⁺.

At high levels, Hg⁺⁺ binds to critical nucleophilic sites, leading to oxidative stress, cell injury and death

Intercellular leak of Hg-thiol complexes

Cadmium

Chronic exposure of nonsmoking humans and animals to cadmium is primarily through food and results in nephrotoxicity.

In the workplace, inhalation of cadmium-containing dust and fumes is the major route of exposure.

Cadmium has a **half-life** of greater than **10 years** in humans and thus accumulates in the body over time. Approximately 50% of the body burden of cadmium can be found in the kidney and nephrotoxicity can be observed when Cd concentrations exceed 50 $\mu\text{g/g}$ kidney wet weight.

Cadmium produces proximal tubule dysfunction (S 1 and S2 segments) and injury characterized by **increases in urinary excretion of glucose, amino acids, calcium, and cellular enzymes**. This injury may progress to a chronic interstitial nephritis. **A very interesting aspect of cadmium nephrotoxicity is the role of metallothioneins. Metallothioneins are a family of low-molecular-weight, cysteine-rich metal-binding proteins that have a high affinity for cadmium and other heavy metals.**

In general, the mechanism by which **metallothionein** is thought to play a role in cadmium and heavy metal toxicity is through its ability to bind to a heavy metal and thereby **render it biologically inactive**. This assumes that the unbound or “free” concentration of the metal is the toxic species. Metallothionein production can be induced by low, nontoxic concentrations of metals.

Following an oral exposure to CdCl_2 , Cd^{2+} is thought to reach the kidneys both as Cd^{2+} and as a **Cd^{2+} -metallothionein** complex formed and released either from intestinal cells or hepatocytes.

The **Cd^{2+} -metallothionein complex is freely filtered by the glomerulus** and reabsorption by the proximal tubule is probably by endocytosis and is limited.

Inside the tubular cells, it is thought that lysosomal degradation of the Cd^{2+} -metallothionein results in the release of “free” Cd^{2+} , which, in turn, induces renal metallothionein production. Once the renal metallothionein pool is saturated, “free” Cd^{2+} initiates injury.

Halogenated Hydrocarbons

Halogenated hydrocarbons are a diverse class of compounds and are used extensively as chemical intermediates, solvents, and pesticides. Consequently, humans are exposed to these compounds not only in the workplace but also through the environment. Numerous toxic effects have been associated with acute and chronic exposure to halogenated hydrocarbons, including nephrotoxicity.

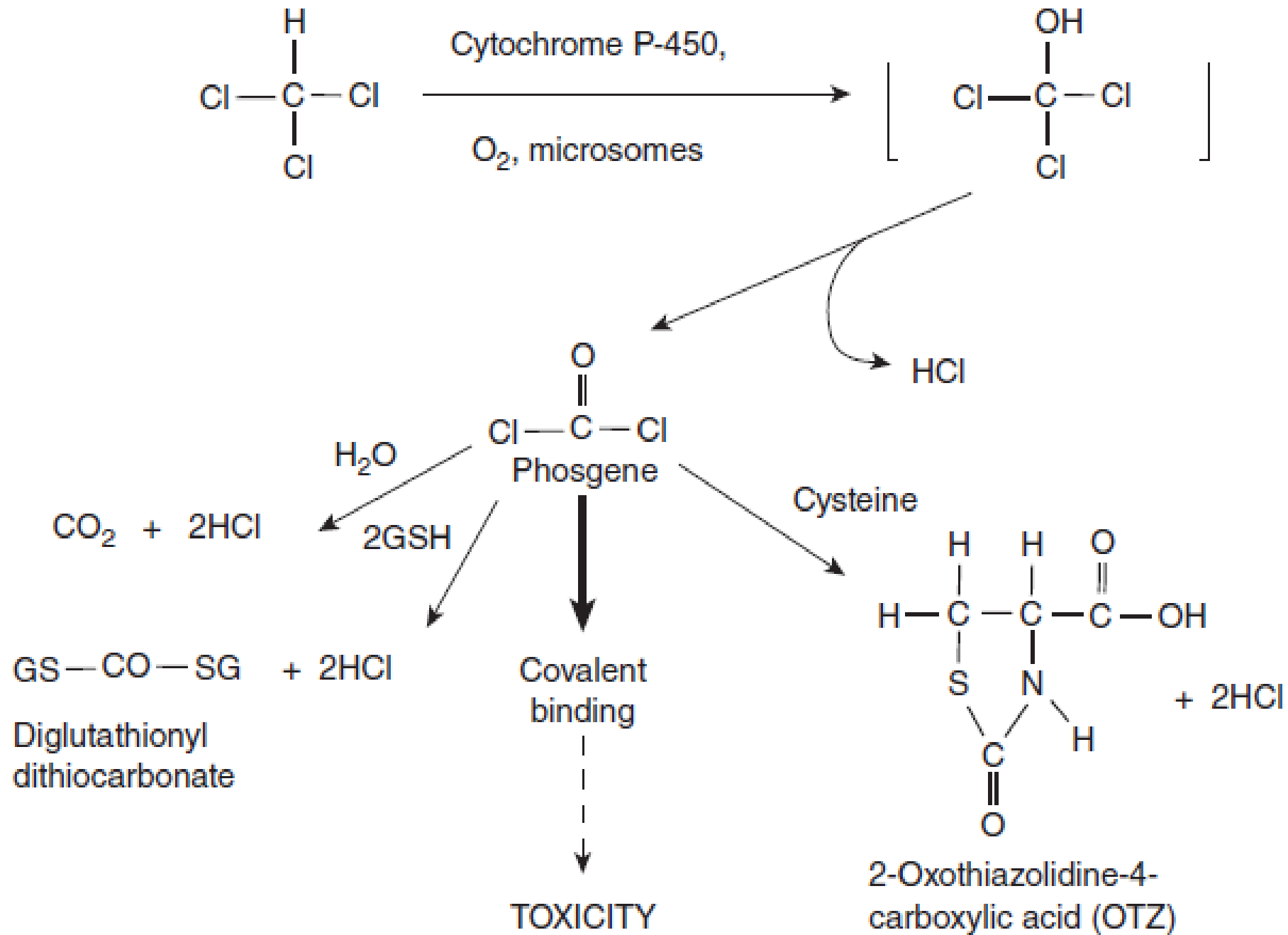
Chloroform

Chloroform produces nephrotoxicity in a variety of species, with some species being more sensitive than others. The primary cellular target is the proximal tubule, with no primary damage to the glomerulus or the distal tubule. **Proteinuria, glucosuria, and increased BUN levels are all characteristic of chloroform-induced nephrotoxicity.** The nephrotoxicity produced by chloroform is linked to its metabolism by renal **cytochrome P450** and the formation of a **reactive intermediate that binds covalently to nucleophilic groups on cellular macromolecules.**

Cytochrome P450 biotransforms chloroform to trichloromethanol, which is unstable and releases HCl to form phosgene. Phosgene can react with (1) water to produce $2\text{HCl} + \text{CO}_2$, (2) two molecules of glutathione to produce diglutathionyl dithiocarbonate, (3) cysteine to produce 2-oxothiazolidine-4-carboxylic acid, or (4) cellular macromolecules to initiate toxicity (see figure 2).

*Figure 2: Proposed mechanism of chloroform biotransformation. Chloroform undergoes cytochrome P450 - catalyzed conversion to trichloromethanol ($\text{CCl}_3 - \text{OH}$), which spontaneously decomposes to form **phosgene**.*

Phosgene is highly reactive and may be detoxified by reacting with sulfhydryl - containing chemicals (cysteine, glutathione [GSH]). Alternately, phosgene can react with sulfhydryl groups on protein, leading to covalent binding and possibly to toxicity.



Therapeutic Agents:

Nonsteroidal Anti-Inflammatory Drugs

NSAIDs such as aspirin, ibuprofen, naproxen, indomethacin, and cyclooxygenase-2 inhibitors (eg, celecoxib) are extensively used as analgesics and anti-inflammatory drugs and produce their therapeutic effects through the inhibition of prostaglandin synthesis. At least **three different types of nephrotoxicity have been associated with NSAID administration.**

AKI may occur within hours of a large dose of a NSAID, is usually reversible upon withdrawal of the drug, and is characterized by decreased RBF and GFR and by oliguria.

When the normal production of vasodilatory prostaglandins (eg, PGE₂, PGI₂) is inhibited by NSAIDs, vasoconstriction induced by circulating catecholamines and angiotensin II is unopposed, resulting in decreased RBF and ischemia. **A number of risk factors (eg, renal insufficiency, congestive heart failure, hepatic cirrhosis, hemorrhage, hypertension, sepsis, diabetes) are known to facilitate the development of AKI following NSAIDs consumption**

In contrast, **chronic consumption** of combinations of NSAIDs and/or APAP (>3 years) results in an often **irreversible** form of nephrotoxicity known as **analgesic nephropathy**.

Impaired urinary concentration and acidification are the earliest clinical manifestations. The primary lesion in this nephropathy is papillary necrosis with chronic interstitial nephritis.

Initial changes are to the medullary interstitial cells and are followed by degenerative changes to the medullary loops of Henle and medullary capillaries.

The mechanism by which NSAIDs produce analgesic nephropathy is not known, but may result from **chronic medullary/papillary ischemia secondary to renal vasoconstriction**.

Other studies have suggested that a reactive intermediate is formed in the cells that, in turn, initiates an **oxidative stress, or binds covalently to critical cellular macromolecules**.

The third, even though rare, type of nephrotoxicity associated with NSAIDs is **an interstitial nephritis** with a mean time of NSAID exposure to development of approximately five months.

This nephrotoxicity is characterized by a diffuse interstitial edema with mild-to-moderate infiltration of inflammatory cells. Patients normally present with **elevated serum creatinine, proteinuria, and nephritic syndrome**.

If NSAIDs are discontinued, renal function improves in one to three months.

Aminoglycosides

Aminoglycoside antibiotics (Figure 3), such as gentamicin, amikacin, and netilmicin, are powerful drugs for the treatment of serious gram - negative infections.

However, about **10%** of patients treated with aminoglycosides will develop **moderate but significant declines in glomerular filtration rate and elevations in serum creatinine concentration.**

The therapeutic utility of aminoglycosides is limited by nephrotoxicity, ototoxicity and neuromuscular junction blockade. Aminoglycoside nephrotoxicity is characterized by **proximal tubular necrosis, proteinuria, and a profound decline in glomerular filtration rate.**

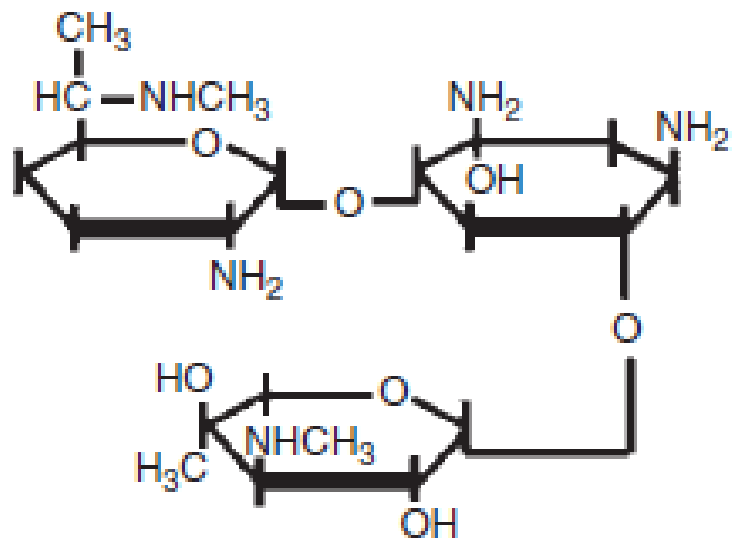
Aminoglycoside antibiotics are **organic polycations** and carry net **positive charges** (Figure 3). These compounds have **relatively low volumes of distribution, and the primary route of elimination is by renal excretion.** Gentamicin, a typical nephrotoxic aminoglycoside, is freely filtered at the glomerulus and appears to be reabsorbed via active transport processes at the proximal tubular brush border.

Renal dysfunction by aminoglycosides is characterized by a nonoliguric renal failure with reduced GFR and an increase in serum creatinine and BUN.

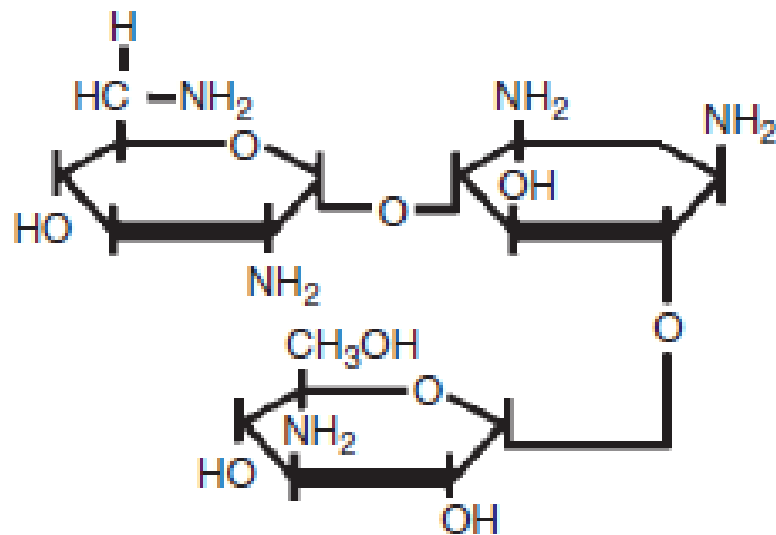
Nonoliguric acute renal failure appears within 5 – 7 days after aminoglycoside therapy is initiated.

Polyuria is an early event following aminoglycoside administration and may be **due to inhibition of chloride transport in the thick ascending limb** (Kidwelle *et al.* , 1994). Within 24 hours, increases in urinary brush-border enzymes, **glucosuria, aminoaciduria, and proteinuria are observed.**

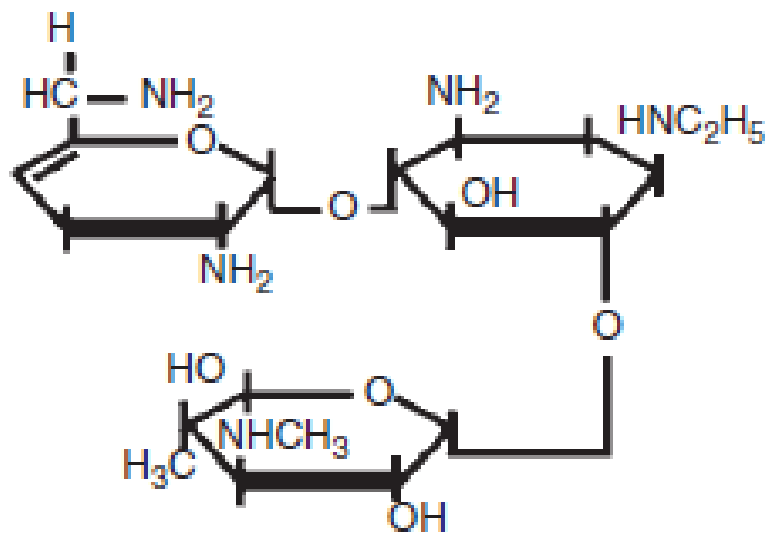
Aminoglycosides are highly polar cations; they are almost exclusively filtered by the glomerulus and excreted unchanged. Filtered aminoglycosides undergo proximal tubular reabsorption by binding to anionic phospholipids in the brush border, followed by endocytosis and sequestration in lysosomes of the S 1 and S 2 segments of proximal tubules.



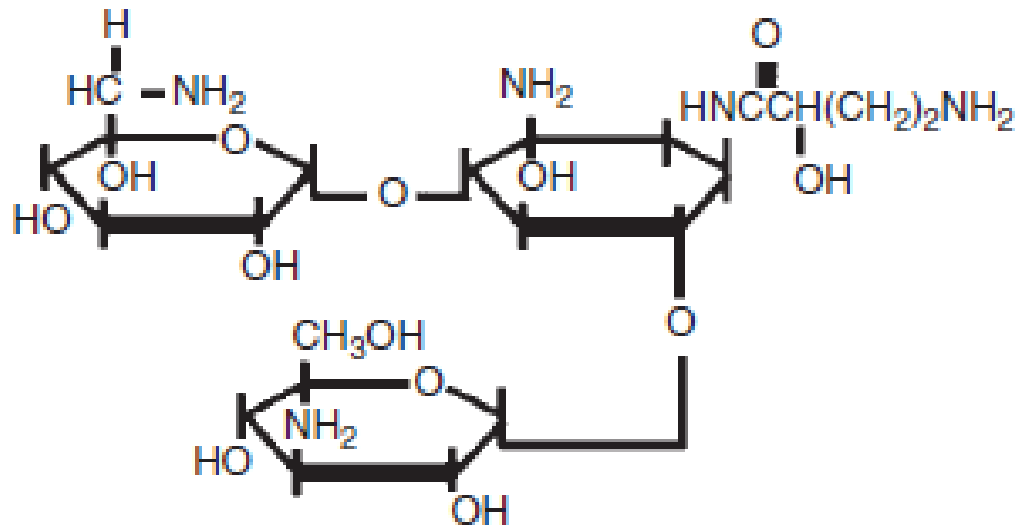
Gentamicin



Tobramycin



Netilmicin

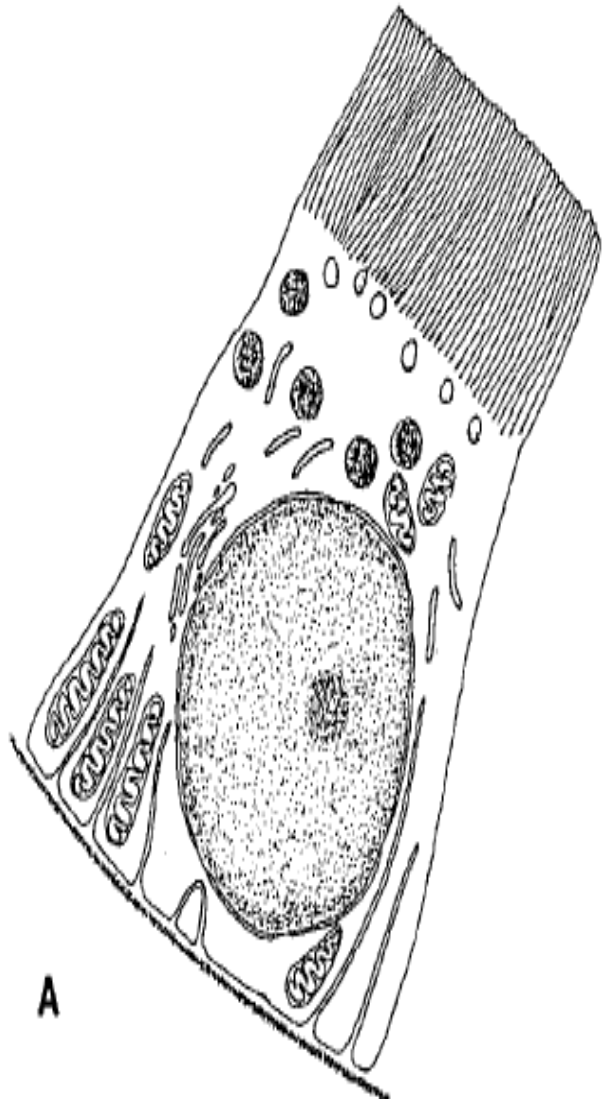


Amikacin

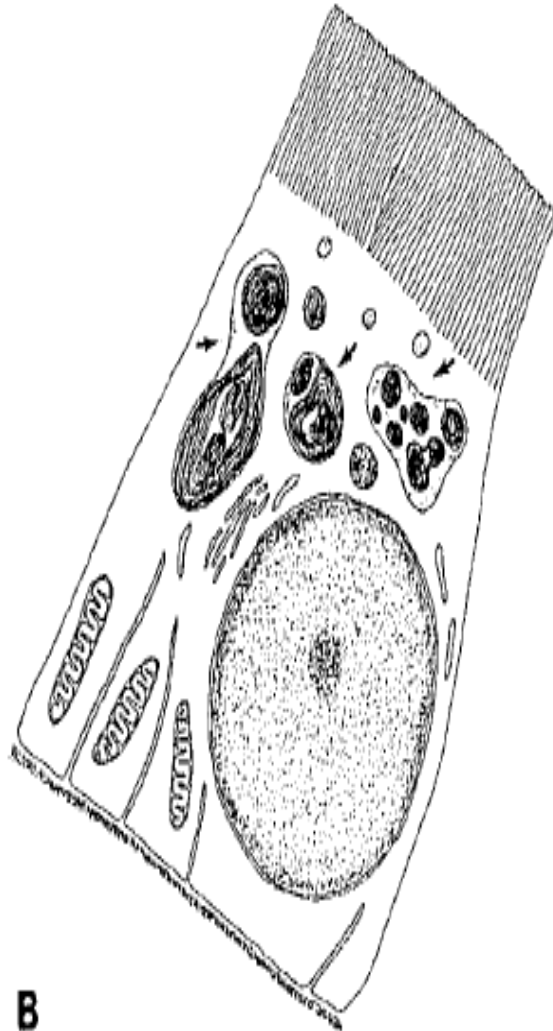
Several mechanisms have been proposed to account for gentamicin cytotoxicity, including:

- (1) lysosomal damage.
- (2) Altered phospholipid metabolism.
- (3) Inhibition of critical intracellular enzymes.
- (4) Inhibition of mitochondrial respiration.
- (5) Lipid peroxidation.
- (6) Misreading of mRNA.

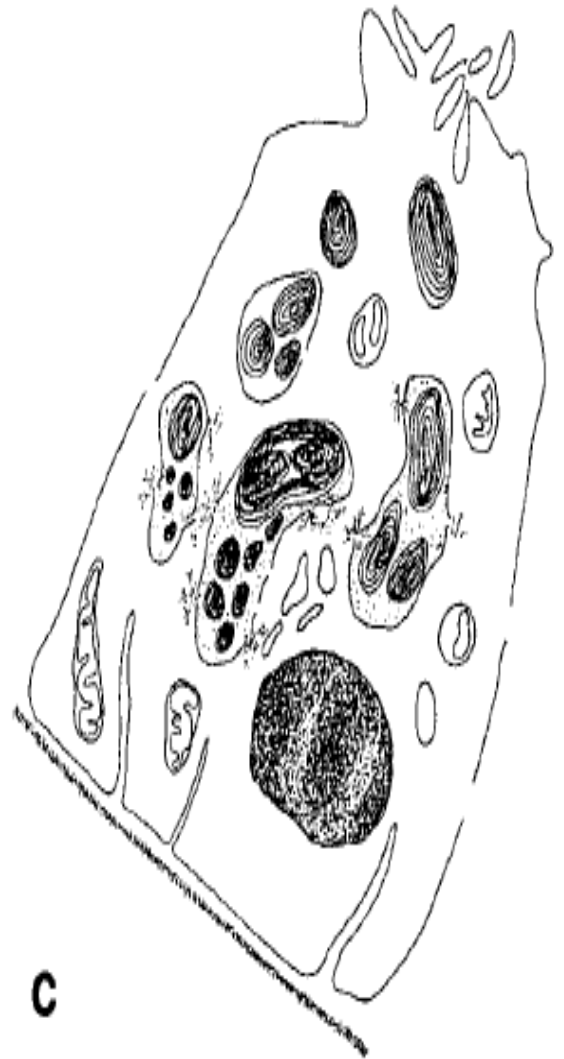
The earliest lesion observed following clinically relevant doses of aminoglycosides is an **increase in the size and number of lysosomes**. These lysosomes contain *myeloid bodies*, which are electron-dense lamellar structures containing undergraded phospholipids. The renal phospholipidosis produced by the aminoglycosides is thought to occur through their inhibition of **lysosomal hydrolases**, such as **sphingomyelinase and phospholipases**. One hypothesis suggests that the lysosomes become progressively distended until they rupture, releasing lysosomal enzymes and high concentrations of aminoglycosides into the cytoplasm (Figure 4). The released lysosomal contents can interact with various membranes and organelles and trigger cell death. Another mechanism of aminoglycoside nephrotoxicity includes a decrease in *K_f* and GFR.



A



B



C

Figure 4: Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment.

(A) Control. Changes detected early on and at low doses

(B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive deposition of polar lipids which adopt a concentric lamellar disposition (myelin-like structures, most commonly referred to as myeloid bodies); the other subcellular structures are usually well preserved. Later changes or changes observed with high doses

(C) include the apparent rupture of lysosomes (with the release of myeloid bodies in the cytosol), extensive mitochondrial swelling and damage, dilatation of the endoplasmic reticulum cisternae, shedding of the apical brush-border villi, pericellular membrane discontinuities, and the occurrence of apoptotic nuclei. These alterations do not necessarily coexist in all cells. للأطلاع

Amphotericin B

One compound that has been associated with **distal tubular injury** is amphotericin B, a polyene antifungal agent used in the treatment of systemic mycoses caused by opportunistic fungi.

Clinical utility of amphotericin B is limited by its nephrotoxicity, characterized functionally by polyuria resistant to antidiuretic hormone administration, hyposthenuria, hypokalemia, and mild renal tubular acidosis.

Amphotericin B is highly lipophilic (Figure 5) and interacts with membrane lipid sterols, such as cholesterol, to disrupt membrane permeability.

Because amphotericin is freely filtered, it achieves high concentrations in distal tubular fluid and easily forms complexes with cholesterol and other lipids present in distal tubular luminal membranes. Amphotericin effectively transforms the “tight” distal tubular epithelium into an epithelium leaky to water, H^+ and K^+ .

Functional abnormalities observed with amphotericin B are attenuated when the antifungal agent is administered as an emulsion formulation whereby amphotericin is incorporated into lipid micelles.

Antifungal activity of emulsion - formulated amphotericin B is equivalent to the standard non - emulsion formulation, whereas polyuria and hyposthenuria are significantly reduced by emulsion formulation.

