MECHANISMS OF NEPHROTOXICITY

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INTRODUCTION

The mammalian kidney is a dynamic and complex organ. Excretion of wastes is a primary function, but the kidney also plays a significant role in the regulation of total body homeostasis. Regulation of extracellular volume and control of electrolyte and acid-base balance are important renal functions. The kidney is a major site for formation of hormones that influence systemic metabolic functions, including erythropoietin, 1, 25 dihydroxy-vitamin D₃, and renin. Recent evidence indicates that the kidney produces several vasoactive prostaglandins and kinins. A toxicological insult to the kidney could affect any or all of these functions.

Few data are available that define specific cellular or subcellular sites of action of nephrotoxicants. Only rarely have receptors for individual agents been identified. Rather, in many cases it appears that several tissue constituents are influenced by a poison. There are two interrelated reasons for this apparent lack of specificity: (1) in contrast to a pharmacological effect which requires interaction with a distinct endogenous receptor, cell damage due to some chemicals may follow interruption of one or several required cellular functions; (2) certain kidney cells may be more susceptible to damage merely because they are exposed to greater concentrations of chemicals than are other cells of the body as the result of this organ’s unique anatomical and functional features.

ANATOMICAL AND FUNCTIONAL BASIS FOR SUSCEPTIBILITY TO CHEMICALS

A consideration of renal responses to potential toxicants is most appropriately

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Based on the functional unit of the kidney—the nephron (Fig. 1). The nephron may be
considered in three portions: the vascular element including afferent and efferent
arterioles, the glomerulus, and the tubular segments. All nephrons have their primary
vascular elements and glomeruli in the cortex. Glomeruli closest to the medulla (juxta-
medulary glomeruli) are associated with nephrons that send their loops of Henle deep
into the medulla, whereas more superficial glomeruli generally form nephrons with
loops of Henle contained within the cortex.

The glomerulus is a site of action of several xenobiotics and is also susceptible to
immunological damage. Following toxic insult, changes in glomerular permeability
may occur, leading to loss of proteins into the urine. Light microscopy indicates that
after certain insults the tissue does not appear to be more porous, but in fact seems to
be somewhat thicker. Recent evidence suggests that the net electrical charge on the
glomerular membrane may be changed, thereby altering the ability of the membrane to
attract or repel charged molecules (Brenner et al., 1977).

Maintenance of normal renal function requires delivery of large amounts of
metabolic substrates and oxygen to the kidney; consequently the kidneys together
receive about 25% of the cardiac output. Because of this high blood flow, many
chemicals in the circulation will be delivered to the kidneys in significant amounts. As

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Fig. 1. The sagittal surface of a bisected kidney is illustrated diagrammatically (lower
left). Numbers 1 through 9 indicate the following: (1) minor calyx, (2) fat in
sinus, (3) renal column of Bertin, (4) medullary ray, (5) cortex, (6) pelvis, (7)
interlobar artery, (8) major calyx, and (9) ureter. The letter A indicates the
renal artery, and the letter V indicates the renal vein. Insert (a) from the
upper pole is enlarged to illustrate the relationships between the juxta-
medullary and cortical nephrons and the renal vasculature. From Tisher, 1976, with
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salt and water are reabsorbed from the glomerular filtrate, the materials remaining are concentrated in the tubular lumen (Fig. 2). Thus, a non-toxic concentration of a chemical in plasma could become toxic in the kidney subsequent to concentration in the urine.

Binding of chemicals to plasma proteins may protect most cells of the body from potentially toxic actions of certain compounds, but a chemical which is actively secreted by the nephron can be removed from plasma binding sites. During the active secretion process, the compound will be accumulated within the cells of the proximal tubule exposing them to very high concentrations. Similarly, a substance that is reabsorbed—even by passive means—from the urine into the blood will pass through the cells of the nephron in a relatively high concentration.

The renal medulla is unique in relation to the nephrotoxicity of chemicals. Since only about 10% of total renal blood flow enters the renal medulla, relatively less drug or chemical might reach this region via the blood than would enter the cortex. However, any chemical in the tubular urine will pass through the loop of Henle and the medullary collecting duct, exposing the cells of the medulla to high concentrations. The collecting duct appears to be relatively insensitive to most nephrotoxicants. Following intoxication with analgesic mixtures, histological evaluation of the medulla

Fig. 2. Scheme of renal handling of a chemical (D). If material is filtered, its concentration in tubular fluid will rise as salt and water are reabsorbed. It may then diffuse down its concentration gradient into the blood. The chemical may be secreted into the tubular cell from the blood (may even be removed from protein binding sites) and into the tubular fluid. The chemical may enter the cell and be metabolically altered (D→X). From Hook and Serbia, 1982, with the permission of the author and Raven Press.
shows that most of the ascending limbs of the loop of Henle have been destroyed, whereas the collecting ducts appear to be unaffected (Axelsen, 1976).

The nephron operates with its three separate components arranged in series, and it is often difficult to isolate an initial lesion when the kidney has been damaged. If renal function is monitored by measuring urine volume and composition, increased volume and decreased osmolality might result from chemical exposure. This could be due to direct damage to tubular cells, altering their ability to reabsorb constituents of the tubular urine. On the other hand, this could be secondary to glomerular damage, causing an increased permeability with excess filtrate entering the tubule and exceeding the reabsorptive capacity. Constriction of the vasculature could decrease delivery of nutrients to the nephron, limiting tubular function and also producing increased urine volume. In the same example, selective vasospasm may lead to redistribution of blood within the kidney. An increase in medullary blood flow would dissipate the renal medullary hypertonicity, again leading to increased urine volume. Elevated BUN (blood urea nitrogen) and plasma creatinine could be due to the formation of crystals which occlude the lumens and prevent excretion of waste materials. Alternatively, increased BUN could be due to selective damage to the glomerulus or vasospasm of renal blood vessels.

BIOCHEMICAL BASIS FOR RESPONSE TO CHEMICALS

Localization of sites of action of nephrotoxics to areas of the tubule other than the proximal nephron may indicate specific biochemical receptors for some types of agents. For instance, the loop of Henle appears to be the site of damage produced by chronic administration of analgesic mixtures (aspirin and phenacetin) and other materials that act in the medulla, such as the fluoride ion. The distal convoluted tubule is a relatively small part of the total nephron yet compounds such as amphotericin have been shown to influence the ability of the kidney to acidify the urine, which is probably a distal tubular event (Gouge and Andriole, 1971).

The toxicity of chemicals may be more specifically evaluated in vitro by utilizing tissue obtained following administration of the compound of interest to the animal or by directly adding the agent to a tissue preparation from untreated animals. This allows a distinction to be made between an effect on the kidney due to direct chemical insult and secondary effects mediated by other systems (e.g., vascular, endocrine) or metabolites produced by other tissues (e.g., liver). The renal cortical slice technique is an in vitro method which has been used extensively to evaluate the influence of nephrotoxicants on renal functions, including the transport of organic ions such as p-aminobenzoic acid (PAH) and the production of glucose and ammonia (Hirsch, 1976).

Using the slice technique, differences in sensitivity along various segments within the proximal nephron have been noted. The proximal convolution is the primary site of reabsorption of glucose and amino acids and seems to be particularly susceptible to certain metals such as chromium. The pars recta (straight portion) has a greater
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capacity to secrete organic compounds, and it is in this area where damage due to mercury, cephaloridine, and other organic compounds first appears.

**ROLE OF RENAL DRUG METABOLISM**

Most compounds and their metabolites are excreted by the kidney without compromising its function. When toxicity does occur, there may indeed be overt changes in physiological function as described above. However, many chemicals can produce biochemical sequelae so slight that no functional effects are detected, yet susceptibility of the organ to the deleterious effects of other agents is altered. An ingested drug or chemical may be excreted unchanged or may be metabolized to non-toxic or toxic derivatives by the kidney or other organs. The pathways involved in such transformations might themselves be targets of toxic chemicals, and the balance between toxification and detoxification upset.

Table 1 illustrates that the concentration of cytochrome P450 and its accompanying catalytic activity is considerably less in the kidney than in the liver. However, comparisons based on homogenization of whole organs might be misleading. If the cytochrome P450 activity in the kidney were localized within specific cells, such as the straight portion of the proximal tubule, then enzyme activity would be relatively high in this region. These cells might therefore be exposed to short-lived reactive intermediates which would ordinarily not reach the general circulation.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cytochrome P450a</th>
<th>Ethoxyresorufin O-deethylasea</th>
<th>Benzphetamine N-demethylasea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.50</td>
<td>2.10</td>
<td>1.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.07</td>
<td>0.02</td>
<td>N. D.</td>
</tr>
</tbody>
</table>

a nmoles / mg protein.
b nmoles product / min / mg protein.
N.D. - not detected.


The toxicity of compounds which produce such metabolites may be increased when drug-metabolizing activity is induced. Chloroform is believed to be metabolized by a cytochrome P450 system in the liver and the hepatic toxicity has been shown to be enhanced by pretreatment of mice with inducing agents such as polychlorinated biphenyls (PBB) or phenobarbital (Kluwe and Hook, 1978). Renal cytochrome P450 activity was also found to be induced by the environmental contaminants polychlorinated biphenyl (PCB) and PBB, although PBB is more potent (Table 2). The nephrotoxicity of chloroform, carbon tetrachloride, and other halogenated hydrocarbons in mice has been found to be enhanced by PBB ingestion (Kluwe and Hook, 1978; Kluwe et al., 1979). Pretreatment with PCB also potentiated the toxicity of carbon tetrachloride
Table 2  Induction of renal arylhydrocarbon hydroxylase (AHH) activity by polybrominated biphenyls (PBB) and polychlorinated biphenyls (PCB)\(^a\).

<table>
<thead>
<tr>
<th>Dietary concentration. ppm</th>
<th>AHH activity, % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBB</td>
</tr>
<tr>
<td>25</td>
<td>150</td>
</tr>
<tr>
<td>100</td>
<td>310</td>
</tr>
<tr>
<td>200</td>
<td>466</td>
</tr>
<tr>
<td>400</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) PBB or PCB was fed to mice (in the diet) for 14 days.
Modified from Kluwe and Hook, 1980, with permission of the author and Springer Verlag New York, Inc.

and trichloroethylene, but reduced the nephrotoxicity of chloroform (Kluwe et al., 1978; Kluwe et al., 1979). This difference in toxicity between PCB and PBB may have been a consequence of qualitative differences in the effects of PCB and PBB on the renal metabolism of chloroform. Alternatively, increased hepatic metabolism following PCB administration may have resulted in decreased delivery of chloroform to the kidney.

Several laboratories are now using the isolated perfused kidney (IPK) to determine the role of drug metabolism in the nephrotoxicity of acetaminophen (paracetamol) and other xenobiotics (Newton and Hook, 1981; Emslie et al., 1981 b). In the liver most acetaminophen is detoxified by metabolism to glucuronide or sulfate conjugates. A small amount appears to go through a P450-dependent mechanism to produce a reactive intermediate which normally is conjugated with glutathione and rendered non-toxic (Brodie and Axelrod, 1948; Potter et al., 1973; Mitchel et al., 1973 a). However, if significant reduction of glutathione occurs (e.g., due to larger doses of acetaminophen or previous exposure to another glutathione-depleting compound), cellular toxicity may ensue (Mitchell et al., 1973 b). The renal conjugation of acetaminophen and/or its reactive intermediate with sulfate, glucuronide, and glutathione has been established using the IPK (Emslie et al., 1981 a; Newton et al., 1982 a) and it is likely that glutathione plays the same protective role in the kidney as in the liver.

It has been possible to determine the effect of various modulators of drug-metabolizing activity on the metabolism and excretion of acetaminophen and its metabolites. Using high performance liquid chromatographic techniques, the glucuronide derivatives of acetaminophen were quantified in the urine and were not significantly changed by pretreatment of the animals with PBB (Fig. 3). Similarly, the sulfate conjugates were measured and were also not affected by PBB. However, the n-acetyl derivative of the cysteine conjugate of acetaminophen (mercapturic acid, which would be formed by the breakdown of the glutathione conjugate) was markedly increased in the urine. This is consistent with a P450-dependent metabolism of acetaminophen, although it should be noted that the same data would have occurred following enhanced glutathione transferase activity.
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Fig. 3. Excretion of the major metabolites of APAP by the IPK. APAP-GLUC, APAP-NAC and APAP SO₃ excretion was quantified in IPKs perfused with 3×10⁻⁵ M APAP from rats treated with PBB or vehicle. Each bar represents the mean ±SEM of four IPKs. * Significantly greater excretion of metabolite compared to IPKs from animals treated with vehicle. From Newton et al., 1982a, with the permission of the author and the Williams and Wilkins Co.

Increasing concentration of acetaminophen in the perfusate produced a dose-dependent decrease in glutathione in all areas of the kidney (Table 3). In kidneys from animals that had been pretreated with the enzyme inducer PBB, the ability of acetaminophen to produce glutathione depletion was markedly increased in the cortex and the medulla, but not in the papilla. Similarly, in kidneys from rats that had been pretreated with the enzyme inhibitor piperonyl butoxide, the ability of acetaminophen to deplete glutathione was markedly reduced in the cortex and medulla. These data strongly indicated that acetaminophen could be metabolized by a P450 system in the kidney.

It has been demonstrated that acetaminophen may be metabolized in the kidney in another manner—deacetylation to p-aminophenol which is recognized to be nephrotoxic (Carpenter and Mudge, 1981; Newton et al., 1982b). It is likely that the metabolism of acetaminophen in the kidney is more complicated than previously assumed. Possibly the compound could be metabolized through a P450-dependent system, or deacetylated to form p-aminophenol which can go through a series of similar reactions, including the formation of a reactive metabolite that would deplete glutathione. Recent evidence has suggested another means of metabolism: co-oxidation of acetaminophen during the formation of prostaglandins (Mattamal et al., 1979). This is an interesting possibility since the enzymes responsible for co-oxidation of substrates with arachidonic acid are found primarily in the renal medulla, an area where P450 does not occur (Table 4). It has been suggested that whereas the mechanisms involving P450 and the formation of
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Table 3  Effects of polybrominated biphenyls (PBB) and piperonyl butoxide pretreatments on depletion of nonprotein sulphydryl groups by acetaminophen (APAP) in isolated perfused rat kidneys.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>APAP in perfusate</th>
<th>% Depletion of nonprotein sulphydryls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3×10^{-8}M</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>11.6±3.6</td>
<td>10.5±6.5</td>
</tr>
<tr>
<td>PBB (150 mg / kg)</td>
<td>29.5±3.0</td>
<td>51.0±7.7</td>
</tr>
<tr>
<td>None</td>
<td>29.8±4.9</td>
<td>34.9±3.3</td>
</tr>
<tr>
<td>Piperonyl butoxide (600 mg / kg)</td>
<td>7.8±3.9</td>
<td>10.9±5.5</td>
</tr>
</tbody>
</table>

Superscript symbols:
- a  Data are expressed as % depletion of nonprotein sulphydryl concentration from the nonperfused kidney of each animal. PBB and piperonyl butoxide alone had no effect. Values represent means±SEM of three to six kidneys.
- b  Significantly different from control (no pretreatment, 3×10^{-8}M APAP), p < 0.05.
- c  Significantly different from control (no pretreatment, 3×10^{-8}M APAP), p < 0.05.

From Kluwe and Hook, 1989, with permission of the author and Springer-Verlag New York, Inc.

Table 4  Oxidation of 1,3-diphenylisobenofuran by NADPH and arachidonic acid dependent pathways in different segments of the kidney.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Co-oxidation (nmol / mg/ min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>10.0±1.1</td>
</tr>
<tr>
<td>NADPH</td>
<td></td>
</tr>
<tr>
<td>Outer Medulla</td>
<td>1.0±0.4</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>3.0±0.6</td>
</tr>
<tr>
<td>NADPH</td>
<td></td>
</tr>
<tr>
<td>Inner Medulla</td>
<td>10.2±1.4</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>NADPH</td>
<td></td>
</tr>
</tbody>
</table>

Modified from Rush and Hook, 1982, with permission of the author and John Wiley and Sons.

p-aminophenol might be responsible for acute renal necrosis, such an interaction with arachidonic acid could be involved in the chronic papillary necrosis produced by acetaminophen (Duggin and Mohandas, 1982).

Another compound of considerable interest is the cephalosporin antibiotic cephaloridine. The clinical usefulness of this agent has been markedly restricted due to its very selective nephrotoxicity. Following systemic administration, cephaloridine accumulated in the kidney to a much higher degree than any other organ; most of it within the cortex (Welles et al., 1966; Tune et al., 1974). Like PAH, cephaloridine was found to be actively transported by the cells of the proximal tubule, and shared many other properties with PAH (Tune and Fernholt, 1973). The transport of cephaloridine was reduced by probenecid as well as high concentrations of PAH. Transport was low in the newborn
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but could be enhanced by substrate stimulation with penicillin, and the relatively low nephrotoxicity was concomitantly increased (Wold et al., 1977). Some of the toxicity is apparently due to retention of cephaloridine within the cells of the proximal tubule, in contrast to PAH, which diffuses into the urine and is excreted readily (Fig. 4).

Little is yet known about the specific biochemical mechanisms of the damage that occurs following cephaloridine administration. Possibly the thiophene ring of cephaloridine could be oxidized by a P450 mechanism similar to that shown for the furans (McMurty and Mitchell, 1977). A species difference exists in the response of cytochrome P450 to phenobarbital, which may be conveniently utilized to evaluate the role of P450 in the toxicity of cephaloridine. In the rat kidney, the administration of phenobarbital has no effect on P450 or associated catalytic activity, whereas in the rabbit phenobarbital will induce renal P450 (Kuo et al., 1982; Uehleke and Greim, 1968). Phenobarbital treatment was found to enhance the nephrotoxicity of cephaloridine in the rabbit: blood urea nitrogen was elevated and the accumulation of PAH and TEA by

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Fig. 4. Schematic representation of the renal transport and intrarenal distribution of p-aminohippurate (PAH) and cephaloridine in the rabbit kidney.

A: PAH is transported by filtration and to a greater extent by secretion. Cortical PAH includes large intracellular and intraluminal components; medullary PAH includes a filtered fraction and a larger secreted fraction. Both cortical and medullary PAH are reduced by probenecid.

B: Cephaloridine is excreted principally by glomerular filtration; in addition, cephaloridine is actively transported into proximal tubule cells with little or no movement from cell to lumen. Cortical cephaloridine is made up of a large intracellular fraction and a small filtered fraction. Medullary cephaloridine is mainly derived from the glomerular filtrate. Thus cortical cephaloridine is markedly reduced by probenecid, whereas medullary cephaloridine is not. From Tine, 1973, with the permission of the author and The University of Chicago Press.

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kidney slices diminished (Table 5). The nephrotoxicity of cephaloridine in rats was not altered by phenobarbital. In both rats and rabbits piperonyl butoxide, a known inhibitor of P450, reduced nephrotoxicity (Table 6).

If cephaloridine were metabolized to an active form, it would be suspected that like acetaminophen, it would react with glutathione, decrease the glutathione concentration in the tissue and produce sulfur-containing metabolites. Administration of cephaloridine resulted in a dose-related depletion of glutathione in rabbit kidney cortex, which was markedly enhanced with phenobarbital pretreatment (Kuo and Hook, unpublished observations). In contrast, in the rat, depletion of renal glutathione was shown but phenobarbital was without effect. These data were consistent with metabolism of cephaloridine to a reactive product that would be conjugated with glutathione. However, no sulfur-containing metabolites of cephaloridine could be found.

Table 5 Effect of phenobarbital treatment on cephaloridine nephrotoxicity in rabbits.a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cephaloridine mg / kg</th>
<th>BUN (mg%)</th>
<th>PAH S/M</th>
<th>TEA S/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0</td>
<td>17</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Control</td>
<td>150</td>
<td>32</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>150</td>
<td>59</td>
<td>3b</td>
<td>6b</td>
</tr>
<tr>
<td>Control</td>
<td>300</td>
<td>93</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>300</td>
<td>146b</td>
<td>1b</td>
<td>1b</td>
</tr>
</tbody>
</table>

a Animals received phenobarbital (60mg /kg, ip) or saline once daily for four days and then a single administration of cephaloridine or saline. All animals were then killed 48 hr. later.

b Significantly different from the control group receiving an equivalent dose of cephaloridine (p < 0.05).

From Rush et al., 1982, with permission of the author and John wiley and Sons.

Table 6 Effect of piperonyl butoxide pretreatment on cephaloridine nephrotoxicity in Sprague-Dawley rats.a

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Cephaloridine (mg / kg)</th>
<th>Kidney Wt / Body Wt (g / kg)</th>
<th>BUN (mg%)</th>
<th>PAH S/M</th>
<th>TEA S/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Oil</td>
<td>0</td>
<td>8.12±0.25</td>
<td>24.2±0.8</td>
<td>25.2±2.0</td>
<td>24.7±2.0</td>
</tr>
<tr>
<td>Piperonyl Butoxide</td>
<td>0</td>
<td>7.73±0.22</td>
<td>24.7±0.8</td>
<td>21.9±0.9b</td>
<td>19.5±1.1b</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>1000</td>
<td>10.14±0.28</td>
<td>45.9±5.0</td>
<td>8.3±2.2</td>
<td>9.8±2.6</td>
</tr>
<tr>
<td>Piperonyl Butoxide</td>
<td>1000</td>
<td>7.85±0.29b</td>
<td>21.3±2.0</td>
<td>22.3±2.1b</td>
<td>14.0±1.1b</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>2000</td>
<td>11.58±0.25</td>
<td>79.1±11.9</td>
<td>4.1±0.4</td>
<td>6.9±0.7</td>
</tr>
<tr>
<td>Piperonyl Butoxide</td>
<td>2000</td>
<td>9.95±0.54b</td>
<td>55.3±5.5</td>
<td>11.3±2.2</td>
<td>11.2±1.5b</td>
</tr>
</tbody>
</table>

a Piperonyl butoxide (1000 mg / kg) was given 30 min. prior to cephaloridine administration. The control groups received corn oil. All animals were killed 24 hr. following cephaloridine administration.

b Significantly different from the corn oil group receiving an equivalent dose of cephaloridine (p<0.05).

Kuo and Hook, unpublished.
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It was subsequently determined that while the concentration of reduced glutathione (GSH) was decreased, that of total glutathione (including GSSG) was elevated: i.e., the total glutathione pool shifted from the reduced to the oxidized state. This could be explained by a mechanism involving oxidative induction of lipid peroxidation. It was possible to test this hypothesis by manipulating the diet. Glutathione peroxidase is dependent upon selenium, and vitamin E is a trapping agent for free radicals generated during lipid peroxidation reactions. Animals were therefore fed diets low in selenium and free of vitamin E, and the nephrotoxicity of cephaloridine was indeed markedly enhanced (Fig. 5).

In summary, the toxicity of cephaloridine appears to be due to its active accumulation and retention within the cells of the proximal tubule. The transport mechanism, and therefore toxicity, can be inhibited by agents like probenecid and can be stimulated by penicillin in newborn animals. Cephaloridine, once intracellularly located, can be loosely bound to a variety of proteins, possibly glutathione transferases and cytochrome P450. These factors contribute to a high intracellular concentration of cephaloridine, leading to NADPH depletion, glutathione depletion, and subsequent lipid peroxidation. Lipid peroxidation, as well as the NADPH depletion, will result in accumulation of oxidized glutathione. All of these effects—along with a possible direct effect of cephaloridine on mitochondria (Tune et al., 1979)—would lead to cellular damage and eventually cellular death.

![Graph](image-url)  

**Fig. 5.** Effects of vitamin E and/or selenium deficiency on blood urea nitrogen in rats after cephaloridine. Values are means ± SEM of seven or more animals. (*) Significantly different from Group 1 receiving an equivalent dose of cephaloridine (P < 0.05). (†) Significantly different from Group 1 receiving saline only (P < 0.05). From Kuo and Hook, unpublished.
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