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Chemical and metabolic aspects of urea-ammonia toxicosis in cattle and sheep

William Eugene Lloyd
Iowa State University

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CHEMICAL AND METABOLIC ASPECTS OF UREA-AMMONIA TOXICOSIS IN CATTLE AND SHEEP

by

William Eugene Lloyd, D.V.M.

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major Subject: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Ames, Iowa

1970
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INTRODUCTION

Urea and ammonium salts are commonly and extensively used in ruminant rations as sources of nitrogen and substitutes for part of the dietary protein. Improper feeding or mistakes in preparation of rations frequently result in poisoning by these compounds. Toxicosis resulting from the oral administration of urea to ruminants is an acute syndrome characterized by hyperammonemia. This indisputable fact has been verified by many investigators. However, researchers have failed to clearly elucidate the mechanisms and the subcellular biochemical changes effected by elevated blood ammonia levels.

Urea toxicosis in ruminants is characterized by increased ammonia and pH values in rumen ingesta. The elevated rumen pH readings may have led earlier investigators to logically assume that toxicosis is associated with symptoms of metabolic alkalosis. In humans the syndrome called "hepatic coma" is common and is characterized by hyperammonemia and alkalosis of either the respiratory or metabolic type. The etiology and pathogenesis of this chronic liver disease syndrome apparently differ from the etiology and pathogenesis of acute ammonia toxicosis resulting from ingestion of urea by ruminants or ammonium compounds by mammals. Nevertheless, knowledge of the hepatic coma liver syndrome may have influenced investigators to assume toxicooses characterized by hyperammonemia are always accompanied
by a state of alkalosis.

Other investigators theorized that poisoning is due to the formation of a toxic intermediate, ammonium carbamate. Although the theory has not been proven, it is still frequently mentioned in literature.

Many clinical reports of urea poisoning in ruminants as seen in veterinary publications seem to lack valid documentation. Indeed, some appear to represent an improper diagnosis.

This research was designed to study some of the effects of toxicosis induced by administering urea orally and various ammonium compounds intravenously. A concerted effort was made (1) to determine the effect of ammonia toxicity on blood pH, and (2) to study the creditability of the carbamate theory. Moreover, additional blood and urine values were evaluated to gain insight into the mechanisms of and possible treatments for ammonia toxicosis.
REVIEW OF LITERATURE

The role of rumen microflora in the digestion of amides and cellulose was suggested by Zuntz (1891). Kellner (1900) conducted feeding trials with amides and ammonium acetate for lambs. Morgen, Kellner, Völtz, Hansen, Honcamp and Ehrenberg with their associates conducted numerous trials from 1907 to 1937 involving the utilization of urea and ammonium salts by ruminants, as reviewed by Stangel (1963). Since then hundreds of publications have been written on the use of non-protein nitrogen (NPN) and ammonium compounds as substitutes for part of the dietary protein for ruminants. However, comparatively little has been published relative to the toxicity of these compounds.

Metabolism of Proteins, Urea and Ammonia by Ruminants

Ammonia is the end-product of intraruminal metabolism of most N-containing feedstuffs. Rumen ammonia production is influenced by the amounts and types of N-containing substrates and enzymatic activities. The kinetics of ruminal ammonia production and utilization are influenced by (1) the types and solubilities of the NPN or protein feedstuffs, (2) types and amounts of soluble carbohydrates present, (3) species and numbers of rumen microorganisms, (4) rumen pH and (5) temperature.
The released ammonia is ideally utilized by rumen microorganisms (Burroughs et al., 1951) and is reported to appear as newly-synthesized protein within 4 to 6 hours (Wegner et al., 1941; Mills et al., 1942). Excess ammonia passes through the epithelial lining of the forestomachs into the portal vascular system.

Bloomfield et al. (1960) reported that ammonia was hydrolyzed from urea in sheep rumens 4 times more rapidly than could be taken up by microorganisms and the excess was lost out of the rumen. The portal ammonia rose to 1.62 mg NH\textsubscript{3}-N (ammonium nitrogen) per 100 ml of blood without an increase in jugular NH\textsubscript{3}-N. Carroll (1961) concluded that rumen fluid has the capacity to hydrolyze urea faster than it can utilize the released ammonia. The excess ruminal ammonia is absorbed through the epithelium of the rumen and reticulum into the portal blood system and is incorporated into urea via the ornithine-urea cycle.

Lewis (1957) and Lewis et al. (1957) showed that blood urea levels positively correlated with rumen ammonia levels but with a lag time of 4 to 8 hours. Portal blood ammonia levels reflected rumen ammonia levels, but peripheral blood levels did not increase above normal until rumen ammonia levels exceeded 60 mmol per liter (ca. 84 mg NH\textsubscript{4}-N per 100 ml).

Urea in blood is returned, in part, to the rumen by
transport across the rumen wall and via saliva (McDonald, 1948; Houpt, 1959; Decker et al., 1960; Decker, 1961; Somers, 1961; Gäärtner, 1963; Juhász, 1965; Packett and Groves, 1965; Cocimano and Leng, 1967; Houpt and Houpt, 1968). Decker (1960), Decker (1961) and Gäärtner (1963) reported that at least 50% of blood urea is recycled to the rumen by an active transfer and reappears as ammonia. The following system is described: rumen ammonia → portal blood ammonia → blood urea → rumen ammonia. Equilibrium for the cycle occurs when rumen ammonia levels are 5 to 8 mg per 100 ml.

**Ureolysis in the Rumen and Urease Activity**

Urea is rapidly hydrolyzed in normal rumen ingesta to ammonia and carbon dioxide by the enzyme urease. Lenkeit and Becker (1938) reported that urea was decomposed within 30 minutes in the rumen with a marked increase in ammonia. Other investigators noted that the completion of urea hydrolysis and peak rumen ammonia levels occur in 30 minutes to 2 hours (Wegner et al., 1941; Mills et al., 1942; Matsumoto et al., 1958; Ryš et al., 1956; Stallcup and Looper, 1958; Le Bars et al., 1957; Holzschuh and Wetterau, 1962; Davis and Stallcup, 1967; Szabó, 1966; Rekib and Sadhu, 1968). Hill et al. (1961) reported that 90 to 95% of \(^{14}\text{C}\) from \(^{14}\text{C}\)-urea that had been administered intraruminally to goats was lost as expired CO\(_2\) in the first 30 to 40 minutes.
Urease catalyzes the following reaction:

\[
\begin{align*}
\text{H}_2\text{N}-\text{C}-\text{NH}_2 + \text{HOH} & \rightarrow 2\text{NH}_3 + \text{CO}_2 \\
\text{urea} & \text{water} & \text{ammonia} & \text{carbon dioxide}
\end{align*}
\]

Luck (1924) described urease activity in rumen fluid and mucosa. Pearson and Smith (1943) showed that the optimum temperature and pH for urease activity were 49° and 7.7, respectively; urease activity was not significantly affected by nitrogen gas, carbon dioxide or air. Stallcup (1954) reported that urea released large amounts of ammonia in the presence of rumen liquor and release was increased by the addition of urease. Green (1955), in an extensive review of urea feeding to ruminants, states the optimum pH for urease is 8.0.

Shimura et al. (1958) showed that urease activity increased within 3 hours after feeding urea in a blind bovine rumen sac and concluded that urease was produced adaptively by protozoa. Underbjerg (1961) described the action of urease in whole soybeans on urea toxicity.

Urease in rumen fluid and mucosa originates predominantly from ruminal bacteria (Jones et al., 1964; Hungate, 1966; Rahman and Decker, 1966; Houpt and Houpt, 1968). Rahman and Decker (1966) concluded that pH 8.5 was optimum for urease and allowed a \( K_m \) of \( 1.5 \times 10^{-3} \) M.

Boda and Varady (1966) reported that urease activity did
not depend on the amount of urea in the diet. Wortham et al. (1968) reported there was no significant correlation between urease levels and rumen ammonia levels in a group of feeder lambs. Apparently adequate urease is normally present to hydrolyze all exogenous and endogenous urea available to ruminants.

Rumen Ammonia Levels and Assimilation by Rumen Microorganisms

Normal rumen ammonia levels, depending on the diet, have been reported by various investigators as 0 to 130 mg NH$_3$-N per 100 ml rumen fluid (McDonald, 1948; Johns, 1955 as quoted in Hungate, 1966; Lewis et al., 1957; Hogan, 1964; Phillipson et al., 1962; Oltjen et al., 1963; Perez et al., 1967; Rekib and Sadhu, 1968; Oltjen et al., 1969).

Rumen ammonia levels are increased by N intake and protein solubility and decreased by the amounts of starch and other readily-fermentable carbohydrates ingested. Wegner et al. (1941) reported that when urea was fed the NH$_3$-N in rumen contents increased from 0.075% to 0.222% (75 to 222 mg per 100 ml) within one hour and the liberated ammonia disappeared within 4 to 6 hours. Some of the ammonia appeared in newly-synthesized protein in the rumen. Mills et al. (1942) reported that urea was only 50% hydrolyzed at 6 hours when a fistulated heifer was fed only hay, but when starch was added hydrolysis was complete in one hour and the liberated ammonia
appeared as newly-synthesized protein at 6 hours. Sirotnak et al. (1953) reported that the optimum pH for amino acid dissimilation by mixed rumen microorganisms was 6.9. Fermentable carbohydrates enhanced the process. Urea and aspartate were attacked 5 times faster than other amino acids.

Annison et al. (1954) fed sheep 8 to 16 g N per day in rations composed of hay and either a highly soluble ground nut meal, casein or ground maize. Rumen ammonia levels peaked in 2 to 4 hours. Animals receiving 16 g N and ground nut meal had rumen NH$_3$-N levels of 70 to 115 mg per 100 ml. Animals receiving 13 g N and casein had values of 55 to 85 mg; those receiving 8 g N and herring meal had values of 25 to 35 mg. When ground maize was given alone rumen ammonia levels were very low.

Chalmers et al. (1954) demonstrated that soluble casein was rapidly deaminated and much of the ammonia was lost by absorption into the blood stream. Peak rumen NH$_3$-N levels were 60 mg per 100 ml in 4 hours. Chalmers and Synge (1954) reported that rumen NH$_3$-N levels ranged from 45 to 60 mg per 100 ml for casein and 30 to 44 mg per 100 ml for herring meal. N retention was better for the latter. Rys et al. (1956) stated that NH$_3$-N levels in rumen fluid of sheep ranged from 27 to 47 mg per 100 ml, depending on the type of diet. Davis and Stallcup (1967) and Szabó (1966) described the influence of protein and urea solubility on rumen ammonia.
Coombe and Tribe (1958) successfully fed very large doses of urea, up to 100 g, to wethers as a 1:4 mixture of urea and molasses. They considered urea toxic if rumen NH₃-N levels exceeded 80 mg per 100 ml. Matsumoto et al. (1958) injected glucose and urea into the rumens of goats and reported that the urea disappeared within 30 minutes and 93% of the sugar in 90 minutes. When they fed a molasses-urea combination gas formation was increased, the pH was 7.3 to 7.4 and VFA (volatile fatty acid) production was quite slow. Rumen NH₃-N levels rose to 49 mg per 100 ml at 2 hours and decreased to 23 mg at 7 hours.

Stallcup and Looper (1958) reported that rumen ammonia levels peaked within 2 hours when feeding a urea-molasses mixture, but peaked in 6 hours when feeding cottonseed and soybean meals and the rises were smaller and declined relatively constantly. Other investigators verified the protective action of readily-fermentable carbohydrates against urea and soluble nitrogen sources (Clark et al., 1951; Preston et al., 1961; Chalupa, 1963; Holzschuh and Wetterau, 1962; Kudryavtsev, 1963; Briggs et al., 1964).

Adaptation of microorganisms to various dietary N levels was demonstrated by Clark et al. (1951). They reported that sheep that had been on diets of lucerne hay tolerated higher doses of urea than animals on diets of poor quality grass hay. They stated that the toxicity of urea depends on the
activity of the ruminal flora, as determined by the basic diet, and the presence of available carbohydrate. Davis and Roberts (1959) reported that cattle that had been adapted to high dietary protein levels would tolerate 7 to 9 times the dosage of urea of animals that had been on low protein diets or that had been fasted 24 to 48 hours. Adaptation was lost within 3 days. Phillipson et al. (1959) showed that starch and glucose were fermented with the formation of acetic and lactic acids, and ammonia was assimilated in part due to an increase in the concentration of bacteria.

Ammonia is essential for the growth of several species of rumen microorganisms (Bryant, 1963; Hungate, 1966). Several investigators have reported that ammonia stimulates the digestion of cellulose or starch by mixed rumen microorganisms (Belasco, 1954; Ewan et al., 1958; Phillipson et al., 1962; Brüggeman et al., 1962; Allison and Bryant, 1963; Chalupa, 1963; Little et al.; 1963; Briggs et al., 1964; Winter et al., 1964; Acord et al., 1966; Caffrey, 1966; Luther et al., 1966).

Rumen pH and Absorption of Ammonia from the Rumen

Several investigators have confirmed that pH values increase with ammonia concentration in the rumen (Clark et al., 1951; Matsumoto et al., 1958; Coombe et al., 1960; Hogan, 1961;
Values up to pH 7.5 have been reported for ruminants that have been given relatively high oral doses of urea that were apparently non-toxic or of a low order of toxicity. Urease activity is enhanced by an increase in pH and the released ammonia increases rumen pH. Hydrolysis of urea by urease thus tends to become a self-augmenting or autocatalytic process to completion.

Rumen pH also influences ammonia metabolism, and the possibility of toxicosis, by governing the passage of ammonia across the epithelium of the forestomachs into the blood. McDonald (1948) studied the absorption of ammonia from the rumen of sheep and its appearance in ruminal veins. He stated normal levels of NH$_3$-N in ruminal veins is ca. 1.5 mg per 100 ml. Hogan (1961) discussed rumen absorption as a function of rumen liquor movement and ammonia concentration. He demonstrated that the transport of ammonia across the rumen epithelium increased with the concentration gradient at pH 6.5 and that the movement of volatile fatty acids (VFA) at that pH increased ammonia transport. Ammonia was not transported to a significant extent at pH 4.5, regardless of concentration, but VFA's were transported relative to their concentration at pH 4.5 or 6.5. The transport of ammonia was not affected by ruminal concentrations of sodium, potassium, carbon dioxide, chloride or lactate, or by the net movement of water into or out of the rumen. He discussed pKa and the relative
concentrations of ammonia.

Warren (1962) reported the pKa of ammonia to be ca. 9.0 and reviewed the passage of ammonia through biological membranes. He stated that the nonionized form predominates in alkaline media and tends to transport rapidly across membranes to a more acid side. Bloomfield et al. (1963), stated the pKa of ammonia to be 8.8 and of VFA's to be 4.8 to 4.9 at 40°. At a normal ruminal pH of 6.4 VFA absorption is favored and NH₃ absorption is minimized; at a pH of 7.55 NH₃ absorption is increased and VFA absorption is depressed.

Absorption of ammonia from the rumen is dependent upon the concentration gradient and pH. Manning (1964) and Visek (1968) reviewed ammonia passage across a biological membrane as a physico-chemical phenomenon dependent largely upon (1) total ammonia (T NH₃) levels, present as both the ionized (NH₄⁺) and nonionized (NH₃) states, and (2) pH. These two factors fix the partial pressure of ammonia (p NH₃), the amount of nonionized ammonia, on each side of a biological membrane and govern the directions and relative passage rates. This explains the transfer of ammonia from rumen to blood and thence to and from cells of the liver, muscle, brain and kidney.
Urea and Ammonia Toxicity
in Ruminants

The response of ruminant animals to oral doses of urea and ammonium salts varies considerably. Variances of toxic doses and response time are high between individuals within a species, but low between species.

Predisposing factors

Whitehair et al. (1955) listed several conditions that may predispose cattle to toxicosis from ingesting urea-containing feeds: (1) starved or fasted animals; (2) presence of animals with "hoggish" appetites; (3) animals not previously fed urea; (4) feeds improperly mixed; and (5) high-roughage rations. Other writers have described similar predisposing factors in ruminants (Clark et al., 1951; Gallup et al., 1953; Davis and Roberts, 1954; Davis and Roberts, 1959; Hornoiu et al., 1959).

Toxic doses

In all species studied dosages of urea varied from a minimum toxic dose of ca. 0.25 g per kg of body weight to a maximum non-toxic dose of 2.0 g per kg.

Cattle Dinning et al. (1948) administered urea to cattle in aqueous solution in doses of 116,272 and 400 g and all animals died within 70 minutes. Gallup et al. (1953) calculated toxic doses to be 0.5 to 1.0 g per kg body weight.
Davis and Roberts (1954) gave doses of 0.345 to 0.454 g per kg body weight with death resulting in 90 to 170 minutes. A small steer, after adapting to high-urea rations, consumed 200 g urea per day in the ration, but died when given 90 g as a drench.

Abonyi et al. (1958) reported doses of 100 to 200 g of urea were toxic to cattle without previous habituation. Davis and Roberts (1959) reported that cattle adapted to low protein diets and fasted 24 and 48 hours would tolerate only 0.18 and 0.23 g per kg body weight, respectively. Adaptation to doses up to 1.57 g per kg body weight was possible, but was lost within 3 days. Holzschuh and Wetterau (1962) stated that up to 600 g urea was tolerated by adult cattle if mixed with cellulose and starch. Rummler et al. (1962a) stated that 0.49 g urea per kg body weight was toxic to cattle when mixed in feeds. Kudryavtsev (1963) reported that cattle withstood 200 g urea in high-carbohydrate rations, but 100 to 150 g administered through a rumen fistula caused intoxication.

Sheep Clark et al. (1951) reported that 15 to 20 g of urea was toxic to sheep, depending on the previous N intake and quality of hay. Repp et al. (1955) fed urea and ammonium salts in doses based on urea equivalent amounts. Toxicity was encountered at 25 and 30 g per 100 lb. (0.55 and 0.66 g per kg) body weight with ammonium propionate and 40 g
per 100 lb. (0.88 g per kg) with urea, ammonium formate and ammonium acetate.

Hornoiu et al. (1959) demonstrated that sheep could tolerate up to 0.5 g and 0.25 g per kg body weight in feed and in solution, respectively. Moreover, they reported doses up to 2.0 g per kg could be tolerated if diluted in a large volume of feed. Le Bars et al. (1957) dosed sheep orally with urea and reported that 0.1 g per kg was nontoxic; 0.3 g diminished ruminal contractions; and 0.5 g completely inhibited rumen motility. Doses of 1.5 g per kg were usually toxic and effects appeared within 5 to 75 minutes. Doses of 1.0 g per kg for two months did not elicit chronic toxicosis. Singer (1969) reported 5 of 7 ewes died when given 1.5 g ammonium chloride per kg of body weight by stomach tube. Similarly, 4 of 8 died with a mixture of the ammonium salts of chloride, sulfate, carbonate and phosphate at dosages of 2.0 g per kg.

**Time of death**

The time necessary to produce death from a toxic dose of urea appeared to depend less upon the dosage levels than upon other predisposing factors, such as the nutritional status and mode of administration. Death time ranged from 30 minutes to several hours (Dinning et al., 1948; Clark et al., 1951; Bullington et al., 1955; Repp et al., 1955;
Symptoms of ammonia toxicosis

The clinical signs of poisoning in ruminants following orally-administered urea and ammonium salts have been described by numerous researchers. They generally include, in order of their appearance, symptoms of dullness, uneasiness, muscle and skin tremors and twitching, salivation, dyspnea, bloating, tetany and death (Osebold, 1947; Dinning et al., 1948; Clark et al., 1951; Gallup et al., 1953; Davis and Roberts, 1954; Bullington et al., 1955; Repp et al., 1955; Meyer and Rustige, 1958; Davis and Roberts, 1959; Szwabowicz, 1962; Oltjen et al., 1963).

The elicitation of symptoms appeared to be similar in all species. Bullington et al. (1955) and Abonyi et al. (1958) reported that cattle also showed hyperesthesia, grunting, groaning and sweating. Clark et al. (1951) and Singer (1969) reported that sheep often regurgitate ruminal contents just prior to death. Kita et al. (1959) reported respiratory paralysis in the terminal stages in the goat. Singer (1969) recorded temperature rises in sheep before death.

Physiopathological changes

Blood ammonia levels appear to directly affect the appearance of symptoms during ammonia toxicosis. Symptoms appeared when blood NH$_3$-N levels reached 0.5 to 1.0 mg per 100
ml (Repp et al., 1955; Lewis et al., 1957; Holzschuh and Wetterau, 1962; Rummler et al., 1962b; Roller, 1966; Singer, 1969). Death occurred in cattle when blood levels exceeded 3.5 to 5.0 mg per 100 ml (Dinning et al., 1948; Davis and Roberts, 1959; Roller, 1966; Rash, 1967; Singer, 1969). Repp et al. stated death occurred in lambs at levels of 2.0 mg per 100 ml.

Acid-base balance Symptoms of alkalosis were associated with blood ammonia levels by Dinning et al. (1948), who administered solutions of urea orally to sheep and yearling steers. Lewis (1960) administered ammonium chloride and ammonium acetate orally to ruminants and reported the former caused an uncomplicated acidosis and the latter caused a respiratory alkalosis. He stated he believed the toxicity was not due to a change in acid-base or electrolyte balance, but rather to the circulating ammonium ion. Drepper (1961) theorized that ammonia intoxication is an alkalosis that causes decreased calcium and magnesium serum levels.

Roller (1966) reported an initial rise in blood pH followed by a decline with a tendency toward metabolic acidosis by the time of convulsions. He theorized that acidosis was the cause of death. Singer (1969) administered various ammonium salts to sheep and reported severe acidosis. He did not feel decreased blood pH values were related to the clinical signs of poisoning. Rash (1967) reported urea orally
administered to ruminants produced a respiratory acidosis.

Blood urea nitrogen

Blood urea-nitrogen increased in cattle and sheep during toxicosis from urea and ammonium salts (Dinning et al., 1948; Davis and Roberts, 1954; Repp et al., 1955; Lewis, 1960; Preston et al., 1961; Singer, 1969).

Rumen physiology

Rumen physiology is affected during ammonia toxicosis. Clark and Lombard (1951) stated that 20 g doses of urea given intravenously to sheep were nontoxic. High oral doses of urea caused ruminal paresis and when the pH reached 7.5 there was a complete ruminal stasis. They concluded stasis was due to the alkaline reaction and not ammonia. They theorized toxicity was not due to urea or ammonia, but was caused by a toxic intermediate. Le Bars et al. (1957) reported that urea administered intravenously at doses of 0.4 g per kg of body weight were well-tolerated by sheep and increased the amplitude of rumen contractions. Ammonium salts given intravenously completely arrested rumen motility which they theorized to be due to the action of ammonium salts on nerve centers.

Rumination declined as the rumen pH rose to 7.0 and became static at pH 7.3 in sheep fed high levels of urea (Coombe et al.; 1960). Hogan (1961) reported that the transport of ammonia from the rumen was not influenced by the organ's contractions. Felinski (1962) demonstrated that orally
administered urea depressed rumen motility. Singer (1969) reported ruminal stasis in sheep given ammonium salts orally. Whitehair et al., (1955), along with other investigators, reported bloating was common in animals fed large quantities of urea. This was apparently due to rumen paresis since large amounts of urea inhibit gas production in the rumen (Jacobson et al., 1942).

Carbamate theory The "carbamate theory" was apparently introduced by Kaishio et al. (1951), who demonstrated that total NPN in the blood was increased after oral administration of urea to cattle and goats. They reported the increased NPN was not due to urea-N, but to some other intermediate, such as ammonium carbamate. The latter, when injected intravenously, caused symptoms similar to those observed in urea toxicosis. King and Hale (1955) administered carbamate salts by intravenous injection, injection into the abomasum and orally to ruminating lambs. They agreed with Kaishio et al. (1951) that ammonium carbamate was the toxic intermediate in urea poisoning.

Sumner et al. (1931) demonstrated that the ammonium carbamate that was formed during ureolysis was rapidly decomposed. Payne (1955) infused dogs with ammonium carbamate and concluded toxicity was due only to the ammonium ion. Soejima et al. (1959), using a sophisticated dilatometer technique, excluded the possibility of the occurrence of
ammonium carbamate in the hydrolysis of urea in the rumen. Wilson et al. (1968) concluded that ammonium carbamate and ammonium carbonate were of the same order of toxicity when given in equimolar amounts to sheep.

**Blood magnesium and calcium**

Blood magnesium and calcium levels have interested several investigators. Intravenous doses of magnesium chloride, calcium chloride and dextrose were given to urea-poisoned sheep and steers by Dinning et al. (1948). The treatment reduced the symptoms of tetany but the animals died. Meyer and Rustige (1958) reported that cows given toxic doses of urea exhibited decreased levels of calcium and magnesium, but deducted that the tetanic symptoms were not due to low serum magnesium levels. Drepper (1961) theorized that ammonia intoxication is an alkalosis which decreases blood calcium and magnesium.

**Other blood changes**

There is a paucity of reports on other blood parameters in urea-poisoned ruminant animals. Roller (1966) reported that packed cell volume (PCV) and serum potassium were increased in cattle; erythrocytes showed lowered sodium and increased potassium content. Singer (1969) reported severe hyperglycemia with levels of 3 to 5 times normal levels in sheep given ammonium salts orally.

**Urine**

Singer (1969) reported albuminuria and hematuria in all sheep poisoned by ammonium salts. Glycosuria
was found in some animals.

**Antidotes**  Acetic acid has been used as an oral antidote for urea poisoning (Clark et al., 1951; Clark and Lom­bard, 1951; Davis and Roberts, 1954; Repp et al., 1955). Acetic acid was effective if 2000 to 3600 ml of 5% solution was given before the onset of tetany. It appeared to reduce the rates of urea hydrolysis and ammonia absorption from the rumen.

**Pathology**

Lesions in sheep that had died of urea poisoning were described by Clark et al. (1951). They included pulmonary congestion and edema, subepicardial and subendocardial hemorrhages, and severe degeneration of the liver and kidney. Perez et al. (1967) reported that lambs given large doses of urea phosphate died several days after treatment. The animals showed very high blood phosphate levels and severe degeneration of the kidneys and liver. It seems probable that the pathological changes were due to hyperphosphatemia rather than to changes induced by the urea moiety. Singer (1969) described various histopathological changes in the lungs, kidneys and thymus of sheep poisoned by ammonium salts.
Ammonia Toxicity in Non-ruminants

Bicknell (1965) reviewed ammonia metabolism in non-ruminant animals and man. The reader is invited to read his excellent review for a more detailed review of literature. He discussed the relationship of ammonia to hepatic coma, a disease in which blood ammonia increases due to a chronic pathological condition of the liver.
MATERIALS AND METHODS

Animals

Cattle utilized in this study were Aberdeen Angus x Holstein-Friesian cross-bred females. The 9 heifers were 6 to 10 months of age with a mean weight of 122.6 kg and a range of 93.0 kg to 181.4 kg.

Adult female sheep, either Shropshire, Hampshire or Columbia-Merino cross-bred, were used. The 20 ewes were 2 to 7 years of age with a mean weight of 61.3 kg and a range of 39.0 kg to 77.6 kg.

Experimental animals were housed in a semi-heated shed containing 1 to 3 animals in each stall. All animals were given water, salt and whole mixed legume-orchard grass hay ad libitum.

Surgical Procedure

A technique was devised whereby polyethylene tubes were implanted in the ureters and exteriorized through the bladder, urethra and vulva of experimental animals. The procedure was performed on most of the animals used in Experiment I. It was used to facilitate the assay of quantitative and qualitative changes in renal function caused by the administration of urea administered in toxic oral doses. The surgical procedure was performed
approximately one week before the experimental procedure.

The general surgical procedure is illustrated by a semi-schematic drawing (Figure 1), using the female bovine as a model. The ureteral cannula was fabricated by forming a collar of suture material near one end of a length of polyethylene tubing. A cannula was surgically implanted in the lumen of each ureter during a laparotomy and secured by ligatures. The cannulas were exteriorized through the urogenital tract.

**Urinary cannula construction**

Cannulas were formed from polyethylene\(^1\) tubing of a medium wall thickness. Tubing sizes ranged from an I.D. (inside diameter) of 0.028 inch (0.711 mm) to 0.066 inch (1.676 mm) and an O.D. (outside diameter) of 0.033 inch (0.838 mm) to 0.095 inch (2.413 mm). Various lengths of tubing were cut, ranging from 0.5 m to 0.8 m. The diameter and length of the cannulas were varied according to the size of the animal for which they were intended. Collars were formed by making 8 to 10 spiral windings of suture material approximately 3 to 5 cm from one end of each cannula as illustrated in Figure 2. Braided silk or polyester fiber\(^2\) sutures of sizes #00

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\(^1\) Intramedic, Clay-Adams, Inc., 141 East 25th Street, New York, N.Y.

\(^2\) Mercilene, Johnson and Johnson, 501 George, New Brunswick, N.J.
Figure 1. Semischematic drawing of semi-permanent cannulation of ureters of the bovine species. Ureteral cannulas (C) appear as dark lines traversing the ureters (U) through the neck of the urinary bladder (B), urethra, vagina (V), and exteriorized through the vagina. Ligatures (L) retain the cannulas within the ureters.

Figure 2. Method of forming retaining collar on the cannula by wrapping suture material around polyethylene tubing.
through #000 were used. Prior to surgical procedures cannulas of various sizes were prepared since there seemed to be a poor positive correlation between the sizes of the animal and lumina of the ureters. Moreover, lumen sizes of the two ureters were often different within a single animal. The assortment of cannulas was stored in a sterilizing solution of chlorhexetidine\textsuperscript{1} until needed.

**Preoperative procedure**

Preoperatively, sheep and cattle were fasted 16 to 20 hours and 24 to 36 hours, respectively. In order to insure a relatively high rate of urine production water and electrolytes were administered to fasting animals that voluntarily reduced their water intake. In this event 6 to 12 liters of water and 10 to 20 g of electrolyte mixture\textsuperscript{2} per 100 kg body weight were given via intraruminal injections through a stomach tube 5 to 8 hours preoperatively.

**Surgical procedure in sheep**

Each ewe was given atropine sulfate approximately one hour preanesthetically at a dosage level of approximately 0.05 mg/kg of body weight in aqueous solution via subcutaneous injection. Anaesthesia was induced primarily by injecting an

\textsuperscript{1}Nolvasan, Fort Dodge Laboratories, Fort Dodge, Iowa.

\textsuperscript{2}ViLec-Sol, Vet-A-Mix, Inc., Shenandoah, Iowa.
aqueous solution of a short-acting barbiturate, thiamylal sodium\(^1\) via a jugular vein to effect. A tracheal tube was inserted and secured after which surgical anaesthesia was maintained by inhalation of halothane\(^2\). The animal was strapped in a supine position on a small animal operating table that could be tipped to elevate the caudal extremities. A ventral abdominal surgical area between the xiphoid process and perineum was clipped and scrubbed, following with 3 applications of 70% ethyl alcohol. The surgical area was draped and sterile technique was used in all surgical procedures.

The abdominal cavity was opened by a midline incision, commencing at the anterior border of the pubis and continuing cranially as far as necessary. The mammary glands were divided by a mid-line incision; the superficial veins and anastomosing vessels were ligated. The caudal extremities of the subject were elevated, allowing the viscera to gravitate cranially.

The bladder was expressed of urine contents and opened by a ventral midline incision. A blunt flexible probe was passed through the opened bladder and cranially into the ureter to estimate the curvature and lumen size. A

\(^1\)Surital, Parke, Davis and Company, Detroit, Michigan.

\(^2\)Fluothane, Ayerst Laboratories, Inc., New York, N.Y.
sterilized plastic cannula, the largest that would readily pass the orifice and flexure, was inserted into the ureter. The proximal extremity of the cannula, the one with the collar affixed, was inserted 4 to 8 cm. The cannula was secured by placing a ligature around the ureter just distal to the collar (Figure 3). Synthetic suture material, either size 0.30 mm or 0.40 mm, was used. Occasionally, it was difficult to insert the proximal end of the cannula into the ureter far enough to secure a ligature around the ureter. In this event, most of the cannula proximal to the collar was removed and the cannula was inserted into the ureteral orifice. It was secured by tying a purse-string suture through the mucosa and surrounding the orifice, just distal to the collar, on the inside of the bladder (Figure 4).

The second cannula was implanted in a similar manner. The distal extremities of the cannulas were inserted through the neck of the bladder and out the vulva. The left cannula was marked by notching the end. After the cannulas were checked for patency as indicated by urine flow, the bladder was closed by the Connell suture method. The parietal peritoneum, the muscle layers and skin were each sutured separately using synthetic suture material.

1 Vetafil Bengen, imported by Dr. S. Jackson, 7801 Woodmont Avenue, Washington, D.C.
Figure 3. Drawing of method used in cannulating ureters of the ovine species. Cannula (C) is shown entering the incised bladder (B) at the ureteral orifice and traversing out through the urethra. Note ligature (L) and ureter (U).
Figure 4. Drawing of optional method used in cannulating ureters of the ovine species. Purse-string suture (L) around ureteral orifice retaining cannula (C) in urethra (U). Note incised bladder (B).
Surgical procedure in cattle

Each heifer was given atropine sulfate and anaesthetized in a manner similar to that described for the ewe. The anaesthetized animal was strapped in a supine position to a tilting large animal operating table. The surgical procedure was identical to that used in the ewe, except the procedure differed after the abdominal cavity was opened.

The bladder was not incised since it was practically impossible to enter the ureters through the opened bladder. The ureters were located along the parietal walls of the abdomen and pelvic cavity before they entered the neck of the bladder. The peritoneum was incised 4 to 12 cm from the bladder to expose a ureter. The lumen of the ureter was opened by making a longitudinal incision approximately 5 mm long through its wall. A blunt flexible probe was passed caudally through the incision into the bladder and removed. The distal end of a cannula of the proper size was passed caudally through the ureteral incision into the bladder and out the vulva. Excess tubing proximal to the collar was cut off, leaving about 5 mm of exposed cannula. An assistant grasped the cannula at the vulva and pulled it caudally through the ureteral incision toward the bladder just far enough so it could be moved back up the ureter by use of forceps. When the collar was in a position cranial to the incision, it was secured by ligatures (Figure 5).
Figure 5. Drawing of method used in cannulating ureters of the bovine species. Cannula (C) has been inserted through the incision in the ureter (U) and retained by ligature (L). Note bladder (B) is not incised.
The incisions in the ureter and the peritoneum were sutured closed. A similar procedure was used on the opposite ureter. The remainder of the surgical procedure was similar to that described for the ewe.

Postoperative procedure

Following recovery from anesthesia, surgical patients were observed every 3 to 6 hours for a period of 24 to 36 hours. The patency of the cannulas was evaluated by observing the urine flow. Water and electrolytes were administered orally when necessary to maintain urine flow. Gentle irrigation and aspiration with normal saline solution in a syringe were used to clear occluded cannulas. Rectal body temperature was checked daily for 5 days. Urine flow was normal in 2 to 4 days in all cases. Figure 6 illustrates functioning cannulas. During the postoperative and pre-experimental period animals had access to water, mixed hay and salt ad libitum.
Figure 6. Photograph illustrating functioning ureteral cannulas in a heifer

Figure 7. Photograph illustrating urine collection from ureteral cannulas into polyethylene bottles
Experiment I. Oral Administration of Urea

Experiments on both cattle and sheep were started early in the working day. Experimental animals of both species were restrained in a small portable chute in a standing position. The heads of the animals were held in an adjustable stanchion.

Clinical procedure

Each experimental animal was observed carefully for external signs and clinical response to treatment. Body temperature was periodically determined by use of a rectal thermometer. The time of each observation and each sampling was recorded. Pre-treatment samples were taken to establish normal values before administration of the urea. Sampling was continued at intervals ranging from 10 to 30 minutes, depending on the development of clinical response; the last samples were obtained at the time of death.

Collection of samples

Urine Urine samples were collected from the surgically implanted ureteral cannulas by securing polyethylene bottles to the tail of the animal as illustrated in Figure 7. The urine volume and collection time were recorded. The pH was determined electrometrically\(^1\) as soon as possible.

\(^{1}\)Zeromatic pH Meter with a combination electrode, Beckman Instruments, Inc., 2400 Wright Ave., Richmond, Calif.
usually within 5 minutes, and each urine sample was identified and immediately placed in ice water pending further analysis for ammonia, urea nitrogen, glucose, calcium, magnesium, sodium, potassium, chloride and phosphorus.

Rumen Rumen fluid samples were aspirated into plastic syringes through a 10 gauge 6" needle which had been inserted through the paralumbar fossa into the rumen. The needle was left inserted but capped between sampling procedures. The pH was determined in a manner similar to the procedure used for urine samples. Each rumen sample was retained in a capped 2.5 ml plastic syringe, identified and immediately placed in ice water pending analysis for ammonia.

Blood Venous blood was withdrawn from a jugular vein through an 18 gauge venous catheter\(^1\). Blood samples for pH and gas analysis were aspirated into 2.5 ml plastic syringes in which the inside surfaces had been moistened with an aqueous solution containing 1,000 units of potassium heparin per ml. Any gas was expelled and the tip of the syringe was immediately capped, marked and immersed in ice water. Blood pH determinations\(^2\) and blood gas

\(^1\)Venocath - 16, Abbott Laboratories, North Chicago, Ill.

\(^2\)Corning Model 12 Expanded Scale Research pH Meter with Corning Model R blood pH electrode and Corning Model BS aspiration service/temperature controller, Corning Glass Works, Corning, N.Y.
analysis\textsuperscript{1} were made as soon as possible and always within 20 minutes.

Whole blood samples were collected in vacuum tubes\textsuperscript{2} containing sodium heparin or potassium EDTA. The samples were identified, placed in an ice water bath, and retained until chemical analyses were completed for ammonia, glucose, packed cell volume (PCV), acetylcholinesterase (ΔpH), citrate, pyruvate and lactate.

Serum samples were prepared by first drawing blood into untreated vacuum tubes. The blood samples were identified and held at room temperature or immersed in a cool water bath; after clotting they were centrifuged. The serum samples were separated and refrigerated pending analysis for ammonia, urea nitrogen, glucose, calcium, magnesium, sodium, potassium, chloride, inorganic phosphorus, total protein, glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT).

Administration of urea

A calculated dose of urea, U.S.P.\textsuperscript{3} ranging from 1.5 to 3.0 g per kg body weight was weighed and dissolved in warm

\textsuperscript{1}IL Micro pH Gas Analyzer, Model 113-52, Instrumention Laboratories, Inc., 9 Galen St., Watertown, Mass.

\textsuperscript{2}Vacutainer Tubes, Becton Dickenson & Co., Rutherford, New Jersey.

\textsuperscript{3}Mallinckrodt Chemical Works, St. Louis, Mo.
water to approximate a 10% solution. The solution was 
administered via intraruminal injection through a stomach 
tube and flushed with an equal volume of water. The solution 
was occasionally introduced into the approximate region of 
the reticulum.

Experiment II. Intravenous Administration 
of Ammonium Compounds

Experimental animals, both cattle and sheep, were 
restrained in a portable chute similar to the method used 
in Experiment I.

Collection of blood samples

Blood catheters were placed in both jugular veins. 
Pre-treatment, treatment and post-treatment samples of whole 
blood were drawn from each experimental animal into 5 ml 
syringes which had been treated with heparin solution as 
described previously. Blood was routinely drawn from one 
specific jugular vein and the treatment solution was injected 
into the opposite jugular vein. The samples were variously 
analyzed for ammonia, pH, acetylcholinesterase, pCO₂, pO₂ 
and [HCO₃⁻].

Administration of ammonium compounds

Solutions ranging from 0.2 N to 1.0 N were administered 
continuously via an intravenous drip apparatus from graduated 
bottles (Figure 8). Compounds that were injected as
Figure 8. Photograph illustrating methods of restraint and intravenous infusion of solutions
treatments included ammonium chloride, ammonium hydroxide, ammonium carbamate, ammonium citrate, and ammonium carbonate. Solutions of sodium hydroxide and hydrochloric acid were injected as controls during other experiments. Treatment solutions were given until the animal fell to a recumbent position and failed to regain a standing position.

Normalcy of the solutions was calculated from dried weights of the salts. Normalcy of ammonium hydroxide, sodium hydroxide and hydrochloric acid was calculated by titrating with normal acids or bases. Ammonium solutions were further tested by use of the microdiffusion method described below.

Chemical Analyses and Postclinical Procedures

Blood packed cell volume

Packed cell volume (PCV) was determined in whole blood samples, utilizing microhematocrit 75 mm open-end tubes and a centrifuge\(^1\).

Ammonia

Ammonia was determined in whole blood, serum, urine and rumen fluid by a modification of the microdiffusion method of Conway (1957). In preliminary experiments, \(^1\) International Hematocrit Centrifuge, International Equipment Co., Boston, Mass.
utilizing the Conway method, it was found that levels of ammonia in blood increased with both incubation time and temperature; recovery rates of added ammonium nitrogen from blood and serum were significantly higher than 100% at the completion of an incubation period of 90 minutes at 37°. This was theorized to be due to deamidation of glutamine and asparagine by the saturated solution of potassium carbonate which was used as a liberating alkali in the outer chamber of the Conway dish. The saturated solution of potassium carbonate was replaced with a borate buffer made by mixing 12.4 g powdered boric acid and 100 mEq sodium hydroxide in a total of 1,000 ml water with a final adjusted pH of 11.0. When the borate buffer was used diffusion of ammonia was nearly completed at 30 minutes and did not increase significantly when incubated for periods up to 120 minutes. Routinely, samples were incubated for 90 minutes at 37° and recovery rates from blood and standard solutions ranged from 92% to 100%.

**Urea nitrogen**

Urea nitrogen in serum and urine was determined by the Chaney and Marback modification (Henry, 1965, pp. 266-267). The method involves the colorimetric determination of ammonia released by the enzymatic hydrolysis of urea. Preformed ammonia, or that ammonia determined by the microdiffusion method described above, appears as an error factor in this
method. The reported values are actually sums of ammonia nitrogen and true urea nitrogen.

**Glucose**

Glucose in blood was measured by the Folin and Wu method (Henry, 1965, pp. 646-648). Glucose in serum was measured by the Hyvarian (1962) method of Dubowski (1962). Kronfeld and Medway in Medway et al. (1969, p. 21) state that erythrocytes of ruminants over 3 months of age contain insignificant amounts of glucose. Blood glucose levels were calculated from serum glucose levels by extrapolation from packed cell volume values. Both methods estimate the levels of all reducing sugars, not exclusively glucose, but were considered adequate to measure significant changes during experiments.

Glucose in urine was measured by the Folin and Wu method (Henry, 1965, pp. 655-656) or estimated by the use of Ames Clinistix¹.

**Blood acetylcholinesterase**

Cholinesterase activity was determined in whole blood by Larson's modification of the method of Michel (Henry, 1965, p. 494).

¹Ames Co., Inc., Elkhart, Indiana.
Calcium, magnesium, sodium and potassium

Serum and urine were analyzed for the cations calcium, magnesium, sodium and potassium by use of an atomic absorption spectrophotometer\(^1\) and the methods of Perkin-Elmer (1967).

Chloride

Serum and urine were assayed for chloride by the titrimetric method of Schales and Schales (1941) as described by the Sigma Co. (1965a).

Inorganic phosphorus

Serum and urine were assayed for inorganic phosphorus by the colorimetric method of Fiske and Subbarow (1925).

SGOT and SGPT

The enzymes SGOT and SGPT were determined in serum samples colorimetrically by the Reitman and Frankel (1957) modification as outlined by the Sigma Co. (1965b) and the Dade Co. (1964).

Serum total protein

Serum total protein was quantitated by the colorimetric method of Weichselbaum (1946).

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\(^{1}\)Perkin-Elmer Atomic Absorption Spectrophotometer Model 303, Perkin-Elmer Corp., Norwalk, Conn.
Blood lactate and pyruvate

Lactic acid and pyruvic acid in blood samples were assayed as outlined by the Sigma Co. (1965c and 1965d), utilizing an ultraviolet spectrophotometer\(^1\).

Blood citrate

Citric acid was estimated in blood samples by the colorimetric method of Camp and Farmer (1967).

Total nitrogen

Total nitrogen in urine was assayed by the improved Kjeldahl method of the Association of Official Agricultural Chemists (1965).

The term "colorimetric method", as used in the preceding descriptions of analyses, was used to denote a procedure in which the quantitative measurement was made by a spectrophotometer and within the visual wave length range. Two models were used\(^2,\)\(^3\).

Postmortem Procedure

Necropsy procedure

Necropsies were performed as soon as possible after death. The brain was removed first, always within 40

\(^1\)Spectronic-600 Ultraviolet Spectrophotometer, Bausch & Lomb, Rochester, N.Y.

\(^2\)Coleman Junior II Model 6120 Spectrophotometer, Coleman Instrument Corp., Maywood, Ill.

\(^3\)Spectronic-20, Bausch & Lomb, Rochester, N.Y.
minutes. The brain was incised transversely exposing the ventricles in four locations and immersed in 10% formalin solution. Sections of lungs, liver, kidneys and other tissues, 5 mm to 8 mm thick, were immersed in 10% formalin. Gross lesions were noted.

Histopathologicprocedure

After fixation, tissues were trimmed, dehydrated in ethyl alcohol and imbedded in paraffin in an Autotechnicon\(^1\). Imbedded blocks of tissues were sectioned and mounted on glass slides. The sections were stained with Mayer's hematoxylin and eosin Y.

\(^1\)The Technicon Company, Chauncey, N.Y.
RESULTS

Experiment I. Oral Administration of Urea

Cattle—clinical procedures and observations

A summary of dosages and outcome of trials on cattle is presented in Table 1. Observations were recorded in units of time (minutes) from the time of the intraruminal infusion of urea solution (0 time), until death (100% time).

Bovine—urea—1 Animal b-1 weighed 181.4 kg and was fasted 16 hours and administered 272.2 g urea (1.5 g/kg) as a 10% solution through a stomach tube into the lower esophagus. She exhibited muscle tremors in 18 minutes, with gradually increasing excitement and ataxia. She fell to a sternal recumbency at 34 minutes and shortly thereafter exhibited tonoclonic spasms in lateral recumbency.

At 49 minutes she was administered 2,000 ml of 5% acetic acid into the rumen via a stomach tube. A rapid intravenous injection of 500 ml of 5% glucose with 5% sodium phosphate in a solution adjusted to pH 7.35 was completed. From 32 minutes until death at 62 minutes the animal displayed hyperexcitability with tetanic and tonoclonic spasms.

Blood ammonia (NH$_3$-N) levels increased from 0.12 to > 1.00 mg/100 ml from the control period (0 time) to 62 minutes. Rumen pH varied from 6.82 to 7.00, but after
administration of the acetic acid dropped to 4.10. At 96 minutes, or 34 minutes post mortem, the rumen and reticulum pH were 6.90 and 8.60, respectively. Necropsy revealed only tracheitis.

Table 1. Summary of trials - urea to cattle

<table>
<thead>
<tr>
<th>Trial No. and Animal No.</th>
<th>Dosage of urea</th>
<th>Disposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine - Urea - I (b-1)</td>
<td>1.50g/kg</td>
<td>Expired in 62 min. (given antidotes)</td>
</tr>
<tr>
<td>Bovine - Urea - II (b-2)</td>
<td>1.50g/kg</td>
<td>Survived</td>
</tr>
<tr>
<td>Bovine - Urea - III (b-3)</td>
<td>1.50g/kg + 1.50g/kg</td>
<td>Survived (dosed 170 minutes apart)</td>
</tr>
<tr>
<td>Bovine - Urea - IV (b-3)</td>
<td>3.00g/kg</td>
<td>Expired in 123 min.</td>
</tr>
<tr>
<td>Bovine - Urea - V (b-4)</td>
<td>2.25g/kg</td>
<td>Expired in 44 min.</td>
</tr>
<tr>
<td>Bovine - Urea - VI (b-5)</td>
<td>1.50g/kg</td>
<td>Expired in 57 min.</td>
</tr>
<tr>
<td>Bovine - Urea - VII (b-6)</td>
<td>1.50g/kg</td>
<td>Expired in 62 min.</td>
</tr>
</tbody>
</table>

aData used in statistical analysis.

Bovine - urea - II Animal b-2 had recovered from topical burns incurred several months earlier. Although apparently healthy, she exhibited a state of health best described as "physiological depression". Ureteral cannulas were surgically implanted. Nine days later she weighed 127.0
kg and was dosed with 190.5 g urea (1.5g/kg) intraruminally.

At 121 minutes the animal was uneasy and had increased salivation. The animal survived and samples of blood, urine and rumen contents were collected until 236 minutes. The data were not used in the statistical analysis, but some parameters appeared to be interesting as comparisons with the pooled data presented later.

Some parameters, at 0 time and 236 minutes were, respectively: blood pH, 7.480-7.540; blood NH$_3$-N, 0.06-0.62 mg/100 ml; blood glucose, 71.5-182.3 mg/100 ml; serum urea-N, 12.7-41.5 mg/100 ml; serum P, 6.7-7.1 mg/100 ml; serum total protein, 6.2-6.4%; urine pH, 7.6-8.3; urine NH$_3$-N, 1.0-6.0 mg/100 ml; urine P, 16.2-1.7 mg/100 ml; urine glucose, 0-0; rumen NH$_3$-N, 16.5-89.5 mg/100 ml; body temperature; 102.2-102.5°F. Analysis of serum for Na, K, Cl, Ca, Mg, GOT, GPT, and analysis of urine for Na, K, Cl, Ca, Mg, urea-N and total N failed to reveal apparently significant changes.

**Bovine - urea - III** Animal b-3 was fitted with surgically implanted ureteral cannulas 7 days before the trial. She weighed 119.0 kg and was given 178.3 g urea (1.5 g/kg) intraruminally. At 62 minutes she exhibited restlessness and salivation and dropped to a sternal recumbency; she arose 10 minutes later. At 170 minutes she was given another 178.3 g dose of urea. At 212 minutes restlessness and grinding of
the teeth were noted. The animal survived and sampling was discontinued at 460 minutes.

Some physiological parameters, at 0 time and either 320 or 460 minutes were, respectively: blood pH, 7.443-7.385; PCV, 37-42%; blood NH₃-N, 0.10-0.36 mg/100 ml; blood glucose, 77.1-107.0 mg/100 ml; serum urea-N, 14.2-28.7 g/100 ml; urine pH, 6.675-7.850; urine NH₃-N, 14.80-41.84 mg/100 ml; urine glucose, 0-0; rumen pH, 6.78-7.10; body temperature, 101.6-102.2°F. Analysis of blood and serum for Na, K, Ca, Mg, P, Cl and total protein, and analysis of urine for Cl, urea-N and total N failed to reveal apparently significant changes.

**Bovine - urea - IV** Animal b-3, the same animal that survived Bovine-urea-III test 13 days previously, was used. She weighed 118.9 kg and was dosed with 356.6 g urea (3.0 g/kg) intraruminally. At 37 minutes the animal exhibited increased salivation followed by ataxia, muscle stiffness, tremors and tics of the ears. She went down in a recumbent position at 46 minutes. At 48 minutes she bellowed and exhibited copious, thick salivation and tics of the nose and shoulder muscles; body temperature was 101.6°F.

At 67 minutes the animal had strychnine-like seizures and hyperesthesia. Prior to death tremors and generalized

^1Data used in statistical analysis.
fasciculations were marked. Anuria occurred at 115 minutes and death at 123 minutes with a body temperature of 108.4°F. The rumen pH and NH₃-N levels (in mg/100 mg) were, respectively: 0 min - QNS, 24.0; 37 min - 8.00, 86.0; 67 min - 8.15, 86.8; 103 min - 7.65, 59.2; 123 min - 8.80, 247.4. Total N analysis of the urine revealed 10.60 and 3.98 mg N/ml at 0 and 37 min, respectively. All other physiological parameters were used in the statistical analysis. Only small epicardial hemorrhages were found at necropsy. 

**Bovine - urea - V¹** Animal b-4 was fitted with surgically implanted ureteral cannulas 7 days before the trial. She weighed 95.3 kg and was dosed intraruminally with 214.4 g urea (2.25 g/kg). Eighteen minutes later she became restless and displayed muscle tremors; at 21 minutes the animal dropped to a sternal recumbency, drooling thick saliva and breathing heavily. Blood appeared in the urine from the left ureteral cannula at 39 minutes; anuria occurred at 42 minutes. She continued exhibiting tonoclonic spasms and dyspnea until death at 44 minutes.

Body temperatures increased from 101.8°F at 10 minutes to 104.6°F at death. Rumen pH and NH₃-N levels (in mg/100 ml) were, respectively: 0 min - 6.76, 7.0; 29 min - 7.65, 96.2; 40 min - 8.82, 203.2. Urine total nitrogen (in mg N/ml urine)

¹Data used in statistical analysis.
was: 0 min - 6.66; 22 min - 5.96; 32 min - 3.44; 42 min - 2.74. Glucose determination of the urine revealed negative results. All other parameters were incorporated in the statistical analysis. Necropsy revealed no gross lesions.

Animal b-5 was fitted with ureteral cannulas 8 days before the trial. She weighed 111.1 kg and was given 166.7 g urea (1.5 g/kg) into the approximate region of the reticulum. At 19 minutes she exhibited slight body tremors and tics of the lips and ears. She became ataxic, and went down in a recumbency at 14 minutes, and then struggled to her feet, showing tetany and hyperexcitability. She struggled considerably and went into a recumbency at 28 minutes. Hematuria from the right kidney appeared at 55 minutes, followed shortly by bilateral anuria. She exhibited tonoclonic convulsions and expired at 57 minutes.

Rumen pH and NH$_3$-N levels (in mg/100 ml) were, respectively: 0 min - 6.85, 10.0; 19 min - 7.55, 24.2; 36 min - 7.50, 17.6; 48 min - 7.55, 27.0; 57 min - 7.65, 45.6. At 195 minutes (138 min post mortem) the pH and NH$_3$-N levels were, respectively: rumen - 7.60, 88.0; reticulum - 8.90, 224.6. Urine total N (in g N/ml) were: 0 min - 11.30; 19 min - 8.50; 37 min - 4.67; 52 min - 2.53; 57 min - QNS. Urine glucose levels were (in mg/100 ml): 0 min - 0; 19 min - 0; 37 min - 23.9; 46 min - 39.5; 57 min - 40.3. All other data

Footnote: Data used in statistical analysis.
were used in the statistical analysis. Necropsy revealed a mild pulmonary congestion.

Bovine - urea - VII Animal b-6 had ureteral cannulas surgically implanted 7 days before the trial. She weighed 93 kg and was dosed with 139.5 g urea (1.5 g/kg) intra-ruminally. At 21 minutes she dropped to a sternal recumbency, displaying muscle tremors and tics of the ears and nose. At 26 minutes she struggled to her feet, muscles tetanic, and dropped down again. She showed tonoclonic spasms until respiration ceased at 58 minutes. At 61 minutes respiration resumed momentarily and at 62 minutes the heart contractions ceased. Anuria apparently did not occur.

Body temperature increased from 102°F at 21 minutes to 104.2°F at death. Rumen pH was: 0 min - 7.15; 19 min - 7.50; 35 min - 6.85; 49 min - 6.70; 60 min - 6.73. Rumen NH₃-N levels were not determined, but levels after death were: rumen - 266.6 mg/100 ml; reticulum - 94.0 mg/100 ml; abomasum - 46.6 mg/100 ml. Urine total nitrogen (in mg N/ml urine) were: 0 min - 2.59; 14 min - 1.90; 26 min - 1.96; 43 min - 2.68; 60 min - 2.81. Urine glucose levels were (in mg/100 ml): 0 min - 0; 14 min - 0; 26 min - 0; 43 min - 68.9; 60 min - 416.4. Other data were incorporated in the statistical analysis. Necropsy revealed only subepicardial hemorrhages and a mild tracheitis.

¹Data used in statistical analysis.
Sheep - clinical procedures and observations

A summary of dosages and outcome of trials on sheep is presented in Table 2.

Ovine - urea - I Animal o-1 was the first animal in which ureteral cannulas were surgically implanted, and one cannula became plugged with mucus, but urine flow was re-established. One week later a rubber rumen cannula was also surgically installed. Two weeks later she was fasted for 12 hours before the day of the trial. She weighed 61.7 kg and was administered 92.5 g urea (1.5 g/kg) as a 10% solution through a stomach tube into the rumen.

At 90 minutes she appeared mildly depressed and at 110 minutes she displayed muscle spasms of the ears and lips. The animal fell to a sternal recumbency at 130 minutes. At 160 minutes she was administered 100 ml of 2% acetic acid solution via intravenous drip and 2,000 ml of 5% acetic acid intraruminally. She arose at 170 minutes, but appeared depressed. Samples of blood, urine and rumen contents were collected until 170 minutes, but the data were not used in the statistical analysis. The animal expired unattended the following evening.

Some parameters at 0 time and 150 minutes were, respectively: blood acetylcholinesterase, 0.351-0.409 ΔpH units; blood NH₃-N, 0.19-1.54 mg/100 ml; serum urea-N, 108-252 mg/100 ml; blood glucose, 87-170 mg/100 ml; blood PCV,
<table>
<thead>
<tr>
<th>Trial No. and Animal No.</th>
<th>Dosage of urea</th>
<th>Disposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovine-Urea-I (o-1)</td>
<td>1.50 g/kg</td>
<td>Expired &gt; 360 min</td>
</tr>
<tr>
<td>Ovine-Urea-IIa (o-3)</td>
<td>1.50 g/kg</td>
<td>Expired in 244 min</td>
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<tr>
<td>Ovine-Urea-IIIa (o-5)</td>
<td>1.50 g/kg</td>
<td>Expired in 128 min</td>
</tr>
<tr>
<td>Ovine-Urea-IVa (o-6)</td>
<td>1.69 g/kg</td>
<td>Expired in 159 min</td>
</tr>
<tr>
<td>Ovine-Urea-V (o-8)</td>
<td>2.00 g/kg</td>
<td>Survived (given antidotes)</td>
</tr>
<tr>
<td>Ovine-Urea-VI (o-9)</td>
<td>1.57 g/kg</td>
<td>Expired ca. 360 min. (given antidotes)</td>
</tr>
<tr>
<td>Ovine-Urea-VII (o-10)</td>
<td>1.50 g/kg</td>
<td>Expired in 193 min. (given antidotes)</td>
</tr>
<tr>
<td>Ovine-Urea-VIII (o-11)</td>
<td>1.00 g/kg</td>
<td>Survived (given antidotes)</td>
</tr>
<tr>
<td>Ovine-Urea-IX (o-12)</td>
<td>ca. 1.50</td>
<td>Expired in 222 min</td>
</tr>
<tr>
<td>Ovine-Urea-X (o-15)</td>
<td>3.00 g/kg</td>
<td>Expired &lt; 870 min (given antidote)</td>
</tr>
<tr>
<td>Ovine-Urea-XI (o-17)</td>
<td>3.00 g/kg</td>
<td>Expired &gt; 643 min (given antidote)</td>
</tr>
<tr>
<td>Ovine-Urea-XIIa (o-18)</td>
<td>2.00 g/kg</td>
<td>Expired in 134 min</td>
</tr>
<tr>
<td>Ovine-Urea-XIIIa</td>
<td>2.00 g/kg</td>
<td>Expired in 114 min</td>
</tr>
</tbody>
</table>

aData used in statistical analysis.
30-43%, serum P, 5.8-7.7 mg/100 ml. Other parameters at 0
time and 190 minutes were, respectively: urine NH₃-N, 0.04-
2.46 mg/100 ml; urine glucose, 0-710 mg/100 ml; rumen pH,
6.74-7.55 before acetic acid administration and 4.40 after
acetic acid administration. Intravenous acetic acid de-
creased blood pH from 7.55 to 7.22. Urine output (in ml/min)
and urine urea-N (in mg/100 ml) changed quadratically and
were, respectively: 0 time - 0.40, 105; 10 min - 0.45, 194;
20 min - 0.60, 290; 30 min - 0.80, 194; 40 min - 1.50, 226;
50 min - 1.80, 258; 60 min - 2.40, 226; 75 min - 1.53, 226;
90 min - 1.20, 290; 120 min - 0.70, 290; 150 min - 0.43,
226; 190 min - 0.40, 194.

Analysis of serum for Ca, Mg, Na, K and Cl, and analysis
of urine for pH, Ca, Mg, Na, K, Cl and P failed to reveal
apparently significant changes. Body temperature increased
from 104 to 104.6°F.

Ovine - urea - II Animal o-3 was fitted with surgically
implanted ureteral cannulas 7 days before the trial. She
weighed 51.3 kg and was given 77.0 g urea (1.5 g/kg) intra-
ruminally. At 60 minutes she exhibited mild depression; at
135 minutes she showed tics of the facial muscles. She fell
to a recumbency at 182 minutes and displayed many convulsions
and generalized tonoclonic spasms before expiring at 244
minutes. Regurgitation of ruminal contents occurred several

¹ Data used in statistical analysis.
minutes before death. Urine glucose levels were 0 at 0 time and 75 mg/100 ml at death. All other physiological parameters were used in the statistical analysis. Necropsy revealed the presence of rumen contents in the trachea and bronchial tube.

**Ovine - urea - III** Animal o-5 was fitted with surgically implanted ureteral cannulas 7 days before the trial. She weighed 52.2 kg and was given 78.2 g urea (1.5 g/kg) intraruminally. At 30 minutes she was mildly depressed; at 48 minutes she exhibited minor spasms of the lips and ears plus body trembling. She went down at 70 minutes and displayed tonoclonic convulsions intermittently until death. She regurgitated at 110 minutes and died at 128 minutes.

All physiological data were used in the statistical analysis. Necropsy revealed no gross lesions except some subepicardial petechiae.

**Ovine - urea - IV** Animal o-6 was surgically fitted with ureteral cannulas 7 days before the trial. She weighed 59.0 kg and was given 100.0 g urea (1.69 g/kg) intraruminally. She displayed tics of the ears at 65 minutes and body tremors at 80 minutes. She went down at 107 minutes, regurgitated at 142 minutes and expired at 159 minutes. Necropsy revealed no gross lesion.

Rumen fluid NH₃-N content at death was 346.5 mg/100 ml.

¹Data used in statistical analysis.
The urine was negative for ketone bodies at 0 time, but contained trace amounts at 70 minutes. All other physiological data were used in the statistical analysis.

Ovine - urea - V Animal o-8 was surgically fitted with ureteral cannulas 7 days before the trial. She weighed 60.0 kg and was given 120 g urea (2.0 g/kg) intraruminally. The animal was in excellent physical condition and urine flow was at a high rate. At 245 minutes she appeared moderately depressed. At 321 minutes she became ataxic and was given 1,000 ml of 5% acetic acid intraruminally. During the next 10 minutes she was given an intravenous injection of 15.3 g glutamic acid in aqueous solution with a final adjusted pH of 5.0. Within 30 minutes she was up and survived the trial.

Sampling was discontinued at 275 minutes and some values at 0 time and 275 minutes were, respectively: blood NH₃-N, 0.10-0.75 mg/100 ml; serum urea-N, 20.0-62.0 mg/100 ml; PCV, 33-40%; urine NH₃-N, 0.90-5.33 mg/100 ml; urine urea-N, 840-1310 mg/100 ml; urine pH, 7.1-8.3. Analysis of serum for glucose revealed 68 mg/100 ml at 0 time, 283 at 65 minutes and 74 at 275 minutes. Analysis of serum for Ca, Mg, Na, K, Cl, P and total protein and analysis of urine for Ca, Mg, Na, K and Cl failed to reveal apparently significant changes. Urine flow remained high throughout the trial.
Ovine - urea - VI  Animal o-9 was surgically fitted with ureteral cannulas one week before the trial. She weighed 63.5 kg and was given 100 g urea (1.57 g/kg) intraruminally. Body tremors were evident at 60 minutes and ataxia and dyspnea at 66 minutes. She went down at 75 minutes and urine flow ceased. At 76 minutes intravenous infusion of a solution from the previous trial was initiated and continued until a total of 750 ml, containing 23.6 g glutamic acid, had been administered. A dose of 2,000 ml of 5% acetic acid was given intraruminally. At 123 minutes the animal arose and urine flow resumed. She went back down at 151 minutes and was given an additional 15 g glutamic acid intravenously. This seemed to increase symptoms of toxicosis and the animal expired at ca. 360 minutes.

Sampling was discontinued at 192 minutes. Some of the parameters at 0 time and 192 minutes were, respectively: blood NH$_3$-N, 0.07-1.26 mg/100 ml, serum urea-N, 16.0-35.0 mg/100 ml; blood glucose, 72-180 mg/100 ml; PCV, 33-38%; urine NH$_3$-N, 1.29-22.38 mg/100 ml; urine glucose, neg-trace; urine pH, 7.4-7.7. Urine flow at 0 time was 3.05 ml/min and ceased at 128 minutes; urine flow resumed to 0.69 ml/min at 141 minutes. Analysis of serum for Ca, Mg, Na, K, Cl, P, and total protein and analysis of urine for Ca, Mg, Na, K and Cl failed to reveal apparently significant changes. The rectal temperature was 104.8°F at 184 minutes.
Ovine - urea - VII  This trial was designed to test the effects of antidotes for urea toxicosis which had been proposed as a result of a literature search. A solution intended for intravenous administration was prepared to contain: monosodium glutamate 30.0 g, anhydrous dextrose 50.0 g, MgSO₄·7H₂O·2.5 g, CaCl₂ 2.00 g, pyridoxine hydrochloride 0.10 g, methyl paraben 1.00 g, propyl paraben 0.20 g, distilled water q.s. to 1,000 ml, with a final pH of 6.7. Dosage was calculated at 15 ml/kg body weight. A solution intended for oral administration contained: monosodium glutamate 60.0 g, anhydrous dextrose 75.0 g, glacial acetic acid 60.0 ml, pyridoxine hydrochloride 0.20 g, water q.s. to 1,500 ml, with a final pH of 4.0. Dosage was calculated at 20 to 25 ml/kg body weight.

Animal o-10 weighed 58.1 kg and was given 87.7 g urea (1.5 g/kg) intraruminally. Depression was exhibited at 46 minutes, trembling at 71 minutes and dyspnea and salivation at 77 minutes. At 80 minutes all 1500 ml of the oral solution was administered intraruminally and administration of the intravenous solution was started. After 300 ml had been administered signs of toxicosis diminished. A total of 975 ml was given, but the animal died at 193 minutes after displaying a series of tonoclonic spasms every 5 to 10 seconds. Necropsy revealed a severe frothy bloat. The rumen contents did not have the typical odor of ammonia and the pH was 5.4.
It was concluded that the oral solution aggravated the urea toxicosis.

**Ovine - urea - VIII** This trial was designed to study the effects of various preservatives at 3 different temperature levels on whole blood ammonia determinations. Animal o-11 weighed 39.0 kg and was given 39 g urea (1 g/kg) intraruminally. Only mild depression, followed by trembling and facial tics, was observed. At 120 minutes blood samples were drawn and 860 ml of 5% acetic acid was given intraruminally. The intravenous solution from the preceding trial was administered by slow infusion for a total of 575 ml. The animal recovered. The blood NH$_3$-N level at 120 minutes was 1.25 mg/100 ml.

**Ovine - urea - IX** This trial was staged to document the symptomatology of urea toxicosis with moving pictures. Animal o-12 was given 100 g urea (ca. 1.5 g/kg) intraruminally and left unrestrained in a small room. She exhibited grating of the teeth at 65 minutes and urinated frequently. She expired at 222 minutes after regurgitating ruminal contents. For approximately 60 minutes preceding death she wandered aimlessly, getting up and down, and often pressed her head into a corner of the room. Analysis revealed the following at 0 time and death time, respectively: blood NH$_3$-N, 0.10-5.88 mg/100 ml; serum NH$_3$-N, 0.12-7.00 mg/100 ml;
serum urea-N, 20.8-42.8 mg/100 ml. Levels of NH₃-N in urine and rumen fluid at death were, respectively 74.0 and 132.2 mg/100 ml.

**Ovine - urea- X** Animal o-15 was surgically fitted with ureteral cannulas 8 days before the trial. She weighed 59.9 kg and was given 179.7 g urea (3.0 g/kg) intraruminally. At 178 minutes she showed tics of the lower lips and muzzle. At 297 minutes the last samples were collected and she was given 1,000 ml of 5% acetic acid intraruminally. At 510 minutes she was apparently normal. At 870 minutes she was dead.

Some parameters at 0 time and 297 minutes were, respectively: blood NH₃-N, 0.16-0.44 mg/100 ml; serum urea-N, 24.2-62.7 mg/100 ml; blood pH, 7.37-7.41; serum K, 4.6-5.5 mEq/l; SGPT, 14-7 units; urine output, 0.154-2.658 ml/min; urine pH, 5.62-6.59; urine Na, 52.6-69.6 mEq/l; urine K, 42.8-49.3 mEq/l; urine Cl, 12.6-5.3 mEq/l; urine urea-N, 980-1440 mg/100 ml.

Serum glucose increased from 89 mg/100 ml at 0 time to 144 at 119 min and decreased to 67 mg/min at 297 minutes. Blood inorganic P increased from 12.3 mg/100 ml at 0 time to 14.8 at 75 minutes and decreased to 12.2 at 297 minutes. Rumen pH was 6.80 at 0 time and increased to 8.50 at 120 minutes. After administration of acetic acid the pH decreased to 4.48; after death the pH was 8.86.
Analysis of serum for Ca, Mg, Na, Cl, SGOT, acetylcholinesterase, total protein and PCV and analysis of urine for Ca, Mg, Na, K, Cl and glucose failed to reveal apparently significant changes. The body temperature remained constant at 104.2°F.

Ovine - urea - XI Animal o-17 was surgically fitted with ureteral cannulas 7 days before the trial. She weighed 69.0 kg and was given 207 g urea (3.0 g/kg) intraruminally. She exhibited only slight bloat and depression throughout the trial. At 480 minutes she was given 500 ml of propylene glycol and ca. 6% water via intraruminal injection. Sampling was continued until 495 minutes and she expired unattended after 643 minutes.

Some values at 0 time and at 495 minutes were, respectively: serum urea-N, 27.8-90.9 mg/100 ml; blood glucose, 40.0-56.0 mg/100 ml; serum inorganic P, 5.6-6.6 mg/100 ml; blood pH, 7.39-7.35. Urine output was nil at 0 time and increased to 0.77 ml/min at 295 minutes.

Ovine - urea - XII Animal o-18 weighed 40.9 kg and was given 81.8 g urea (2.0 g/kg) intraruminally. Only blood samples were obtained. At 40 minutes she displayed tics of the ears and at 60 minutes she showed body tremors. Tremors continued until she fell to a recumbency at 93

\[^1\text{Data used in statistical analysis.}\]
minutes. She expired at 134 minutes after much struggling, profuse salivation and tonoclonic spasms. Rumen ammonia levels at 2 different locations after death were 153.5 and 241.5 mg/100 ml. All other data were used in the statistical analysis.

Ovine - urea- XIII

Animal o-19 weighed 41.5 kg and was given 83 g urea (2.0 g/kg) intraruminally. Her treatment and response were similar to o-18 in the previous trial and she expired in 114 minutes. Composite analysis of 3 rumen fluid samples revealed 298.0 mg NH$_3$-N/100 ml. All other data were used in the statistical analysis.

**Statistical analysis of data**

Data for each parameter were plotted in a regression form, utilizing an IBM 360/65 computer and a programming system (Chamberlain and Jowett, 1968). Observations for each parameter were plotted as dependent variables on the y-axis and regressed on time, which was plotted on the x-axis.

During preliminary or trial plots time was plotted as (1) actual time in minutes, (2) log time, (3) square root of time and (4) percent time to death. There were considerable differences between the death times of individual animals.

$^1$Data used in statistical analysis.
animals and the data did not fit the first three modes of plotting. The last method was used to facilitate a regression analysis. The x-axis, or time, was thereby standardized, ranging from 0% to 100%. The pretreatment or control observation for each animal was plotted at 0 time and the observation at death was plotted at 100% time. All other observations were interpolated at percentage points between 0 and 100.

Regression analyses were made on all data for which quantitative observations were secured. An adjustment was made for individuality of animals, with an appropriate loss of degrees of freedom. Analyses were made for linear (lin) regression of y on x and followed by quadratic (quad) regression of y on x in all cases. Blood and serum parameters in cattle trials were further estimated, using PCV as a covariant (cov) after adjusting for linear and quadratic effects. This was to test the effect of changes in hemoconcentration, as reflected by PCV values.

The computer regression plots appear in the Appendix section. Graphs for cattle and sheep appear in Appendix A and Appendix B, respectively. A single common regression line appears on each graph in Appendix A as a series of dashes (-); each animal is represented by a separate symbol [(•), ('), (•*), (+)]. On each graph illustrating blood and serum values the common regression line (y1) is plotted as a
series of plus signs (+). The regression line \((y_2)\) with the covariant, PCV, is plotted as a series of asterisks (*) and has been adjusted so that the a-intercept at 0 time has the same value as \(y_1\) at the a-intercept; each observation for all animals is plotted as a dot (•). Graphs in Appendix B contain a single common regression line represented by a series of asterisks (*); each observation for all animals is represented by a dot (•).

Some graphs in both appendices contain numbers rather than symbols. In the event there is more than one observation or regression line symbol plotted at the same point on the graph the identities of the plots are lost. The plots then appear as an arabic numeral representing the number of simultaneous plot points.

Each plotted regression line represents a smooth curve of the least-square fitting of all observations of all animals. It is the best common regression line that can be predicted from the available information. With few observations the line may not be a good estimator as indicated by a lack of significance (ns). Indeed, a lack of significance can be due to (1) a lack of significant change of the parameter from 0 time to death or (2) a small number of observations with a high standard deviation. When curvilinear effects are present the curve-fitting may exaggerate by extrapolation so that values at either end of the regression line may not
accurately represent true mean values.

An analysis of variance table was printed for each plot and contained a sum of squares, degree of freedom, mean square, standard deviation and F value for each variable. Summaries of the statistical analyses of data from cattle and sheep appear in Table 3 and Table 4, respectively. The F values were tested for significance levels by the use of values obtained from Snedecor and Cochran (1965).

**Physiopathologic observations: blood and serum**

**Packed cell volume**
Blood packed cell volume (PCV) of cattle (Graph 1) increased from 32.7 to 38.0% in a linear (lin) fashion (lin P<.005) from the control observation (0 time) to 100% time (death). It increased in sheep (Graph 36) from 29.3 to 33.3% (lin P<.250). The regression line was used as a covariant (cov) on each regression analysis of blood and serum parameters of cattle.

**Blood and serum ammonia**
Blood ammonia nitrogen ($\text{NH}_3$-$\text{N}$) values of cattle (Graph 2) increased from 0.10 to 3.80 mg/100 ml (lin P<.005, cov P<.100). Blood $\text{NH}_3$-$\text{N}$ levels of sheep (Graph 37) increased in a linear and quadratic (quad) fashion (lin P<.005, quad P<.005) from 0.431 to 4.139 mg/100 ml. Due to a curvilinear computermetric extrapolation the former value did not accurately represent the true mean control value of 0.170 mg/100 ml.
Table 3. Statistical results of lethal doses of urea to cattle

<table>
<thead>
<tr>
<th>Physiological Parameter</th>
<th>( T_L ) ( ^a ) F Value</th>
<th>DF</th>
<th>SD ( ^e )</th>
<th>( T_Q ) ( ^b ) F Value</th>
<th>DF</th>
<th>SD ( ^e )</th>
<th>PCV ( ^c ) F Value</th>
<th>DF</th>
<th>SD ( ^e )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood PCV</td>
<td>34.28**</td>
<td>14</td>
<td>1.44</td>
<td>0.02</td>
<td>13</td>
<td>1.49</td>
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<td>-</td>
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<tr>
<td>Blood Ammonia</td>
<td>151.05**</td>
<td>14</td>
<td>0.473</td>
<td>0.43</td>
<td>13</td>
<td>0.483</td>
<td>4.25</td>
<td>12</td>
<td>0.432</td>
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<tr>
<td>Serum Ammonia</td>
<td>420.11**</td>
<td>9</td>
<td>0.227</td>
<td>0.84</td>
<td>8</td>
<td>0.230</td>
<td>1.79</td>
<td>7</td>
<td>0.219</td>
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<tr>
<td>Serum Urea-N</td>
<td>35.78**</td>
<td>14</td>
<td>6.31</td>
<td>10.81**</td>
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<td>4.84</td>
<td>2.95</td>
<td>12</td>
<td>4.51</td>
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<tr>
<td>Blood Glucose</td>
<td>98.97**</td>
<td>14</td>
<td>26.2</td>
<td>0.56</td>
<td>13</td>
<td>26.6</td>
<td>0.37</td>
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<td>Blood pH ( ^{+} )</td>
<td>41.13**</td>
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<td>40.10**</td>
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<td>Blood ([H^+])</td>
<td>29.30**</td>
<td>14</td>
<td>2.11</td>
<td>46.15**</td>
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<td>1.03</td>
<td>1.75</td>
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<td>Blood Cholinesterase</td>
<td>57.87**</td>
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<td>0.026</td>
<td>11.10**</td>
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<td>Serum Calcium</td>
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<td>13</td>
<td>0.248</td>
<td>14.00**</td>
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<td>Serum Magnesium</td>
<td>38.01**</td>
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<td>4.82*</td>
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<tr>
<td>Serum Sodium</td>
<td>28.11**</td>
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<td>18.47**</td>
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<td>Serum Potassium</td>
<td>46.18**</td>
<td>14</td>
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<td>20.52**</td>
<td>13</td>
<td>0.535</td>
<td>2.50</td>
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</table>

\( ^a \) Linear regression on time (x).
\( ^b \) Quadratic regression on time (x).
\( ^c \) PCV Packed cell volume (covariant).
\( ^d \) Degrees of freedom (denominator only, all numerators have a value of 1).
\( ^e \) Standard deviation (s).

*Significance level < .05.
**Significance level < .01.
\(^\dagger \times 10^{-5}\).
| Physiological Parameter | $T_L^a$ | | | $T_Q^b$ | | | PCV$_c$ | |
|-------------------------|--------|----------|--------|-------|--------|-------|--------|
|                         | F Value | DF$^d$ | SD$^e$ | F Value | DF$^d$ | SD$^e$ | F Value | DF$^d$ | SD$^e$ |
| Serum Chloride          | 0.00    | 14      | 1.08   | 0.01    | 13      | 1.12   | 4.63    | 12      | 0.99   |
| Serum In. Phos.         | 16.06** | 14      | 0.780  | 21.60** | 13      | 0.496  | 9.34**  | 12      | 0.387  |
| Serum GOT               | 39.46** | 14      | 10.2   | 0.01    | 13      | 10.6   | 7.34*   | 12      | 8.7    |
| Serum GPT               | 34.12** | 14      | 4.91   | 0.39    | 13      | 5.02   | 0.27    | 12      | 5.17   |
| Serum Tot. Protein      | 22.77** | 14      | 0.224  | 2.26    | 13      | 0.215  | 13.04** | 12      | 0.155  |
| Body Temperature        | 11.68*  | 5       | 1.38   | 0.25    | 4       | 1.50   |         |         |        |
| Urine Output            | 1.13    | 19      | 6.77   | 8.45**  | 18      | 5.73   |         |         |        |
| Urine Ammonia           | 18.17** | 14      | 5.41   | 4.26    | 13      | 4.88   |         |         |        |
| Urine Ammonia/min       | 2.74    | 14      | 1.100† | 0.68    | 13      | 1.113++|         |         |        |
| Urine-Urea-N            | 12.17** | 18      | 0.178++| 0.70    | 17      | 0.179++|         |         |        |
| Urine-Urea-N/min        | 0.93    | 18      | 15.20  | 16.72** | 17      | 11.10  |         |         |        |
| Urine [H$^+$]           | 2.86    | 18      | 5.61†  | 0.01    | 17      | 5.77†  |         |         |        |
| Urine [H$^+$]/min       | 1.39    | 18      | 14.90‡ | 0.00    | 17      | 15.33‡ |         |         |        |
| Urine Calcium           | 0.06    | 18      | 0.275  | 1.57    | 17      | 0.271  |         |         |        |
| Urine Calcium/min       | 4.67*   | 18      | 12.4   | 0.90    | 17      | 12.4   |         |         |        |
| Urine Magnesium         | 0.22    | 18      | 1.30   | 0.47    | 17      | 1.31   |         |         |        |
| Urine Magnesium/min     | 3.19    | 18      | 33.3   | 6.27*   | 17      | 29.3   |         |         |        |
| Urine Sodium            | 17.68** | 18      | 9.63   | 0.64    | 17      | 9.73   |         |         |        |
| Urine Sodium/min        | 3.48    | 18      | 0.418‡ | 3.78    | 17      | 0.389‡ |         |         |        |
| Urine Potassium         | 11.33** | 18      | 1.80   | 0.03    | 17      | 1.85   |         |         |        |
| Urine Potassium/min     | 2.65    | 18      | 0.255‡ | 6.51*   | 17      | 0.223‡ |         |         |        |
| Urine Chloride          | 6.39*   | 18      | 10.4   | 0.49    | 17      | 10.5   |         |         |        |
| Urine Chloride/min      | 6.13*   | 18      | 0.358‡ | 3.48    | 17      | 0.335‡ |         |         |        |

$^\dagger\dagger x 10^3$.

$^\dagger x 10^{-3}$. 
Table 4. Statistical results of lethal doses of urea to sheep

<table>
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<tr>
<th>Physiological Parameter</th>
<th>( \text{T_L}^a )</th>
<th>( \text{T_Q}^b )</th>
<th>( \text{PCV}^c )</th>
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<tbody>
<tr>
<td></td>
<td>Value</td>
<td>DF</td>
<td>SD</td>
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<tr>
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<td>3.13</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Serum GOT</td>
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\( ^a \) Linear regression on time (x).

\( ^b \) Quadratic regression on time (x).

\( ^c \) PCV Packed cell volume (covariant) not used.

\( ^d \) DF Degrees of freedom (denominator only, all numerators have a value of 1).

\( ^e \) SD Standard deviation (s).

* Significance level < .05.

** Significance level < .01.
Table 4 (Continued)

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<td>Blood pO$_2$</td>
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<td>Urine Phosphorus</td>
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<td>Rumen pH</td>
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</table>
Serum ammonia levels of cattle (Graph 3) increased from 0.120 to 3.414 mg NH$_3$-N/100 ml (lin P<.005, cov P<.250). Ammonia levels in serum were ca. 20% higher in the three animals tested than levels in blood. Animal b-3 in Bovine-urea-IV trial had very high blood NH$_3$-N levels, but the serum NH$_3$-N levels were not determined. The common regression line of serum ammonia would undoubtedly have been steeper if data from animal b-3 had been included.

Serum urea nitrogen  Serum urea-N levels of cattle (Graph 4) ranged from 14.6 mg/100 ml at 0 time to a peak of 33.5 at 75% time and decreased to 30.8 at death (lin P<.005, quad P<.010, cov P<.250). Levels in sheep (Graph 38) increased linearly (lin P<.005) from 31.5 to 92.7 mg/100 ml. One animal ranged from 53 to 157 and undoubtedly raised the regression line higher than normally expected.

Blood glucose  Blood glucose values of cattle (Graph 5) increased from 77.9 to 240.4 mg/100 ml (lin P<.005). In sheep (Graph 39) values increased curvilinearly (lin P<.005, quad P<.005) from 79.6 to 214.0 mg/100 ml.

Blood pH and hydrogen ion concentration  Cattle and sheep blood pH values decreased curvilinearly. Cattle blood pH (Graph 6) ranged from 7.438 at 0 time to 7.477 at 15 to 25% time and decreased to 6.962 at death (lin P<.005, quad P<.005). Cattle hydrogen ion concentration (Graph 7) is an
inverted image of the pH graph and values ranged from $3.65 \times 10^{-5}$ mEq/l to $2.90 \times 10^{-5}$ mEq/l to $11.30 \times 10^{-5}$ mEq/l (lin $P<.005$, quad $P<.005$). Sheep blood pH (Graph 40) ranged from 7.385 at 0 time to 7.468 at 35% time and 7.166 at death (lin $P<.005$, quad $P<.005$).

**Blood acetylcholinesterase**  
Cattle blood acetylcholinesterase values (Graph 8) increased linearly from 0.628 to 0.747 ΔpH units (lin $P<.005$, cov $P<.010$). Sheep values (Graph 41) changed curvilinearly from 0.343 at 0 time to 0.336 at 20 to 35% time to 0.393 at death (lin $P<.100$, quad $P<.250$).

**Serum calcium**  
Serum Ca levels of cattle and sheep increased curvilinearly. Cattle serum Ca values (Graph 9) increased from 4.94 to 5.70 mEq/l (lin $P<.005$, quad $P<.100$, cov $P<.005$). Sheep serum Ca values (Graph 42) ranged from 5.06 mEq/l at 0 time to 4.92 at 20 to 40% time and 5.54 at death (lin $P<.005$, quad $P<.005$).

**Serum magnesium**  
Serum Mg levels of cattle and sheep increased in a curvilinear manner similar to serum calcium. Cattle serum Mg values (Graph 10) increased from 1.82 to 2.22 mEq/l (lin $P<.005$, quad $P<.005$, cov $P<.050$). Sheep serum magnesium values (Graph 43) ranged from 2.04 mEq/l at 0 time to 1.89 at 25 to 40% time and 2.43 at death (lin $P<.005$, quad $P<.005$).
Serum sodium

Serum Na values of cattle (Graph 11) decreased and increased in a curvilinear manner. Values were 142.8 mEq/l at 0 time, 141.8 at 25 and 30% time and 153.8 at death (lin P<.005, quad P<.005). Sheep serum Na values (Graph 44) increased linearly from 143.1 to 147.3 mEq/l (lin P<.100).

Serum potassium

Serum K levels of cattle and sheep decreased and increased in a curvilinear manner. Cattle serum potassium (Graph 12) ranged from 5.45 mEq/l at 0 time to 5.22 at 15 and 20% time and 9.04 at death (lin P<.005, quad P<.005, cov P<.250). Sheep serum K levels (Graph 45) ranged from 6.30 mEq/l at 0 time to 5.52 at 15 to 40% time and 11.36 at death (lin P<.005, quad P<.005).

Serum chloride

Serum Cl levels of cattle (Graph 13) did not change significantly but dropped with the PCV co-variant (cov P<.100). Sheep serum Cl levels (Graph 46) decreased from 132.2 to 113.6 mEq/l (lin P<.025).

Serum inorganic phosphorus

Serum inorganic P levels decreased and increased in a curvilinear manner. Cattle serum P levels (Graph 14) ranged from 4.85 mg/100 ml at 0 time to 4.46 at 20 to 40% time and 6.67 at death (lin P<.005, quad P<.005, cov P<.010). Sheep serum P levels (Graph 47) ranged from 5.82 mg/100 ml at 0 time to 4.80 at 30 to 50% time and 7.30 at death (lin P<.005, quad P<.005).
Serum glutamic oxaloacetic transaminase (SGOT) activity of cattle (Graph 15) increased linearly from 87.6 to 127.6 units (lin P<.005, cov P<.025). SGOT activity of sheep (Graph 48) changed quadratically from 130 to 174 units (quad P<.050).

Serum glutamic pyruvic transaminase (SGPT) activity of cattle (Graph 16) increased linearly from 15.86 to 33.84 units (lin P<.005). SGPT activity of sheep (Graph 49) changed quadratically, ranging from 40.2 units at 0 time to 22.4 at 50 to 60% time and 34.0 at death (quad P<.250).

Serum total protein Serum protein of cattle (Graph 17) increased linearly from 6.43 to 7.25% (lin P<.005, quad P<.250, cov P<.005). Serum protein of sheep (Graph 50) changed quadratically, ranging from 6.62 at 0 time to 6.14 at 35 to 50% time and 6.96 at death (quad P<.005).

Blood pCO₂ Blood pCO₂ of sheep (Graph 51) displayed a curvilinear change, ranging from 38.9 mm mercury at 0 time to 30.6 at 35% time and 60.2 at death (lin P<.050, quad P<.005).

Blood pO₂ Blood pO₂ of sheep (Graph 52) changed curvilinearly, ranging from 41.5 mm mercury at 0 time to 42.4 at 10 to 30% time and 18.4 at death (lin P<.050, quad P<.250).
Blood bicarbonate  Blood bicarbonate levels of sheep (Graph 53) decreased curvilinearly, ranging from 23.5 mEq/l at 0 time to 24.6 at 15 to 40% time and 15.6 at death (lin P<.025, quad P<.100).

Blood lactic acid  Blood lactate values of sheep (Graph 54) increased linearly from 43.4 to 90.6 mg/100 ml (lin P<.005).

Blood pyruvic acid  Blood pyruvate values of sheep (Graph 55) increased in a curvilinear manner from 1.55 mg/100 ml at 0 time to 1.99 at 70 and 75% time to 1.81 at death (lin P<.250, quad P<.250).

Blood citric acid  Blood citrate values of sheep (Graph 56) decreased in a curvilinear manner, ranging from 3.38 mg/100 ml at 0 time to 1.81 at 60% time and 2.86 at death (quad P<.250).

Physiopathologic observations: urine

Urine production  Urine output from the ureteral cannulas of cattle and sheep increased and decreased in a quadratic manner. Urine output of cattle (Graph 18) ranged from 2.92 ml/min at 0 time to 14.03 at 50 to 65% time and 7.36 at death (quad P<.010). Urine output of sheep (Graph 57) ranged from 0.51 ml/min at 0 time to 2.02 at 40 to 50% time and 0.08 at death (lin P<.250, quad P<.025).
Urine ammonia  Urine NH₃ levels of cattle (Graph 19) increased from 2.564 to 17.936 mg NH₃-N/100 ml, or 1.830 to 12.805 mmoles/l (lin P<.005, quad P<.100). Urine NH₃ of sheep (Graph 58) increased in a non-significant curvilinear manner.

Urine NH₃ excretion rates of cattle (Graph 20) increased curvilinearly, ranging from 117.2 μg NH₃-N/min at 0 time to 1,148.8 at 60% and 1,055.0 at death (lin P<.250).

Urine urea nitrogen  Urine urea-N levels of cattle (Graph 21) decreased from 626 to 276 mg/100 ml (lin P<.005).

Urine urea-N excretion rates of cattle (Graph 22) changed in a quadratic manner similar to urine production rate. It ranged from 15.61 mg/min at 0 time to 44.10 at 50 to 60% time to 23.92 at death (quad P<.005).

Urine pH and hydrogen ion concentration  Urine hydrogen ion concentration of cattle (Graph 23) increased (lin P<.250) and urine pH decreased. Urine pH of sheep (Graph 59) increased from 7.42 to 8.50 (lin P<.025).

Urine hydrogen ion excretion rates of cattle (Graph 24) increased linearly from 0.33 x 10⁻⁵ to 5.53 x 10⁻⁵ μEq/l (lin P<.250).

Urine calcium  Urine Ca levels of cattle (Graph 25) changed quadratically from 1.72 mEq/l at 0 time to 1.46 at 50% time and 1.63 at death (lin P<.250). Urine Ca levels of
sheep (Graph 60) did not change significantly.

Urine Ca excretion rates of cattle (Graph 26) increased from 6.62 to 25.15 μEq/min (lin P<.05).

**Urine magnesium** Urine Mg levels of cattle (Graph 27) did not change significantly. Urine Mg levels of sheep (Graph 61) increased linearly from 3.18 to 5.81 mEq/l (lin P<.025).

Urine Mg excretion rates of cattle (Graph 28) changed curvilinearly, ranging from 12.2 μEq/min at 0 time to 76.6 at 55 to 65% time and 51.4 at death (lin P<.100, quad P<.025).

**Urine sodium** Urine Na values of cattle (Graph 29) increased linearly from 40.13 to 65.25 mEq/l (lin P<.005). Urine Na values of sheep (Graph 62) changed curvilinearly, ranging from 3.90 mEq/l at 0 time to 26.42 at 60 and 65% time and 17.41 at death (quad P<.250).

Urine Na excretion rates of cattle (Graph 30) changed curvilinearly, ranging from 164.3 μEq/min at 0 time to 837.6 at 55 and 60% time and 613.2 at death (lin P<.100, quad P<.100).

**Urine potassium** Urine K values of cattle (Graph 31) increased linearly from 38.0 to 41.7 mEq/l (lin P<.005). Urine K levels of sheep (Graph 63) changed curvilinearly, ranging from 75.3 mEq/l at 0 time to 114.2 at 50 to 80% time to 104.4 at death (lin P<.100, quad P<.100).

Urine K excretion rates of cattle (Graph 32) changed
curvilinearly, ranging from 149.8 μEq/min at 0 time to 583.6 at 55 to 65% time to 377.0 at death (lin P<.250, quad P<.0250).

**Urine chloride** Urine Cl values of cattle (Graph 33) increased linearly from 49.3 to 65.4 mEq/l (lin P<.025, quad P<.250). Urine Cl levels of sheep (Graph 64) did not change significantly.

Urine Cl excretion rates of cattle (Graph 34) changed in a curvilinear manner, ranging from 153.8 μEq/min at 0 time to 804.0 at 65 to 75% time and 685.8 at death (lin P<.025, quad P<.100).

**Urine phosphorus** Urine P levels of sheep (Graph 65) did not change significantly.

**Physiopathologic observations: body temperature and rumen pH**

**Body temperature** Body temperatures of cattle (Graph 25) increased from 102.4 to 106.4°F (lin P<.025).

**Rumen pH** Rumen pH of sheep (Graph 66) increased curvilinearly from 7.02 to 8.50 (lin P<.005, quad P<.050).

**Histopathology**

Histopathologic examination of organs from both species failed to reveal the extensive changes reported by some previous investigators. The most consistent changes were observed in the kidneys. Congestion was frequently observed, especially in the medullary region (Figures 9 and 10).
Initial stages of nephrosis were present in some areas with dilated tubules and swollen epithelial cells (Figure 11). The glomeruli often were congested and swollen; others often contained an unidentified amorphous or hyaline material within Bowman's capsule (Figures 12 and 13).

The liver appeared normal or moderately congested (Figure 14). The lungs usually contained some areas of congestion (Figure 15). The spleen was moderately congested. Some areas of the brain and brain stem frequently contained foci of extravascular erythrocytes highly suggestive of antemortem hemorrhage (Figure 16).
Figure 9. Section of congested ovine kidney with arrows pointing to dilated blood vessels. x 150

Figure 10. Section of congested bovine kidney with arrow pointing to blood vessel dilated with blood cells. x 375
Figure 11. Section of ovine kidney with dilated tubules and swollen epithelial cells suggestive of early nephrosis. x 925

Figure 12. Section of ovine kidney with swollen glomerulus and dilated tubules. x 375
Figure 13. Section of bovine kidney with hyaline-like droplets in the Bowman's capsule. x 375

Figure 14. Section of ovine liver with mild congestion. x 375
Figure 15. Section of ovine lung with congestion. x 375

Figure 16. Section of ovine cerebral cortex with extravascular collection of erythrocytes. x 375
Experiment II. Intravenous Administration of Ammonium Compounds

Summaries of experiments on cattle and sheep are presented in Table 5 and 6, respectively.

**Cattle - observations**

Animals b-7 and b-8 weighed 110 kg and 93 kg, respectively. They were given 1.0 N NaOH solutions intravenously until each animal appeared to display increased but shallow respiration rates and symptoms of alkalosis. Animal b-7 and b-8 received 858 and 792 mEq, respectively, within 40 minutes.

Five days later b-7 and b-8 were infused with 0.5 N solutions of NH\(_4\)OH and NH\(_4\)Cl, respectively. Animal b-7 showed hypersensitivity and fell to a recumbency at 45 minutes after 368 mEq of NH\(_4\)OH had been given. She was apparently normal at 75 minutes. Animal b-8 displayed slight muscle stiffness and tremors after 150 mEq of NH\(_4\)Cl had been given at 15 minutes. The symptoms increased in intensity until she went down at 31 minutes after 338 mEq NH\(_4\)Cl had been administered. She arose and was very hyperexcitable and tetanic. At 61 minutes she was still somewhat ataxic.

Two days later the previous experiment was replicated. Animal b-7 withstood 346 mEq NH\(_4\)Cl in 31 minutes and b-8 218 mEq NH\(_4\)OH in 32 minutes. Both animals displayed hyperexcitability and tremors before going down.
Table 5. Summary of trials in Experiment II: intravenous administration of compounds to cattle

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<th>mEq Infused</th>
<th>Blood pH</th>
<th>Blood Cholinesterase ΔpH</th>
<th>Blood NH₃ (mg/100 ml)</th>
<th>pCO₂ (mm Hg)</th>
<th>PO₂ (mm Hg)</th>
<th>[HCO₃⁻] (mEq/l)</th>
<th>Blood Citrate</th>
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<tr>
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ᵇ112 mMoles total dose equivalent to 224 mEq ammonia.
Table 6 (Continued)

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<th>Animal No.</th>
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<th>mEq Infused</th>
<th>Blood pH</th>
<th>Cholinesterase ΔpH</th>
<th>Blood NH₃ (mg/100 ml)</th>
<th>pCO₂</th>
<th>pO₂</th>
<th>[HCO₃⁻] (mEq/l)</th>
<th>Blood Citrate (mg/100 ml)</th>
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\(^{119.5}\) mMoles total dose equivalent to 239 mEq ammonia.
Three weeks later b-7 was given 165 mEq ammonium citrate as a 1 N solution within a period of 9 minutes before she went down, displaying tetany, and struggling.

Animal b-9 weighed 112 kg and received 173 mEq ammonium citrate as a 0.5 N solution before going down at 12 minutes after displaying considerable struggling and excitement.

Two days later b-9 withstood 330 mEq NH₄CO₃ as a 0.5 N solution before going down at 30 minutes after a period of hyperexcitability.

Three weeks later b-9 was infused with 473 mEq NH₄OH, as a 0.86 N solution, in 44 minutes before the animal went down. The samples were used to make blood gas analysis.

**Sheep - observations**

Animal o-13 was administered 1,000 ml of 0.2 N NaOH (200 mEq) intravenously in a period of 42 minutes with no apparent reactions. Fourteen days later she was given 0.2 N NH₄OH until she fell to a recumbency at 23 minutes after 102 mEq had been administered. During the same day animal o-14 was given 1,000 ml of 0.4 N NaOH (total of 400 mEq) within 34 minutes. She displayed rapid, shallow respirations with dyspnic expirations, which were concluded to be symptoms of alkalosis.

One day after the preceding trials o-13 and o-14 were infused with 0.2 N solutions of NH₄Cl and NH₄OH, respectively. Animal o-13 displayed mild tremors and
excitement after 80 mEq of NH$_4$Cl had been administered within 10 minutes and went down after a total of 101 mEq NH$_4$Cl had been given within 20 minutes. She salivated profusely and remained in a deep coma with irregular breathing at 30 minutes. At 40 minutes she was up, breathing rapidly. Animal o-14 displayed increasing tremors, salivation and excitement and went down in tonoclonic spasms at 25 minutes after 130 mEq of NH$_4$OH had been administered. She was up at 35 minutes.

Five days after the preceding trials o-13 and o-14 were infused with 0.2 N solutions of NH$_4$OH and NH$_4$Cl, respectively. Animal o-13 displayed trembling after 50 mEq and 75 mEq of NH$_4$OH had been infused in 17 and 23 minutes, respectively. She became ataxic after 90 mEq had been given in 25 minutes and thrashed in excitement and fell to a recumbency at 33 minutes after 112 mEq NH$_4$OH had been infused. She displayed tics of the ears and nose and tonoclonic convulsions. At 62.5 minutes she appeared normal. Animal o-14 displayed hyperexcitability, tics of the lips and general trembling after 80 mEq of NH$_4$Cl had been administered at 12.5 minutes. She then displayed ataxia and tonoclonic spasms and went down at 20 minutes after 109 NH$_4$Cl mEq had been infused. At 50 minutes she appeared normal.

Two days after the preceding trials o-13 and o-14 were given solutions of 0.2 N NH$_4$Cl and 0.2 M ammonium carbamate
(equivalent to 0.4 M ammonia). Animal o-13 displayed slight
trembling at 20 minutes after 95 mEq NH₄Cl had been infused.
At 22 minutes she displayed chewing and tics of the nose;
at 23 minutes hypersensitivity; at 24 minutes ataxia and
weaving. At 25 minutes she went down after 112.5 mEq NH₄Cl
had been administered. She was apparently normal at 55
minutes. Animal o-14 showed slight trembling at 14 minutes
after 75 mmoles ammonium carbamate had been administered and
tics of the lips at 17 minutes after 85 mmoles. At 20
minutes she displayed ataxia and at 24 minutes she went down
after 119.5 mmoles ammonium carbamate (equivalent to 239
mmoles ammonia) had been given. She was essentially normal
at 54 minutes.

Animal o-15 weighed 68.2 kg, was given 0.5 N ammonium
citrate and displayed tremors and struggling before she went
down at 12 minutes after having received 125 mEq ammonium
citrate.

Two days later o-15 withstood 120 mEq of NH₄CO₃, as a
0.5 N solution, before going down at 9 minutes after dis­
playing tics of the nose, generalized tremors and ataxia.

One week later o-14 withstood 125 mEq NH₄OH before going
down at 8 minutes. A 10% solution of a ketoglutaric acid
was given intravenously until the animal arose. Both solu­
tions were given concurrently and after the animal had received
500 ml a ketoglutaric acid she had received 215 mEq NH₄OH in
42 minutes.

Animal o-16 was given 227 mEq NH₄Cl, as a 0.35 N solution in 34 minutes before going down. The data were used for blood gas analyses.

Three weeks later o-16 was given 100 mEq NH₄OH, as a 0.5 N solution, before going down at 8 minutes. The data were used for blood gas analysis.

Animal o-8 was given a solution of ammonium carbamate 8 days after surviving an oral dose of urea. The purpose of the experiment was to assay cholinesterase activity change. A total dose of 112 mmoles (equivalent to 224 mmoles ammonia) was given as 5 to 10 ml doses of a 25% solution within a total period of 27.5 minutes.
DISCUSSION

Physiopathologic Observations

The results of this study agree with those of other investigators who state that the development of symptoms depends on blood ammonia levels. Levels increased linearly to death (Graphs 2 and 37). Levels in excess of 1 mg NH$_3$-N/100 ml were associated with tics and muscle spasms in sheep and tetany and excitement in cattle.

Blood NH$_3$-N levels in cattle at death in Experiment I ranged from 3.10 to 5.54 mg/100 ml with a mean value of 3.77 mg/100 ml (2.69 mmoles/l). Blood NH$_3$-N levels in sheep at death ranged from 3.28 to 6.24 mg/100 ml with a mean value of 4.50 mg/100 ml (3.21 mmoles/l). The higher values were usually associated with longer death times.

In Experiment II it was noted that practically all animals became ataxic and fell when blood NH$_3$-N levels ranged from 1.0 to 2.0 mg/100 ml. It appeared that animals receiving ammonium chloride withstood higher blood ammonia levels than those receiving the alkalinizing compounds (Tables 5 and 6). This was probably related to the pKa of ammonia and the fact that ammonia tends to diffuse from the blood stream to cells of the vital organs more rapidly when the blood pH is high. Ataxia occurred sooner when ammonium salts were infused more rapidly; higher doses (>2 mEq/kg) were tolerated when doses were infused more slowly. This is
undoubtedly due to detoxification through the ornithine-urea cycle since, by assays of the serum of these animals, it was determined there were rapid increases in urea nitrogen. Serum ammonia levels appear to be the most reliable estimate since they portrayed a very high linear F value (Table 2, Graph 3).

Rumen ammonia levels are responsible for, but do not correlate well with, the elevated blood ammonia levels. Ureolysis is faster and pH higher when the urea is not buffered in a large volume of rumen fluid. Urea toxicosis is most acute when the urea is present in a small region of the rumen-reticulum. The reason for this can best be explained by use of a hypothetical but logical comparison. Assume that the pKa of ammonia is 9.0 and a dose of 1.50 g/kg urea is (a) mixed homogeneously in the rumen-reticulum or (b) infused into a small region that is exposed to 10% of the rumen-reticulum surface are.

Case (a) \( \text{TNH}_3 = 80 \text{ mg/100 ml}; \text{pH} = 7.4 \)

\[
\text{pH} = \text{pKa} + \log \frac{\text{NH}_3}{\text{NH}_4}\quad \text{(Henderson-Hasselbalch eq.)}
\]

\[
7.4 = 9.0 + \log \frac{\text{NH}_3}{\text{NH}_4}
\]

\[
\log \frac{\text{NH}_3}{\text{NH}_4} = -1.6; \quad \text{antilog} -1.6 = \frac{1}{39.81}
\]
\[
\frac{\text{NH}_3}{\text{NH}_4^+} = \frac{1.96}{78.04}; \quad \text{pNH}_3 \approx 2.0
\]

\[
\text{pNH}_3 \times \text{surface area} \times X = 2.0 \times X
\]

Case (b) \(\text{TNH}_3 = 240 \text{ mg/100 ml}; \quad \text{pH} = 9.0\)

\[
\text{pH} = \text{pKa} + \log \frac{\text{NH}_3}{\text{NH}_4^+} \quad \text{(Henderson-Hasselbalch eq.)}
\]

\[
9.0 = 9.0 + \log \frac{\text{NH}_3}{\text{NH}_4^+}
\]

\[
\log \frac{\text{NH}_3}{\text{NH}_4^+} = 0; \quad \text{antilog} \ 0 = 1 = \frac{1}{1}
\]

\[
\frac{\text{NH}_3}{\text{NH}_4^+} = \frac{120}{120}; \quad \text{pNH}_3 = 120
\]

\[
\text{pNH}_3 \times \frac{\text{surface area}}{10} \times X = 12.0X
\]

Therefore, ammonia would theoretically diffuse 6.0 times faster in Case (b) than in Case (a). Researchers have apparently assumed that the major pathway for ammonia absorption is through the rumen-reticulum epithelial wall. It would be of value to ascertain what portion is diffused through the lungs after being eructated and inhaled by the mechanism elucidated by Dougherty et al. (1962).

In this study rumen ammonia levels varied greatly in different areas of the rumen-reticulum, especially in cattle. One analysis is not valid as a diagnostic indicator unless it
is a composite sample. Values of 80 to 100 mg NH₃-N/100 ml are probably of diagnostic significance if they represent mean values from at least 3 different locations. Rumen ammonia levels of sheep did not vary as much among different locations. Slower ureolysis in sheep apparently delayed the onset of toxicosis. The autocatalytic action of ureolysis may have deflected a linear blood ammonia regression, based on actual time, to an increasing curvilinear effect regressed on percent death time.

The increase in PCV seen in cattle (Graph 1) and to a lesser extent in sheep (Graph 36) agrees with the results of Roller (1966) and must be a direct result of diuresis. The resulting hemoconcentration would therefore increase the effect of ammonia, potassium, inorganic phosphorus and other substances which may be potentially toxic.

Urea-nitrogen levels in serum increased to approximately 2.0 times in cattle (Graph 4) and 2.8 times in sheep (Graph 38) from control values to peak values when ammonia nitrogen is subtracted from the values. Peak serum urea-nitrogen levels were 2 to 6 times control levels in animals that survived oral doses of urea regardless of the control values. Animals which survived had very high levels of serum urea-nitrogen and a high urine production rate.

Urea formation in the liver is a major pathway whereby ammonia is detoxified. The animals that are able to detoxify
high doses of ammonia must have high levels of urea cycle enzymes due to adaptation plus a ready source of the necessary substrates. The urea cycle is an energy-consuming process, requiring the consumption of 3 moles of ATP whenever 2 moles of ammonia form 1 mole of urea. This may explain why blood urea levels failed to increase and even decreased as time progressed. Regression lines of sheep data appeared curvilinear on an actual time regression.

Blood glucose levels increased at death to ca. 3-5 times normal values (Graph 5, Graph 39). The cause of hyperglycemia is difficult to explain. It does not appear to be due to epinephrine release since it does not correlate well with the excitement state of the animal. Moreover, intravenous administration of ammonium chloride in adrenalectomized rabbits causes hyperglycemia.

It would be valuable to assay insulin and glucagon serum levels during ammonia toxicosis, even without the benefit of knowing a cause-effect relationship. Glucagon activates phosphorylase by a direct action on the liver to increase hepatic cyclic AMP levels through activation of adenyl cyclase. Inorganic phosphate is released in the process and ATP is consumed. Some amino acids cause rapid secretion of glucagon. Glucagon stimulates the conversion of amino acids and lactic acid to glucose. Glucagon increases potassium release from the liver, which may be the result of
phosphorylase activity in promoting glycogenolysis.

Insulin action is antagonistic to glucagon. It decreases cyclic AMP formation and glucose release and increases potassium and phosphorus uptake (Harper, 1969). Furthermore, it decreases urea formation. In ammonia toxicosis it would appear that the action of glucagon is stronger than the action of insulin. Fuiz et al. (1965) suggested that in animals with elevated ammonia production renal glucose production may account for a major proportion of total glucose synthesis. He also reported that gluconeogenesis was significantly increased at pH 7.1 and depressed at pH 7.7, suggesting that both hyperammonemia and acidosis may be responsible for hyperglycemia.

Singer (1969) postulated that hyperglycemia is due to the inhibition of the citrate cycle by fumaric acid released from the urea cycle. He cites instances in which blood urea nitrogen correlated closely with blood glucose levels. In this study blood glucose appeared to correlate more closely with blood ammonia and blood hydrogen ion concentration than with blood urea nitrogen. Animals that survived toxic doses of urea varied considerably in their glucose tolerance. Some had high blood glucose levels when the sampling was discontinued; others displayed a transient rise in blood glucose levels that had returned almost to normal when sampling was discontinued. Glycosuria was the usual occurrence in both
species in the later stages of toxicosis, but there appeared
to be a high variation in the glucose renal threshold.

Acid-base studies disprove the theory that toxicity from
urea in ruminants is due to alkalosis. Indeed, a distinct
acidosis exists during the time when acute symptoms of toxi-
cosis exist. There is a small but definite increase in blood
pH to 25 to 35% death time followed by a curvilinear decrease
to death in both species (Graphs 6 and 40). This agrees with
the report of Roller (1966).

The results of Experiment II correlate well with those
observed in Experiment I. Intravenous administration of
ammonium hydroxide (NH₄OH) usually produced a slight increase
in pH followed by a decline (Table 6). The intravenous
administration of NH₄OH should simulate toxicosis from orally-
administered urea to ruminants. It seems plausible that more
samplings of the animals that were given the ammonium salts
would have detected a transient pH increase before the usual
decline. All ammonium salts appeared to cause a pH decrease
which was least evident with NH₄OH and most pronounced with
ammonium chloride (NH₄Cl). The observed state of acidosis
confirms the results of Singer (1969).

Sodium hydroxide (NaOH) increased the pH ca. 0.2 pH units
when given at milliequivalent doses of 2 to 3 times the doses
of ammonium compounds. At these levels the NaOH-treated
animals were still standing and alert, in spite of being
alkalotic, while the animals treated with ammonium salts were recumbent. This also disproves the alkalosis theory of urea toxicosis. The animals receiving sodium hydroxide intravenously (Tables 5 and 6) displayed a tendency toward decreased blood ammonia levels. This lends support to the principle of the Henderson-Hasselbalch equation which states that ammonia is more apt to diffuse from the blood stream when the pH is raised.

A visual comparison of blood pH values with \( pCO_2 \), \( pO_2 \) and bicarbonate reveals striking apparent correlations. Blood \( pO_2 \) (Graph 52) and blood \( [HCO^-_3] \) (Graph 53) appear to correlate positively with blood pH (Graph 40); blood \( pCO_2 \) (Graph 51) seems to correlate negatively with blood pH. The ratios of \( [H_2CO_2]:[HCO^-_3] \) were as follows: 0 time, 1:20.1; 35% time (peak pH), 1:26.8; death, 1:8.6. In converting \( pCO_2 \) to \( [H_2CO_2] \) a conversion factor of 0.03 was used. There was apparently a small degree of compensation, so it was concluded the pH change was largely the result of an uncompensated respiratory acidosis. This agrees with the results of Bicknell (1965) and Rash (1967). However, even though the statistical treatment of the blood gas analysis revealed significance, only two sheep were involved. The results of the blood gas analysis on three animals administered ammonium compounds intravenously do not appear so conclusive (Tables 5 and 6). There seems to have been an attempt to
compensate for the pH changes. The acidosis probably serves to protect the vital organs from the action of ammonia.

Acetylcholinesterase studies did not substantiate the carbamate theory in urea toxicosis of ruminants. This agrees with the conclusions of Wilson et al. (1968). Carbamate insecticides are cholinesterase inhibitors and depress ΔpH. In this study ΔpH was actually increased in urea toxicosis (Graphs 8 and 41). A study of cholinesterase graphs, pH graphs, the hydrogen ion graph and Tables 5 and 6 show ΔpH is influenced by blood pH. An increase in ΔpH is undoubtedly a result of the inability of blood to buffer the acetic acid, released during the test, due to depressed bicarbonate levels. The high negative correlation between ΔpH and pH indicate a pure physico-chemical relationship rather than changes based on acetylcholinesterase activity.

Serum calcium levels increased in both cattle and sheep (Graphs 9 and 42), in a small but significant mode. It is postulated this is due to increased hydrogen ion concentrations and the effect of lactic acid levels on the mobilization and chelation of calcium.

Serum magnesium levels increased in both cattle and sheep (Graphs 10 and 43). Magnesium, like potassium and phosphorus, is predominantly an intracellular ion. Extracellular increases of magnesium may be due to a "leaking" through the cell membrane or mobilization with glucose and
lactate. As with the case of calcium, there appears to be a high correlation between magnesium and hydrogen ion concentration.

Serum sodium levels increased in both cattle and sheep (Graphs 11 and 44). If hemoconcentration is considered, sodium levels do not appear to change.

Serum potassium increased in both cattle and sheep (Graphs 12 and 45) about two-fold to possible cardiotoxic levels. Roller (1966) reported an increase in serum potassium and a concomitant increased potassium and lowered sodium content of erythrocytes. This would disprove the theory that changes in serum potassium and sodium are due to an intracellular shift in erythrocytes. Regression plots of potassium seem to possess a high negative correlation with pH plots and a high positive correlation with the antilog of pH ([H⁺]). This is to be expected as hyperkalemia is usually associated with acidosis. Bicknell (1965), however, reported that serum potassium levels were much higher in pigs after infusion of ammonium salts than after infusion of hydrochloric acid, so the hyperkalemia does not appear to be a pure function of acidosis.

Serum chloride levels of cattle decreased only when the effect of hemoconcentration is considered (Graph 13). This could be explained by the increased excretion of the chloride ion in the urine (Graph 33) in electrochemical
balance with the increased excretion of the cations: \( \text{NH}_4^+ \), \( \text{Ca}^{++} \), \( \text{Mg}^{++} \), \( \text{K}^+ \), \( \text{Na}^+ \) and \( \text{H}^+ \). Sheep serum chloride levels decreased significantly (Graph 46), but not urinary excretion of chloride (Graph 64). This can only be explained by an intracellular chloride shift, especially into erythrocytes. The serum chloride values of sheep appear to be higher than normal limits, which may have been caused by an inaccurate chloride standard.

Serum inorganic phosphorus (P) levels decreased and increased curvilinearly in both cattle and sheep (Graphs 14 and 47). They seemed to correlate moderately well with \([\text{H}^+]\), without implying a cause-effect relationship. This could be explained by movement of P from the cell in a manner similar to that of K and Mg. Much energy is expended in urea synthesis in the liver and the activation of phosphorylase with a release of inorganic P. Indeed, the increase of serum inorganic P would suggest a lack of energy utilization and a reduction of oxidative phosphorylation.

The enzymes, SGOT and SGPT, increased linearly in cattle (Graphs 15 and 16), and appeared to correlate moderately well with serum total protein (Graph 17). The enzymes are not notably organ-specific, but increases may signify damage to cell membranes of the heart, liver or muscles. Serum total protein increased even when adjusting for hemoconcentration. Whether the increase of all 3 parameters signify a loss of
intracellular elements or increased protein synthesis is not clear. Singer (1965) reported an increase in serum amino acids, but did not report serum protein values.

SGOT, SGPT and serum total protein of sheep all decreased and increased with quadratic significance (Graphs 48, 49, and 50), in spite of a small number of observations. The apparent correlations of the 3 parameters, and especially the reversibility of the changes, represent an enigma to the author.

Blood lactate of sheep increased linearly over two-fold (Graph 54) from 4.8 to 10.1 mEq/l. This explains part of the replacement of the bicarbonate ion which was reduced curvilinearly from 23.5 to 15.6 mEq/l. Keul et al. (1967) reported in humans that ca. 95% of the decrease in standard \([\text{HCO}_3^-]\) was linked to an increase in lactate and pyruvate.

Lactic acid increases in blood during exercise, but it cannot be said in this study that hyperlactacidemia is due to excitement. The lactate increases correlate well with blood and serum levels of ammonia and glucose, but not with the onset of symptoms characterized by muscle contractions. If it is true that ammonia interferes with oxidative phosphorylation hyperlactacidemia could occur without muscular exertion. The blood pyruvate increase (Graph 55) would be expected.

Blood citrate levels decreased and increased in a low
order of significance (Graph 56), but the number of observations was small. This is predominantly a mitochondrial substrate, but seems to correlate with SGOT, SGPT and serum total protein changes.

The change in urine production rate, as evaluated by urine output from the ureteral cannulas, is an interesting phenomenon. Urine production increased rapidly within minutes after oral administration of urea in all animals with surgically fitted cannulas (Graphs 18 and 57). The production rates increased rapidly in a regressional quadratic curve until 40 to 50% death time in sheep and 50 to 65% death time in cattle. Thereafter urine production rate decreased rapidly and ceased > 2 minutes before death in all cases except animal b-6 in Bovine-urea-VII trial.

The computer regression plot failed to reflect the true dynamic change in urine production, especially just preceding death. Very frequent sampling, though impractical, would have rectified this error. Each urine sample represented mean parameter values for the collection period rather than absolute values at the termination of the collection period.

The rapid incline of the regression plot of urine production appears to correlate better with blood ammonia levels rather than with blood urea-N levels. Urea in the blood stream is considered a powerful diuretic. Gäertner (1963) reported that rumen ammonia elevations in goats
decreased serum antidiuretic hormone (ADH) and increased renal free water clearance. This mechanism would conserve N in a deficiency and aid in detoxification during urea toxicosis. The reduction and final complete cessation of urine production are undoubtedly due in part to increased ADH production induced by hemoconcentration. Huber (1969), however, reported that renal flow and filtration rate were significantly depressed by elevated blood lactate levels and depressed pH.

Urine flow is the best single clinical evaluation of the animal's state of toxicosis. Animals that survived trials invariably had very high urine production rates. Conversely, anuria was usually a premonitory sign of impending death.

Urine ammonia levels were influenced by blood ammonia levels, but it appeared excretion was limited somewhat by urine pH. In cattle, levels of NH$_3$-N in urine (Graph 19) significantly increased with a concomitant increase in urine [H$^+$] (Graph 23). The rate of NH$_3$-N excretion (Graph 20) seems to have been limited after attainment of a certain level, but with a low order of statistical significance.

In sheep, levels of NH$_3$-N in urine (Graph 38) appear to be limited by an increase in urine pH (Graph 40). This is conjectural, also due to a low order of significance. The analysis was not performed, but excretion rate of NH$_3$-N in sheep (NH$_3$-N x urine production rate) appears to have been
severely depressed when regressed on time. These findings seem to verify the fact that ammonia is excreted by the kidney primarily as the ammonium ion and one mole of ammonia requires one hydrogen ion by the following reaction:

\[ \text{NH}_3 + \text{H}^+ \rightarrow \text{NH}_4^+ . \]

Urine urea-N levels of cattle (Graph 21) decreased in a highly significant linear manner and excretion rate of urea-N (Graph 22) correlated well with the quadratic change in urine production. The reason for these changes is not evident to the author. Pragmatically, it appears that urea excretion was related to urine production rate rather than to blood urea concentrations. It is regrettable that urine urea-N determinations were not performed in sheep trials, since they would have helped in determining the influence of urine pH and species variations.

The total N levels in cattle urine samples correlated well with urine urea-N values. The urea-N values reported actually included N from both urea and ammonia. The reported urine urea-N values were ca. 85% of the total N values derived from the Kjeldahl analysis. This meant that 15% of the total N was excreted as other nitrogenous compounds.

It is interesting to note that ammonia excretion represented a small part of the total N excretion, but values of urine NH$_3$-N did reflect the state of hyperammonemia better than did urine urea-N levels. As a percent of reported urine
urea-N, NH\(_3\)-N represented 0.41% of urea-N at 0 time and 7.25% at death. As a percent of reported urine urea-N excretion rate, NH\(_3\)-N represented 0.75% of urea-N at 0 time and 4.41% at death. Teleologically, it appears the animal body is more eager to rid the blood stream of the highly toxic ammonia than it is of the less toxic urea.

Acid-base studies of urine are confusing when the two species are compared. Cattle urine revealed depressed pH and increased \([H^+]\) with low statistical significance; sheep urine revealed increased pH with good significance. However, animals of both species that survived oral doses of urea displayed an apparently significant increase in pH. One questions whether the increase is due to hydroxyl ion from the sustained urine ammonia excretion or to other anions such as bicarbonate.

Excretion of H\(^+\) is a competitive process and is influenced by the other cations. The law of electrochemistry demands that an anion be excreted concomitantly. It appears that in this study chloride excretion of cattle (Graph 64) increased to balance the H\(^+\) and other cations. In sheep it appears that bicarbonate in the alkaline urine may have been an important anion and buffer.

A study of the urine cations and anions does not lead to ready conclusions (Tables 7 and 8). A pH decrease in cattle urine appeared to increase concentrations of ammonia
Table 7. Some urine constituents of cattle

<table>
<thead>
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<th>Parameter</th>
<th>0 time</th>
<th>50% time</th>
<th>100% time</th>
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<tr>
<td>NH$_4^+$</td>
<td>1.57</td>
<td>3.59</td>
<td>12.14</td>
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<tr>
<td>NH$_3$</td>
<td>0.26</td>
<td>0.34</td>
<td>0.67</td>
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<tr>
<td>Urea$^b$</td>
<td>437.</td>
<td>259.</td>
<td>178.</td>
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<tr>
<td>Ca$^{++}$</td>
<td>1.72</td>
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<td>1.63</td>
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<td>Mg$^{++}$</td>
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<td>5.21</td>
<td>6.09</td>
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<td>Na$^+$</td>
<td>40.13</td>
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<td>K$^+$</td>
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<td>Cl$^-$</td>
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<td>pH</td>
<td>8.22</td>
<td>7.98</td>
<td>7.74</td>
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</table>

$^a$Adjusted for pH changes.

$^b$As mEq of ammonia (TNH$_3$).

Table 8. Some urine constituents of sheep

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 time</th>
<th>50% time</th>
<th>100% time</th>
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<td>NH$_4^+$</td>
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<td>4.18</td>
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<td>NH$_3$</td>
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<td>1.32</td>
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<td>Ca$^{++}$</td>
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<td>Mg$^{++}$</td>
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<td>K$^+$</td>
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<tr>
<td>Cl$^-$</td>
<td>15.62</td>
<td>12.24</td>
<td>5.48</td>
</tr>
<tr>
<td>HPO$_4^{2-}$ + H$_2$PO$_4^-$</td>
<td>1.24</td>
<td>1.04</td>
<td>0.89</td>
</tr>
<tr>
<td>pH</td>
<td>7.42</td>
<td>7.69</td>
<td>8.50</td>
</tr>
</tbody>
</table>

$^a$Adjusted for pH changes.
and chloride. Urine excretion rates of these two ions also increased greatly. A pH increase in sheep urine seemed to decrease concentrations of ammonia and chloride. Chloride may be the favored anion for ammonia excretion.

Calcium and magnesium concentrations in urine did not change significantly in cattle and sheep. However, the urinary excretion rates of both ions increased moderately in cattle, possibly due to the pH changes.

Potassium concentrations in cattle urine did not increase significantly, probably due to the competitive action of the hydrogen ion for passage through membranes. This accounts for the increase of potassium concentration in the alkaline urine of sheep. The urinary excretion rates of potassium increased significantly in cattle, probably due to elevated serum levels.

The elevated concentrations of sodium in urine of both cattle and sheep plus the increased excretion rate of the ion in cattle must be a means to maintain urine osmolarity. The identities of the balancing anions are a mystery.

Phosphorus content in sheep urine did not change significantly in the analysis in spite of increased serum levels. In one unreported trial urine phosphorus levels decreased to about 15% of control levels. The low urine phosphorus levels may be a result of alkaline urine or complexation. Of course, urinary phosphorus excretion is
not normally high in ruminant animals.

Toxic Doses of Urea and Time of Death

Cattle

In trials with cattle, lethal doses of urea ranged from 1.50 to 3.00 g/kg body weight and the time of death after administration ranged from 44 to 123 minutes with a mean time ($\bar{x}$) of 69.6 minutes (Table 1). Non-lethal doses were 1.50 g/kg for one animal and 3.00 g/kg in divided doses 170 minutes apart for another animal.

Sheep

Sheep used in the statistical analysis died after receiving urea in doses of 1.50 to 2.00 g/kg body weight and the time of death ranged from 114 minutes to 244 minutes with a mean ($\bar{x}$) time of 155.8 minutes (Table 2). Minimum non-lethal doses ranged from 1.00 to 2.00 g/kg, but the animals were given antidotes.

Other animals lived up to 870 minutes before succumbing to doses of 1.5 to 3.0 g/kg after receiving antidotes. Animals o-15 and o-16 died < 870 minutes and > 643 minutes, respectively, after receiving antidotes. Acetic acid was given per os in addition to intravenous infusion of proposed antidotes. Both animals appeared normal one hour after treatment, but had elevated rumen pH and ammonia levels.
at death. The acetic acid had apparently diffused from the rumen and allowed ureolysis and ammonia diffusion to resume.

Development of Symptoms

Cattle

Symptoms developed rapidly in cattle, progressing from uneasiness to facial twitching, hyperexcitability, salivation, bloating and tetany. Many animals showed strychnine-like seizures and occasionally resembled animals poisoned by chlorinated hydrocarbon insecticides with extreme hyperexcitability. Animals usually died after severe intermittent tonoclonic spasms. Body temperature readings ≤ 108.6°F were observed in these animals.

Sheep

Symptoms in sheep developed more slowly and included a period of depression followed by twitching of the ears and facial muscles. Bloating, salivation and dyspnea were common, but most sheep in the experiments did not display the extreme hyperexcitability and severe tetany; animals infused with ammonium salts more commonly displayed hyperexcitability and tetany. Most animals regurgitated rumen contents a few minutes prior to death and all displayed tonoclonic spasms preceding death. Hyperthermia was not as marked in sheep as in cattle.
Possible causes of symptoms

Fasted ruminants and those on low quality roughages (Clifford et al., 1967) are apparently more susceptible to urea toxicosis due to high rumen urease levels, a moderate pH and low levels of substrates and assimilating microorganisms. In this study "physiologically depressed" animals, those that appeared dull and phlegmatic, had relatively high control serum urea-N levels. These animals would withstand relatively high oral doses of urea. Perhaps the rumens of these animals contained low populations of urease-containing microorganisms. Inhibited ureolysis would allow efficient detoxification of blood ammonia when associated with increased activity of urea cycle enzymes and substrates.

Animals that were well hydrated and displayed high urine production rates withstood lethal doses of urea longer than did dehydrated animals. The urine flow is probably a mechanism whereby high levels of blood ammonia that has escaped detoxification in the liver are excreted. Urine ammonia excretion is certainly not a "front line" method of ammonia detoxification. The reduced urine flow is probably due to hyperlactacidemia, acidosis and increased levels of ADH.

Rumen atony and bloat are, according to other investigators, caused by alkalosis of the rumen and
hyperammonenia. The cause of regurgitation in sheep is not explained, but most animals displayed moderate degrees of reticulitis and ruminitis.

The development of muscle spasms and tics can only be explained empirically as a toxic action on the central nervous system. This may also explain why respirations are shallow and there appears to be only partial compensation of the acidosis.

The muscle stiffness, tetany and ataxia are apparently not due to an inhibition of acetylcholinesterase activity. The administration of calcium and magnesium salts reduced the symptoms of tetany, but not the local muscle spasms. This happens in spite of elevated levels of these elements in the serum. Lactic acid is known to cause muscle stiffness and is also thought to chelate calcium, and perhaps magnesium.

Symptoms of tetany are associated with hypomagnesemia. During toxicosis there is a moderate increase of magnesium and large increases in ammonia and inorganic phosphorus in serum. The theory is proposed that magnesium may be partially bound by increased serum protein and amino acids, perhaps chelated by increased lactic acid and perhaps complexed as MgNH$_4$PO$_4$, which has a solubility product (Ksp) of 2.5 x 10$^{-13}$.

During toxicosis serum levels commonly attain values of:
\[ \text{NH}_4^+ = 4.2 \text{ mg/100 ml (3.0 mmoles/\ell)} \]
\[ \text{Mg}^{++} = 3.0 \text{ mEq/\ell (1.5 mmoles/\ell)} \]
\[ \text{In. P} = 7.0 \text{ mg/100 ml (2.25 mmoles/\ell)} \]

(With \( pK_3 = 11.8 \), \( \text{PO}_4^{3-} = \text{ca.} \ 9.0 \times 10^{-8} \text{ M} \))

Solving for the ion product: \( 3.0 \times 10^{-3} \text{ M} \text{NH}_4 \times 1.5 \times 10^{-3} \text{ M} \text{Mg}^{++} \times 9.0 \times 10^{-8} \text{ M} \text{PO}_4^{3-} = 4.0 \times 10^{-13} \). This value exceeds the Ksp of \( \text{MgNH}_4\text{PO}_4 \). It must be admitted that blood serum does not present an excellent model of an inorganic equilibrium system, due to the existence of metal ion-binding, especially of the divalent ions. Nevertheless, the principle seems plausible in serum, and especially in urine.

Hyperthermia is undoubtedly caused by the extreme muscle exertion.

**Possible causes of death**

Several investigators have stated that symptoms and death from urea toxicosis in ruminants are due to the toxic action of ammonia. The statement rhetorically oversimplifies the situation, even if a target organ is named as the site of action. Indeed, death from any cause must be a complicated syndrome with a many-faceted pathogenesis. One questions which parameters are significant in the biochemical and structural alterations in disease and whether the changes are irreversible and will lead to death of the organism. Above
all, it is difficult to determine whether a significant change is a cause or an effect.

Finally, the conditions which are responsible for death of the animal from ammonia toxicosis are speculative. Serum potassium levels near the time of death exceed accepted cardiotoxic levels. Chelation of part of the serum calcium would enhance potassium inhibition of the myocardium. The electrocardiograms of Wilson et al. (1968) and Rumsey et al. (1969) lend support to this theory. Rash (1967) reported cardiac fibrillation in poisoned animals.

The cardiotoxic action evolves even in decerebrated animals. Nevertheless the action of ammonia by direct or indirect means on the respiratory center must be considered, since respirations often cease before terminal heart fibrillation. There seems to be only moderate compensation or response to the acidosis by hyperventilation. Of course, the hypoxia and hypercapnia seen as toxicosis develops may be due to decreased action of either the heart (bradycardia) or the lungs.

Regardless of the exact mechanisms, there is good evidence that there is inhibition of aerobic metabolism. The development of hyperlactacidemia, indicative of increased anaerobic glycolysis, suggests this. Nishhi et al. (1964) in mitochondrial studies reported that ammonia inhibited isocitrate dehydrogenase and malic dehydrogenase action and
glutamate formation from α-ketoglutarate. There was a great reduction in total pyridine nucleotide, which seemed to be converted to an unknown substance. The citrate cycle was inhibited with a resultant decrease of oxidative phosphorylation. Prior et al. (1968) stated they felt that depletion of reduced pyridine nucleotides in liver and blood may cause significant adverse changes in intermediary metabolism when high concentrations of ammonia are introduced. Energy for reduction of the oxidized forms of the pyridine nucleotides is derived from the citrate cycle via oxidative phosphorylation. Inhibition of the citrate cycle would deplete the reduced pyridine nucleotides and ATP.

Possible reasons for pathological changes

Very little morphological pathology was evident. The subepicardial hemorrhages can pragmatically be considered agonal in nature. The cause of lesions suggestive of hemorrhages in the central nervous system is questionable, as is the cause of the appearance of hematuria. Decreased levels of ATP in blood will inhibit clotting action of thrombocytes. An inhibition of oxidative phosphorylation may be the cause of small hemorrhages in many diseases.

Congestion of the liver and lungs may well be passive congestion due to decreased heart action. The engorgement of the kidneys may be both active and passive. Certainly the kidneys appear to be the site of the most evident
histopathological changes. Of course, nephrosis and damage to kidney tubule epithelial cells appear to be surprisingly reversible or repairable.
A state of alkalosis is not the cause of death from orally administered urea in ruminants. Indeed, acidosis develops, but it is probably not the cause of death. Ammonium carbamate was disproved as a toxic principle, since acetylcholinesterase activity was not depressed.

It is proposed that the primary cause of death is the inhibition of the citrate cycle and of oxidative phosphorylation. Death may be due to cardiac block from hyperkalemia and chelation of serum calcium plus involvement of the respiratory center.

Lethal doses of urea for a ruminant species varies considerably under the same environmental conditions. In this study cattle died sooner ($\bar{x} = 69.6$ minutes) than sheep ($\bar{x} = 155.8$ minutes). Ruminal urease levels and rumen fluid buffering power apparently vary due to variation in feedstuffs and microorganism populations. Doses of urea that are well-mixed in the rumen contents are less toxic than similar doses confined to a small area of the rumen-reticulum. There is an apparent variation in animals' abilities to render blood ammonia non-toxic by reforming urea. Animals with high urine production rates resist toxicosis better than dehydrated animals.

Symptoms of urea toxicosis in both species include
depression, uneasiness, twitching of the ears and facial muscles, bloating, salivation, dyspnea, tonoclonic spasms and death. Tetany, hyperexcitability and hyperthermia are common in cattle; regurgitation is common in sheep. Symptoms from the intravenous administration of ammonium compounds are similar to symptoms from orally-administered urea.

Sodium glutamate appeared to be a better intravenous antidote than a ketoglutaric acid. Further study is needed in the development of antidotes. Injections of arginine and mixtures of aspartic acid and ornithine are proposed as antidotes, since they have been effective in non-ruminant animals. It is proposed also that an effective and efficient intravenous antidote could contain formalin with calcium, magnesium, sodium and chloride salts. Intraruminal injections of 5% acetic acid is effective if given soon enough or as an adjunctive therapy with large amounts of cold water.

Diagnosis of urea toxicosis is best made by evaluating serum or blood ammonia, levels > 2.0 mg NH₃-N/100 ml being significant. Levels exceeding 100 mg NH₃-N per 100 ml rumen fluid are probably diagnostically significant if from a composite sample.

Further study is needed of the cellular and humoral fractions in the evaluation of enzyme and hormonal relationships during ammonia toxicosis. It will be worthwhile to learn what specific enzymes and biochemical pathways are
affected so that the exact mechanism of ammonia toxicosis can be ascertained. Knowledge regarding the interrelationships of ammonia metabolism and the adrenal hormones, insulin and glucagon will be valuable. A great economic value will be gained by agriculture and mankind when practical methods are developed that will reduce the rate of ureolysis, whereby toxicity will be minimized and utilization of feed urea maximized.
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APPENDIX A: CATTLE REGRESSION GRAPHS
Graph 1. Bovine blood packed cell volume (lin P<.005, quad ns)
Graph 2. Bovine blood ammonia (lin P<.005, quad ns, cov P<.100)
Graph 3. Bovine serum ammonia (lin P<.005, quad ns, cov P<.250)
Graph 4. Bovine serum urea nitrogen (lin $P<.005$, quad $P<.010$, cov $P<.250$)
Graph 5. Bovine blood glucose (lin P<.005, quad ns, cov ns)
Graph 6. Bovine blood pH (lin P<.005, quad P<.005, cov ns)
Graph 7. Bovine hydrogen ion concentration (lin $P<.005$, quad $P<.005$, cov $P<.250$)
Graph 8. Bovine blood acetylcholinesterase activity (lin P<.005, quad ns, cov P<.010)
Graph 9. Bovine serum calcium (lin P<.005, quad P<.100, cov P<.005)
Graph 10. Bovine serum magnesium (lin P<.005, quad P<.050, cov P<.050)
Graph 11. Bovine serum sodium (lin P<.005, quad P<.005, cov ns)
Graph 12. Bovine serum potassium (lin P<.005, quad P<.005, cov P<.250)
Graph 13. Bovine serum chloride (lin ns, quad ns, cov P<.100)
Graph 14. Bovine serum inorganic phosphorus (lin P<.005, quad P<.005, cov P<.010)
Graph 15. Bovine serum glutamic oxaloacetic transaminase (lin $P<.005$, quad ns, cov $P<.025$)
Graph 16. Bovine serum glutamic pyruvic transaminase (lin P<.005, quad ns, cov ns)
Graph 17. Bovine serum total protein (lin P<.005, quad P<.250, cov P<.005)
Graph 18. Bovine urine production (lin ns, quad P<.010)
Graph 19. Bovine urine ammonia (lin P<.005, quad P<.100)
Graph 20. Bovine urine ammonia excretion rate (lin P<.250, quad ns)
Graph 21. Bovine urine urea nitrogen (lin P<.005, quad ns)
Graph 22. Bovine urine urea nitrogen excretion rate (lin ns, quad P<.005)
Graph 23. Bovine urine hydrogen ion concentration (lin P<.250, quad ns)
Graph 24. Bovine urine hydrogen ion excretion rate (lin ns, quad ns)
Graph 25. Bovine urine calcium (lin ns, quad $P<.250$)
Graph 26. Bovine urine calcium excretion rate (lin P<.050, quad ns)
Graph 27. Bovine urine magnesium (lin ns, quad ns)
Graph 28. Bovine urine magnesium excretion rate (lin $P < .100$, quad $P < .025$)
Graph 29. Bovine urine sodium (lin P<.005, quad ns)
Graph 30. Bovine urine sodium excretion rate (lin P<.100, quad P<.100)
Graph 31. Bovine urine potassium (lin P<.005, quad ns)
Graph 32. Bovine urine potassium excretion rate (lin $P < .250$, quad $P < .025$)
Graph 33. Bovine urine chloride (lin $P < .025$, quad ns)
Graph 34. Bovine urine chloride excretion rate (μEq/min) excreted in urine.

Graph 34, Bovine urine chloride excretion rate (μEq/min) excreted in urine.

P<.025, quad P<.100
Graph 35. Bovine body temperature (lin P<.025, quad ns)
APPENDIX B: SHEEP REGRESSION GRAPHS
Graph 36. Ovine blood packed cell volume (lin P<.250, quad ns)
Graph 37. Ovine blood ammonia (lin P<.005, quad P<.005)
Graph 38. Ovine serum urea nitrogen (lin P<.005, quad ns)
Graph 39. Ovine blood glucose (lin $P < 0.005$, quad $P < 0.005$)
Graph 40. Ovine blood pH (lin P<.005, quad P<.005)
Graph 41. Ovine blood acetylcholinesterase activity
   (lin P<.100, quad P<.250)
Graph 42. Ovine serum calcium (lin \( P < .005 \), quad \( P < .005 \))
Graph 43. Ovine serum magnesium (lin P<.005, quad P<.005)
Graph 44. Ovine serum sodium (lin P<.100, quad ns)
Graph 45. Ovine serum potassium (lin \( P < .005 \), quad \( P < .005 \))
Graph 46. Ovine serum chloride (lin P<.025, quad ns)
Graph 47. Ovine serum inorganic phosphorus (lin P<.005, quad P<.005)
Graph 48. Ovine serum glutamic oxaloacetic transaminase (lin ns, quad P<.050)
Graph 49. Ovine serum glutamic pyruvic transaminase (lin ns, quad P<.250)
Graph 50. Ovine serum total protein (lin ns, quad P<.005)
Graph 51. Ovine blood pCO₂ (lin P<.050, quad P<.005)
Graph 52. Ovine blood $pO_2$ (lin $P<.050$, quad $P<.250$)
Graph 53. Ovine blood bicarbonate (lin P < .025, quad P < .100)
Graph 54. Ovine blood lactic acid (lin P<.005, quad ns)
Graph 55. Ovine blood pyruvic acid (lin $P<.250$, quad $P<.250$)
Graph 56. Ovine blood citric acid (lin ns, quad P<.250)
Graph 57. Ovine urine production (lin $P < .250$, quad $P < .025$)
Graph 58. Ovine urine ammonia (lin ns, quad ns)
Graph 59. Ovine urine pH (lin P<.025, quad ns)
Graph 60. Ovine urine calcium (lin ns, quad ns)
Graph 61. Ovine urine magnesium (lin $P < .025$, quad ns)
Graph 62. Ovine urine sodium (lin ns, quad P<.250)
Graph 63. Ovine urine potassium (lin P<.100, quad P<.100)
Graph 64. Ovine urine chloride (lin ns, quad ns)
Graph 65. Ovine urine phosphorus (lin ns, quad ns)
Graph 66. Ovine rumen pH (lin $P<.005$, quad $P<.050$)