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B. J. Smith, DVM, PhD; B. G. Anderson, PhD; S. A. Smith, MS, DVM; D. J. Chew, DVM

SUMMARY

A sublethal dose of ethylene glycol was administered orally to 3 groups of dogs; dogs of a control group were given distilled water instead. Renal cortical biopsy samples were obtained from dogs of experimental and control groups at various times after treatment. Tissue was examined by use of light microscopy and transmission electron microscopy. In dogs of the control group, the light and electron microscopic appearances of tissue were within normal limits at all sample collection hours. In dogs of the experimental groups, renal corpuscular structure remained within normal limits by use of light and electron microscopy throughout the study, though morphologic change was seen in other structures of the cortex. Light microscopic lesions first appeared at 12 hours, and were similar to those reported in the literature. Ultrastructural lesions were first observed in the 5-hour samples, and similar to the light microscopic lesions, were most common in the proximal convoluted tubules (PCT). Initial PCT cellular changes included vacuolization of cells and distention of the parabasal extracellular spaces; PCT cellular lesions seen in later-hour samples included formation of apical buds and cellular rupture. Internalization or sloughing of the PCT brush border was not observed. Distal convoluted tubules (DCT) were frequently dilated and/or packed with cellular debris. A few DCT cells had degenerative or necrotic changes. In PCT and DCT, abnormal cells were frequently flanked by normal or nearly normal cells. During later hours, a few cells with types of changes first observed in early hours continued to be observed, implying ongoing response of cells to the toxin.

Although oliguric acute renal failure induced by antifreeze or ethylene glycol (EG) intoxication in domestic animals and human beings has been the subject of numerous investigations, most studies concerning EG intoxication address the clinical and biochemical course of natural or experimentally induced intoxications, diagnostic procedures, or suggested treatment regimens. Excellent reviews of this literature are available.[1,2] Renal morphologic changes associated with EG intoxication are the principal subject of a few investigations.[3-5] Most descriptions of renal structural change in domestic and laboratory animals must be extracted from literature aimed principally at clinical or biochemical aspects of EG intoxication. These descriptions address structural change mainly at the histologic level, with ultrastructural changes not extensively described. Further, progressive development of renal lesions in individual animals has not been widely reported.

Nephrotoxic metabolites of EG can be detected in the serum of intoxicated dogs as early as 3 hours after EG ingestion.[6] Changes in serum biochemical profile implying renal damage are apparent at 18 to 24 hours,[6,7] and those indicating diminished renal excretory function are established at 48 hours.[8] Functional aberrations in the initial phases of the intoxication suggest the possibility of relevant structural alterations developing earlier than previously reported histologic changes. Thus, examination of renal tissue by use of light and electron microscopy in the early hours of EG intoxication was warranted in an attempt to document such structural lesions.

Primary goals of the study reported here were to characterize, in individual dogs, the existence and progression of renal cortical ultrastructural changes from 0 to 30 hours after EG ingestion, and to correlate ultrastructural changes with concurrent histologic examination results.

Materials and Methods

Dogs and drug selection—Twelve adult male mixed-breed dogs weighing 15 to 20 kg were used. Halothane[a] was used for brief anesthesia during the pretreatment biopsy sample collection and sodium pentobarbital[b] (30 mg/kg of body weight, IV) was used for prolonged posttreatment anesthesia. Reagent-grade EG[c] (99+% pure) was used as the toxic agent; an antiemetic agent[d] was also used.

Pretreatment biopsy—To establish that the renal ultrastructure of each dog was normal prior to the study, a renal cortical biopsy sample was obtained from all 12 dogs 1 day prior to treatment. Left-flank laparotomy was performed, and a transverse cortical wedge biopsy sample was obtained from the cranial pole of the left kidney. The kidney biopsy site was closed by apposition of the cut edges of the kidney, using moderate pressure for 5 to 8 minutes. Three simple interrupted sutures were placed in the renal capsule. The flank incision was sutured, and the dog was allowed to recover.

Treatment—Dogs were assigned at random to 4 groups of 3 dogs each. One group of dogs was designated as control group and groups 2, 3, and 4 were designated as experimental groups.

Food was withheld from all dogs overnight prior to treatment. Immediately before treatment, each dog was given 3 ml total dose of the antiemetic, equivalent to 12 mg of prochlorparazine

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[a] Halocarbon Laboratories Inc, Hackensack, NJ.
[b] The Butler Co, Columbus, Ohio.
[c] Fisher Scientific, Fair Lawn, NJ.
[d] Norden Laboratories, Lincoln, Neb.

and 0.84 mg of isopropamide, SC. A catheter was then placed in a cephalic vein, and the dog was administered maintenance volumes (24 to 40 ml/kg/d) of 0.9% saline solution. Drinking water was provided ad libitum.

Treatment of the experimental groups of dogs consisted of administration by stomach tube of 3 ml of EG/kg of body weight, followed by flushing of the tube with an equal volume of distilled water. Dogs of the control group were similarly treated, except that distilled water was used in place of EG.

Evaluation of posttreatment condition—After administration of EG, the attitude and condition of each dog were evaluated every 2 hours up to induction of anesthesia, to guard against development of undesirable clinical signs of EG intoxication. The factors evaluated included general attitude, responsiveness, and character of respiration, as well as development of emesis and CNS and/or cardiopulmonary effects. Preparation was made to induce general anesthesia, using sodium pentobarbital, if evidence of suffering or pronounced discomfort were observed.

Posttreatment biopsy—One dog of the control group was randomly assigned to the sample collection regimen of a corresponding experimental group, as follows: control dog A followed the same biopsy schedule as did group-2 dogs; control dog B followed that of group-3 dogs; and control dog C followed that of group-4 dogs. Biopsy specimens were obtained from the left kidney of dogs of group 2 and control dog A at 2, 5, and 8 hours after treatment. Dogs of group 3 and control dog B were biopsied at posttreatment hours 12 and 18, and dogs of group 4 and control dog C were biopsied at posttreatment hours 24 and 30.

Fifteen minutes prior to obtaining the first posttreatment biopsy sample, each dog was anesthetized by IV administration of sodium pentobarbital. Anesthesia was maintained for the 6-hour sample collection period with supplemental doses of sodium pentobarbital given IV. Heating pads were used when necessary to maintain normal body temperature. The original left flank incision was reopened and a cortical biopsy sample was obtained from a site at least 1.5 cm caudal to that of the pretreatment biopsy sample. The biopsy site in the kidney was closed as described. Edges of the flank incision were apposed with sutures, and the area was covered with moist sterile abdominal packs. Subsequent biopsy samples were obtained in similar manner from a site at least 1.5 cm caudal to the previous biopsy site. After the final biopsy sample was obtained, dogs were euthanatized by administration of an overdose of sodium pentobarbital.

Microscopy—Tissue to be evaluated by light microscopy was fixed in Bouin solution, embedded in paraffin and sectioned (5 μm), using routine histologic methods. From each biopsy specimen, a separate slide was prepared and stained, using periodic acid-Schiff and hematoxylin and eosin methods.

For electron microscopic evaluation, a 1-mm thick slice of tissue was immersed for 3 to 5 minutes in cold phosphate-buffered fixative containing 2% formaldehyde and 2.5% glutaraldehyde in 0.04*M* phosphate buffer, minced into 1-mm cubes in cold fixative, and placed in fresh cold fixative for 3 to 5 hours on ice. The tissue was rinsed in cold phosphate buffer (pH 7.4), further fixed in 2% aqueous osmium tetroxide for 2.5 hours on ice, rinsed again with cold buffer, dehydrated in a graded ethanol series, and embedded in resin.[o] Blocks were sectioned and contrasted with aqueous uranyl acetate and aqueous lead citrate for examination.

Results

Condition of dogs—At no time did any dog have undesirable clinical evidence of pain. Dogs remained alert and responsive with good appetite, until general anesthesia was administered prior to collection of the first posttreatment biopsy sample. Polyuria and polydipsia developed, but IV administered saline solution and ad libitum drinking water permitted the dogs to readily satisfy their thirst. Dogs of group 3 had mild ataxia when removed from their cages at posttreatment hour 24, but this was mild enough for the dogs to easily ambulate from the holding areas to the preparation room of the surgical suite without assistance. The subsequent induction of general anesthesia precluded the possibility of any further pain.

Control group 1—Pretreatment biopsy samples obtained from each dog of the control group had normal renal cortical light microscopic and ultrastructural appearances. Similarly, all posttreatment biopsy samples obtained from each dog after administration of distilled water had normal renal cortical histologic structure and ultrastructure.

Experimental groups 2, 3, and 4—The pretreatment biopsy sample obtained from dogs of each experimental group had normal renal cortical histologic structure and ultrastructure.

After EG administration, morphologic changes were observed at the light microscopic and ultrastructural levels. Histologic changes observed in renal cortical structure are given in Table 1, and ultrastructural changes in proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) are given in Tables 2 and 3, respectively.

After administration of EG, the brush border of the PCT

TABLE 1—Number of dogs in ethylene glycol-intoxicated groups with light microscopic lesions in the renal cortex*

	Interstitial edema	Interstitial inflammation	Tubular dilation	Cellular degeneration or necrosis	Crystal deposits
Group 2					
2 h	0	0	0	0	0
5 h	0	0	0	0	0
8 h	0	0	0	0	0
Group 3					
12 h	1	0	1	1	1
18 h	2	0	2	2	1
Group 4					
24 h	2	1	2	2	1
30 h	2	1	2	2	1

* Each group was composed of 3 dogs; the control group did not have lesions; glomerular structure was within normal limits in all samples.

TABLE 2—Number of dogs in ethylene glycol-intoxicated groups with ultrastructural changes in the proximal tubules*

	Increased cytoplasmic vacuoles	Distension of parabasal extracellular spaces	Cellular necrosis or rupture	Mitochondrial change	Luminal cytoplasmic buds
Group 2					
2 h	0	0	0	0	0
5 h	1	0	0	0	0
8 h	2	2	2	0	0
Group 3					
12 h	3	1	3	3	3
18 h	3	3	3	3	3
Group 4					
24 h	3	3	3	3	2
30 h	3	3	3	3	3

* Each group was composed of 3 dogs; dogs of the control group did not have lesions.

[o] Ted Pella Inc, Tustin Calif.

TABLE 3—Number of dogs in ethylene glycol-intoxicated groups with ultrastructural changes in the distal tubules*

	Increased cytoplasmic vacuoles	Distension of parabasal extracellular spaces	Cellular necrosis or rupture	Mitochondrial change	Luminal cytoplasmic buds
Group 2					
2 h	0	0	0	0	0
5 h	0	0	0	0	0
8 h	0	0	1	0	0
Group 3					
12 h	0	0	3	0	0
18 h	0	0	3	0	0
Group 4					
24 h	0	0	3	2	0
30 h	0	0	3	3	0

* Each group was composed of 3 dogs; dogs of the control group did not have lesions.

Fig 1—Section of renal cortex at 30 hours after ethylene glycol (EG) ingestion. The brush border of proximal convoluted tubules (P) is present, though mildly decreased in height in some areas because of tubular dilatation. Sloughing of brush borders is not evident. Periodic acid-Schiff stain; bar = 100 μm.

was preserved at the light and electron microscopic levels throughout the study (Fig 1). Renal corpuscular structure at the light microscopic and ultrastructural levels (Fig 2) was within normal limits in the biopsy samples obtained at each sample collection.

Light microscopic alterations—Histologic lesions were first observed in the specimens obtained at postingestion hour 12. Tubular dilatation was the most common change. Tubular cells affected by mild to moderate degenerative lesions were scattered through the cortex, with loss of cellular detail and hydropic degeneration (Fig 3). Tubular cell necrosis with preservation of basement membranes was occasionally seen. Crystals, presumably calcium oxalate, were observed in 4 biopsy samples (Fig 4). Of these 4, 2 were from a single dog of group 3, and the remaining 2 were from a single dog of group 4. The birefringent crystals were less readily visible under standard microscope lighting than under polarized light. Crystals were observed mainly within tubular lumina, observed more frequently in PCT than in DCT. Tubular epithelial cells were flat and sometimes ruptured in areas where large clumps of crystals filled the tubular lumen. Though biopsy samples with crystals had the most severe lesions,

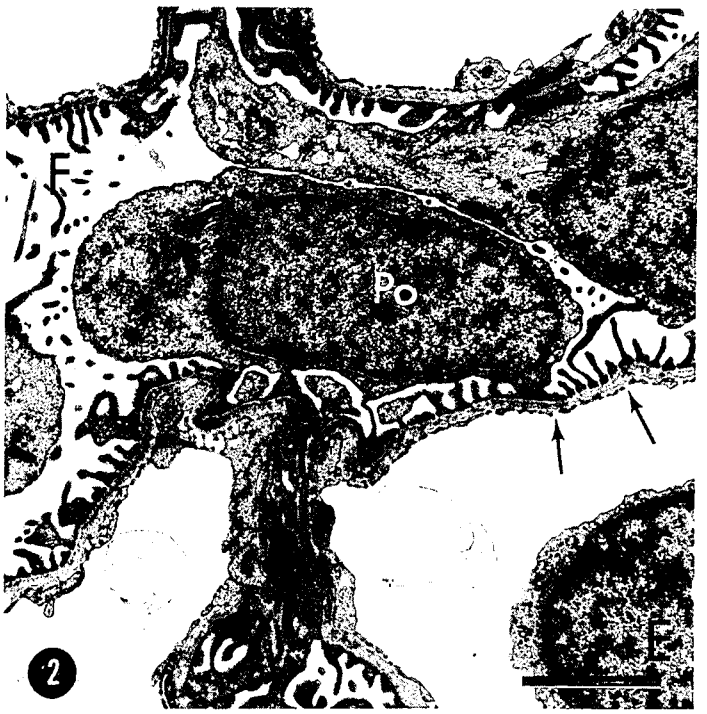

Fig 2—Electron micrograph of a glomerulus at 30 hours after EG ingestion. Structure is within normal limits. Endothelial (E) and podocyte (Po) nuclei are seen. Foot processes (F) are separate and not fused. Fenestrae (arrows) in endothelial cells are distinct. Bar = 2 μm.

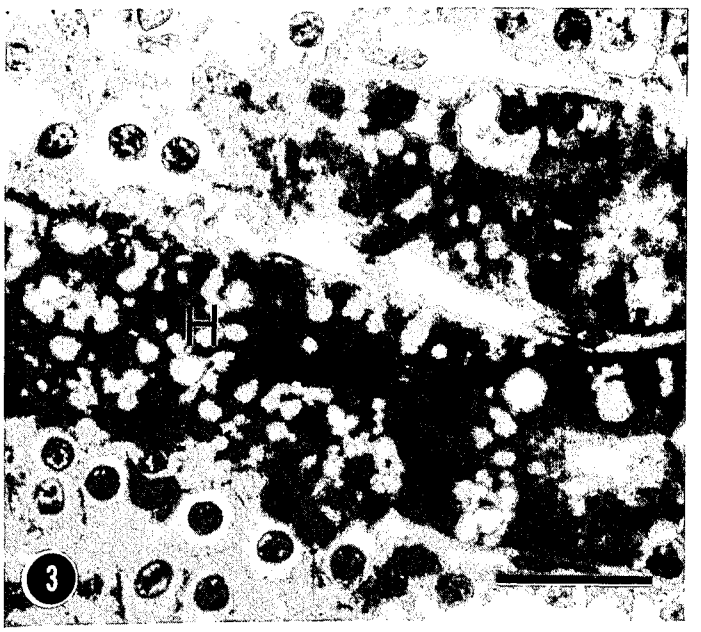

Fig 3—Section of renal cortex at 30 hours after EG ingestion showing hydropic degeneration (H) of proximal convoluted tubules. Hematoxylin and eosin (H&E) stain; bar = 25 μm.

interstitial edema, tubular dilatation, hydropic degeneration, and tubular cell necrosis also were observed in biopsy samples free of crystals.

Electron microscopic alterations—Initial ultrastructural abnormalities were observed as early as 5 hours after EG ingestion and consisted of numerous vacuoles distributed evenly through the cytoplasm of PCT cells (Fig 5). Affected cells were scattered, with no discernible pattern, through the cortex. This lesion preceded the initial

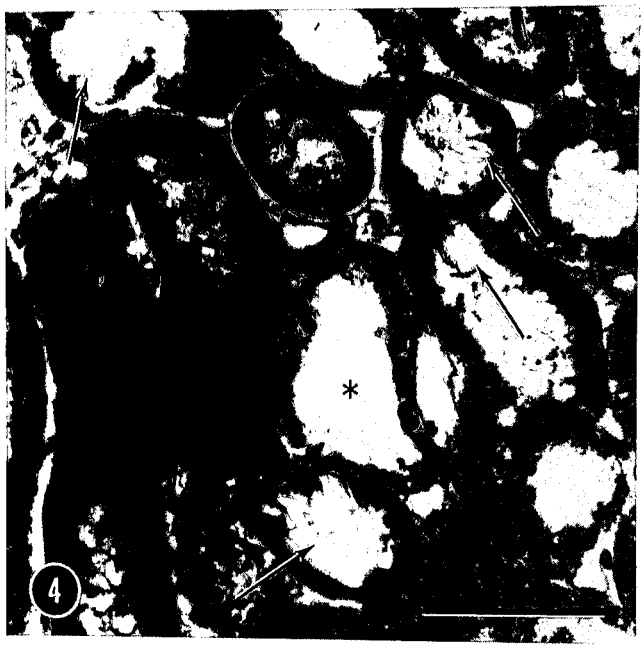

Fig 4—Section of renal cortex at 18 hours after EG ingestion, photographed with partially polarized light. Asterisk indicates a dilated distal convoluted tubule. Crystals, presumably calcium oxalate (arrows), are visible as refractile objects within the lumina of proximal convoluted tubules. H&E stain; bar = 50 μm.

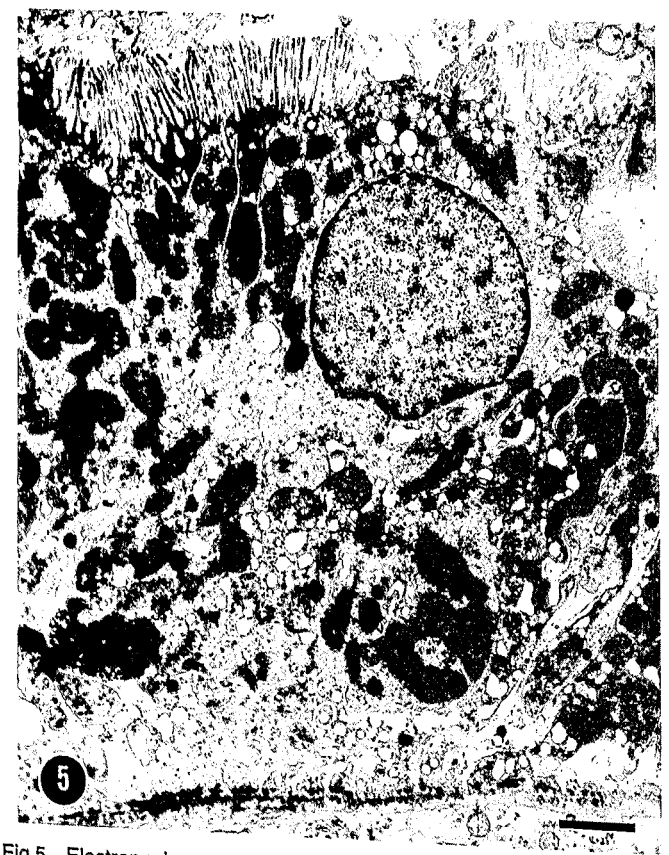

Fig 5—Electron micrograph of proximal convoluted tubular cell at 30 hours after EG ingestion. Diffuse small vacuolization is seen throughout the cell. Bar = 2 μm.

histologic changes by 7 hours; vacuolization was most prominent in 8-hour samples, but continued to be observed less frequently in each of the later samples.

Some PCT cells had distentions of the extracellular space between the basal infoldings (Fig 6). These spaces were confirmed to be extracellular by their direct contact with the basal lamina. Though most of the cytoplasm had been forced out of the intracellular spaces between the membranes, mitochondria were trapped between some of the infoldings. In many of these tubules, the normal round tubular contour was disturbed, with the basal lamina assuming an irregular, convoluted shape. This lesion was first observed in 8-hour samples, and continued to be observed at each subsequent sample collection hour, being most prominent in the 8- and 12-hour samples. At 18 hours, the distention of the extracellular parabasal spaces was, in some instances, modified into extremely large distentions (Fig 7 and 8).

Lysis of PCT cells was evidenced by cellular organelles lying free within tubular lumina, and by discontinuities in the apical membrane and collapse of PCT cells. Ruptured cells were seen in tubules in which intact cells had other abnormalities, as well as in tubules in which other cells appeared nearly normal. Rupture of PCT cells was first observed at 8 hours, becoming more prominent at each later sample collection hour.

Mitochondria in many PCT cells had increased density (Fig 9 and 10). This change was first observed at 12 hours, and became progressively more prominent in later-obtained samples.

Cytoplasmic buds, occasionally containing organelles, formed from the apical surface of some PCT cells and projected into the tubular lumen (Fig 9 and 10). The surface of the buds was devoid of microvilli. In many instances, the cytoplasm at the basal aspect of these cells was rarefied, with dispersion of the cytoplasmic elements. These buds appeared in 18-hour and each later-obtained sample, but were most prominent at 18 and 24 hours.

The range of structural change in DCT cells was more

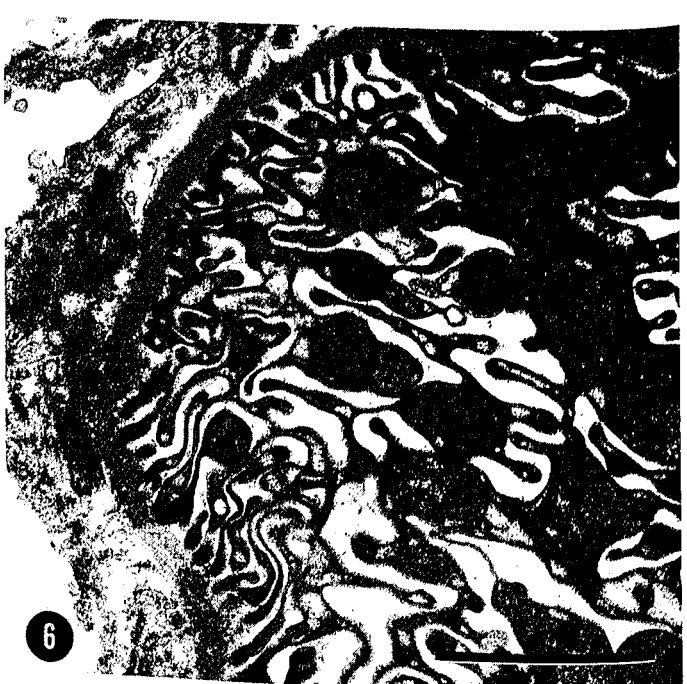

Fig 6—Electron micrograph of base of a proximal convoluted tubular cell at 8 hours after EG ingestion. Parabasal extracellular spaces are distended with organelles and small amounts of cytoplasm trapped between the displaced membranes. The base of the tubule has assumed an abnormal sinuous configuration. Bar = 2 μm.

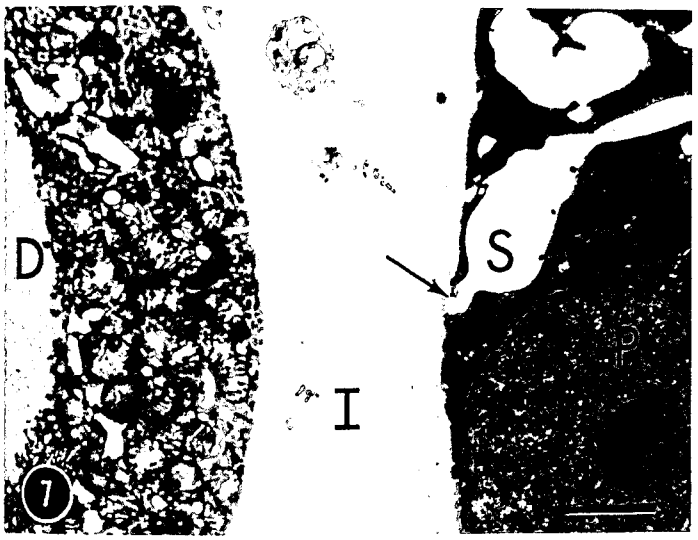

Fig 7—Electron micrograph of interstitium and bases of a proximal (P) and a distal (D) convoluted tubule at 30 hours after EG ingestion. Interstitial edema (I) is present. Proximal convoluted tubular mitochondria show poorly distinguishable cristae. The distended parabasal spaces (S) of proximal convoluted tubules are confirmed to be extracellular by direct contact with the basal lamina (arrow). The distal convoluted tubule is dilated and degenerate, with compaction of cellular organelles. Bar = 2 μm.

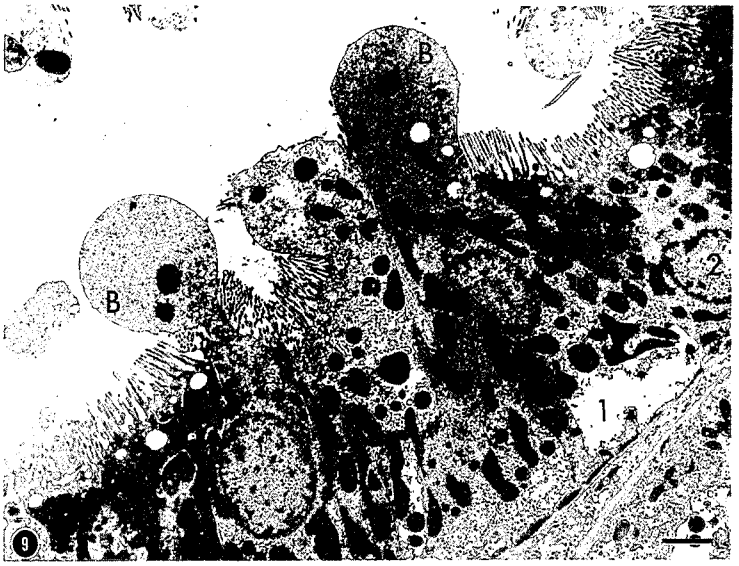

Fig 9—Electron micrograph of proximal convoluted tubule at 18 hours after EG ingestion. Apical cytoplasmic buds (B) devoid of microvilli protrude into the tubular lumen. Organelles are included in some of the buds. The cytoplasm at the base of one of the cells (1) is greatly rarefied, and the entire cell is in the process of sloughing into the lumen. Adjacent cell (2) has more normal appearance. Bar = 2 μm.

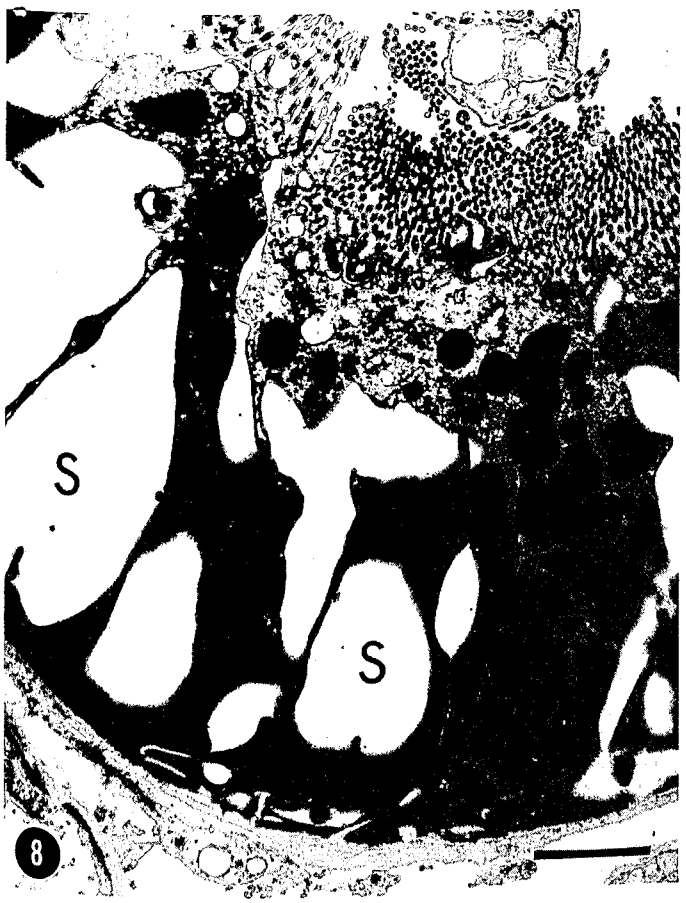

Fig 8—Electron micrograph of proximal convoluted tubular cells at 18 hours after EG ingestion. Parabasal and lateral extracellular spaces (S) are widely distended. Bar = 2 μm.

Fig 10—Electron micrograph of proximal convoluted tubule at 18 hours after EG ingestion. A large apical cytoplasmic bud (B) has formed on one cell. Large distensions of the parabasal extracellular spaces (S) are seen in some cells. One cell (1) shows an accumulation of small cytoplasmic vacuoles similar to that seen in early samples. Mitochondria (M) show indistinct internal structure. Bar = 2 μm.

restricted than in PCT cells. Some DCT cells had fine cytoplasmic vacuolization similar to that observed in PCT cells in 12 hour and later-obtained samples. Necrosis of individual DCT cells was observed in 12 hour and later-obtained samples, either as collapsed, shrunken cells or as cells sloughing into the lumen. Such cells were seen either adjacent to cells of more normal appearance (Fig 11), or in a grouping of degenerative cells (Fig 12). Ruptured DCT cells were observed in 12-hour and later-obtained samples. Each of these DCT lesions became more prominent in succeeding samples.

Discussion

Fixation by immersion was required to detect progressive development of lesions in individual dogs. Immersion fixation has previously been used successfully for canine renal pathology.[9]

Fig 11—Electron micrograph of distal convoluted tubule at 18 hours after EG ingestion. A shrunken necrotic cell (N) shows clumping of chromatin and mitochondrial destruction. Both adjacent cells appear more normal. Bar = 2 μm.

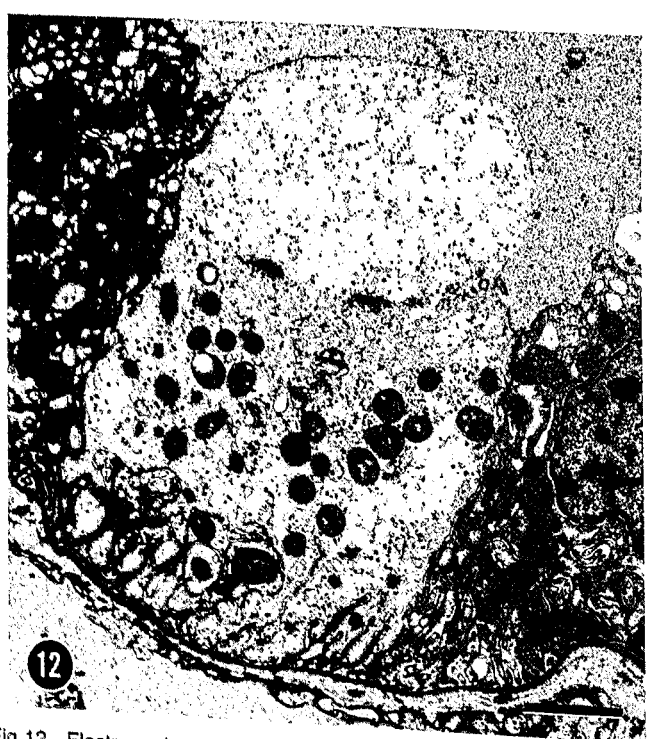

Fig 12—Electron micrograph of distal convoluted tubule at 24 hours after EG ingestion. A swollen, pale cell appears to be sloughing into the tubular lumen. The other tubular cells show degenerative changes, but do not appear necrotic. Bar = 2 μm.

Male dogs were selected for use because of ease of urinary catheterization. The use of all male dogs was deemed appropriate, because a sexual difference in response to EG intoxication in dogs is not known. Although sexual differences in the susceptibility of other species to intoxication by EG or its metabolites have been reported,[10,11] the biochemical and renal histologic response to EG intoxication in these and other studies is essentially identical irrespective of sex, once intoxication has developed. Halothane was used for anesthesia when recovery was required, because of quick recovery time, minimal nephrotoxic effects,[12] and economic considerations. Sodium pentobarbital was used for nonrecovery surgery, because it does not result in appreciable ultrastructural change in the canine renal parenchyma for up to 9 hours during surgical-plane anesthesia.[13] Because experimentally induced intoxication with reagent-grade EG is essentially indistinguishable clinically and histologically from spontaneous or experimentally induced intoxication with commercial antifreeze,[1,14,15] reagent-grade EG was used to avoid possible effects of additives in various antifreeze preparations, such as phosphorus-containing antirust compounds.[8]

The minimal lethal dose of EG in dogs is reported to be 6.6 ml/kg, but death has also been reported with dosage as low as 4.2 ml/kg.[14,15] The dosage administered in this study was set at 3.0 ml/kg in an attempt to maximize the likelihood of renal structural change, while minimizing undesirable CNS and cardiovascular side effects and preventing undue discomfort to the dogs. Administration of this dose of EG accomplished both goals. Structural changes were induced in the renal cortex, without inducing pain and suffering in the dogs. The ready responsiveness of the dogs, their interest in food, and their willingness to play up to the time of anesthesia were interpreted as indicating lack of pronounced discomfort. During the later phases of treatment, induction of general anesthesia prevented suffering in the dogs.

Originally, the experimental design had included determination of serum biochemical profiles and blood gas values.[f] Alterations in these variables followed a predictable course, with values changing as would be anticipated after EG intoxication. However, the magnitude of change was not statistically significant. The authors speculate that had the dogs been permitted to survive longer, the alterations would have continued their trends and eventually become statistically significant (though not necessarily lethal). Their failure to change significantly over the course of the study likely was related to the short duration of the investigation and the low dose of EG administered.

Structural change at the histologic and ultrastructural levels involved mainly the PCT and DCT, with PCT affected more frequently and by a wider array of structural changes than was DCT. Affected areas of the cortex were separated by more normal-appearing tissue.

During this study, histologic lesions were first seen at postingestion hour 12. Light microscopic lesions seen this early after EG administration have previously been reported only in animals that died acutely from the effects of a lethal dose of EG.

[f] Smith BJ. *Early morphological and biochemical effects of ethylene glycol on the canine renal cortex.* PhD Dissertation, Department of Veterinary Anatomy, The Ohio State University, 1986.

The types, distribution, and progression of light microscopic lesions observed in the dogs of this study, were similar to those that have been classically reported for EG intoxication in domestic animals and human beings. Minimal renal corpuscular change,[4,5,14] dilated proximal and distal convoluted tubules,[5,16] hydropic degeneration of PCT cells,[5,15] and epithelial cell necrosis[3,17] are well-known sequelae to EG intoxication.

The lack of crystal formation prior to 24 hours likely was related to the duration required to develop calcium oxalate crystals and the relatively low dose of EG given to the dogs. The widespread development of other renal cellular lesions, in the absence of calcium oxalate crystals, is to be expected because several metabolites of EG induce direct cytotoxic effects on renal cells irrespective of the concurrent presence or absence of calcium oxalate crystals.[6,11,16]

Transmission electron microscopy revealed lesions in biopsy tissue that had appeared within normal limits when examined with the light microscope. However, ultrastructural lesions were predictably most widespread and severe in dogs that also had histologic lesions. Ultrastructural changes developed in a pattern similar to histologic lesions, with lesions similarly distributed within the cortex, and PCT being more affected than DCT.

A spectrum of ultrastructural change was observed in later-obtained samples. In addition to the more advanced lesions of cellular rupture and cytoplasmic budding, samples obtained from 18 hours onward also occasionally had lesions, such as fine vacuolization of PCT, which were first observed in the early obtained (5- and 8-hour) samples. This implies continuing response by individual cells to the toxin. Similar observations have been made in studies of other forms of acute renal failure.[18,19]

Juxtaposition of normal and markedly abnormal cells within the cortex appears to be a characteristic of acute renal failure in general.[18,20,21] Reasons for such single-cell response remain open to conjecture. It may be hypothesized that individual cells in the PCT may not function at the same level at any given point in time. More active cells could possibly sustain greater damage from a toxic or ischemic insult, whereas cells that are less active at the time of insult might be less affected. Such cells might survive to repopulate the nephron, as has been reported after sublethal injury in animal models of toxic[22,23] and ischemic[24,25] forms of acute renal failure.

Results of this study concurred with those of other investigations[5,14,26] in characterizing EG intoxication as primarily inducing PCT damage. A similar pattern of predilection for proximal tubular injury has been described from experimental studies of models of ischemic[27] and other nephrotoxic[28,29] forms of acute renal failure, as well as in human case reports, though differences exist in the cytologic effects.[21,30] The predilection of the PCT to injury in multiple forms of acute renal failure may be related to their high level of metabolic activity, as well as to their role in metabolizing and transporting xenobiotics. Because of their great energy requirements, PCT have numerous mitochondria. The metabolites of EG induce direct toxic effects on isolated mitochondria.[31] The numerous mitochondria in PCT may render them highly susceptible to insult by various mitochondrial toxins, thus predisposing them to injury in various forms of acute renal failure.

Distention of intercellular or parabasal spaces has been observed in other forms of acute renal failure,[29,32,33] but has not been discussed at length. Twenty-four hours after exposure to uranyl nitrate, rat kidneys had changes similar to the large parabasal distentions seen here after EG intoxication.[29] In our study, multiple small distentions were seen as early as 8 hours, whereas larger distentions appeared first in the 18-hour samples. This suggests that multiple small distentions may be an early response, which progresses by coalescing into fewer, larger distentions. These distentions are likely related to changes in fluid movement through the injured kidney.

The observation of cytoplasmic protrusions from the apical surfaces of PCT cells differed from findings of previously reported ultrastructural studies of EG intoxication. Apical buds were not observed in rats with EG intoxication.[26] However, biopsy samples obtained from human beings 5 or more days after EG intoxication did have this feature.[33] Apical buds have also been observed in ultrastructural studies of other forms of acute renal failure. Some authors[18,34] have dismissed such changes as artifact related to biopsy, whereas others[20,33] have ascribed importance to them. In our study, the lack of buds in any control sample, or in the intoxicated dogs prior to 18 hours, suggests that their development is a response to EG intoxication.

In all biopsy samples from each dog, renal corpuscular structure, as seen by light and transmission electron microscopic observation, was within normal limits. Severe alterations in the structure of podocytes can develop in other animal models of acute renal failure.[35,36] The lack of renal corpuscular changes in the dogs of this study may be a particular characteristic of EG intoxication itself, or may be related to the time of procurement of samples. Studies of other forms of acute renal failure that reported change in podocyte structure evaluated samples obtained 48 hours or later after intoxication. In this study, the latest sample was obtained at 30 hours after EG ingestion. Thus, the lack of renal corpuscular change in this study could be a function of the sample collection schedule of the study. On the basis of our results, the possibility cannot be eliminated that structural change in the renal corpuscle may develop in more protracted instances of EG intoxication.

Alterations of mitochondrial structure seen in the dogs of this study were unlike those previously described in experimental models or reports of acute renal failure in people. In this study, mitochondrial swelling was not a prominent feature. Instead, many mitochondria of PCT cells from 12 hours onward had increased density. Certain metabolites of EG have toxic effects on mitochondrial function.[31] Structural changes in mitochondria seen in dogs of this study, therefore, may be related to the functional changes induced in mitochondria by EG intoxication.

The lack of widespread loss of the brush border in dogs of our study is uncommon among the well-studied models of acute renal failure. Spangler et al[9] reported a progressive decrease in brush border staining at the light microscopic level in dogs given gentamicin, though microvilli were intact ultrastructurally. Sloughing or internalization of the brush border has been described in numerous other studies of acute renal failure, in animal models[28,37,38] and in human clinical cases.[18,21,34] Misidentification of such denuded PCT (pseudodistal tubules)[18] as DCT is prevented by observation

of other structural features of the cells. Sloughed microvilli are seen within 8 to 12 hours after administration of mercuric chloride,[22,28,29] whereas in dogs of this study, sloughing was not observed through 30 hours after EG administration. Histologic findings in domestic animals or human beings with EG intoxication days or weeks after intoxication[5,14,39] and a report of ultrastructural findings in human renal biopsy samples obtained up to 22 days after EG ingestion[33] also did not involve loss of the brush border. Further, reports of histologic examination of renal tissues from animals ingesting or being administered higher doses of antifreeze or EG do not describe loss of the brush border. Thus, preservation of the brush border appears to be related to EG itself as the toxic agent, rather than to a toxic vs ischemic mechanism of renal injury, time elapsed since intoxication, or dose of EG administered.

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